

How to optimize patch testing with diphenylmethane diisocyanate

MALIN FRICK-ENGFELDT, MARLÉNE ISAKSSON, ERIK ZIMERSON AND MAGNUS BRUZE

Department of Occupational and Environmental Dermatology, Malmö University Hospital,
Lund University, S-205 02 Malmö, Sweden

We have previously shown that patch test preparations of polymeric diphenylmethane diisocyanate (PMDI) are more stable than preparations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI). This study was conducted to (i) investigate whether PMDIs yield as many positive reactions as 4,4'-MDI, (ii) study concurrent reactions to 4,4'-MDI and 4,4'-diaminodiphenylmethane (4,4'-MDA), and (iii) follow the course of positive reactions during 4 weeks. It was shown that PMDIs detect as many positive reactions as 4,4'-MDI. Thus, they are better patch test agents being more stable than preparations of 4,4'-MDI. We recommend that PMDIs with a monomer content of at least 35% is used in 2.0% petrolatum (pet.) (i.e. monomer patch test concentration approximately 0.7%). It was shown that reactions to 4,4'-MDI and PMDIs appear late and we recommend readings on both day (D) 3/4 and D7. 4,4'-MDA was shown to be a good marker for 4,4'-MDI and patch testing with 4,4'-MDA in 0.25% pet. can be used instead of PMDI. Concomitant reactions to 4,4'-MDI and 4,4'-MDA are probably not caused by conversion of 4,4'-MDI into 4,4'-MDA by reaction with water. Another explanation is a path of reactions leading to ureas and MDI conjugates with skin constituents, which are hydrolysed into 4,4'-MDA. This complex process depends upon several factors and might explain why positive MDI reactions appear after D7.

Key words: allergic contact; CAS 101-68-8; CAS 9016-87-9; dermatitis; diphenylmethane-4,4'-diisocyanate; MDI; PMDI; polymeric MDI. © Blackwell Munksgaard, 2007.

Accepted for publication 19 April 2007

Isocyanates are highly reactive substances that bear attributes associated with contact allergens, i.e. ability to react with proteins in the skin and thereby induce an immunological state. However, there are relatively few reports found in the literature that describe contact allergy from isocyanates. It has been proposed that the low frequency of reported cases of contact allergy from diphenylmethane diisocyanate (MDI) could partly be because of inadequate patch test diagnostics depending on the fact that (i) commercially available patch test preparations polymerize relatively rapidly, leaving a too low amount of residual monomer in the preparations for allergic reactions to be elicited and (ii) allergic reactions sometimes are observed not only on day (D) 3/4 but also on D7 or later (1–3).

Commercially available patch test preparations of MDI consist of 1 monomeric isomer called diphenylmethane-4,4'-diisocyanate (4,4'-MDI). We have shown that even though the expiry date had not yet passed, the concentration of 4,4'-MDI in commercial test preparations, obtained from 8 European and 4 American dermatology depart-

ments, was so low that patch testing with the same could not be considered reliable (1). Furthermore, 4,4'-MDI might be a poor representative of the products that patients are in fact exposed to because within industry, technical-grade isocyanate products often are used. Technical MDI products, often referred to as polymeric MDI (PMDI) generally contain a complex mixture of isocyanates where 4,4'-MDI predominates followed by oligomers containing predominately 3–6 aromatic rings but also smaller amounts of oligomers containing a higher number of rings. The amount of 2,2'-MDI and 2,4'-MDI in these mixtures is often low (4). Because PMDI might be a better representative of the products patients are in fact exposed to, we decided to investigate and compare the stability of petrolatum (pet.) patch test preparations of 4,4'-MDI and PMDI over time when stored under 3 different conditions, namely at room temperature, in a refrigerator, and in a freezer (2). It was concluded that the pet. preparations of the PMDI investigated were better suited to use when patch testing

patients exposed to MDI because they were more stable and more homogenous than those of 4,4'-MDI (2).

The present study was initiated to:

- (1) study whether the proposed chemically more stable and more homogenous test preparations of PMDI yielded as many positive reactions as test preparations of 4,4'-MDI (2);
- (2) investigate the, in literature often reported, concurrent reactions to 4,4'-MDI and its corresponding amine 4,4'-diaminodiphenylmethane (4,4'-MDA) (3, 5–10);
- (3) follow the development and course of positive patch test reactions to the investigated substances because, from our experience, these reactions might appear late (after D7), which has also been shown when patch testing with, e.g. gold sodium thiosulfate, corticosteroids, and methacrylates (11–13).

Materials and Methods

Subjects

12 patients, 7 females and 5 males (mean age 41 years, range 17–61) from the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden, previously known to have a delayed hypersensitivity to 4,4'-MDA, participated in the study. 4 of the 12 (patients 1–4) also had a previously known contact allergy to 4,4'-MDI and/or products containing MDI (Table 1).

Patch test preparations

3 products containing MDI substances were patch tested. 2 products contained PMDI (i.e. mixtures consisting of both monomeric as well as oligomeric MDI), and 1 product contained only 2 isomers of monomeric MDI (Table 2). All patients were patch tested with dilution series in 7 steps (2.0, 0.63, 0.20, 0.063, 0.020, 0.0063, and 0.0020%, w/w) of 4,4'-MDI from TCI Europe N.V. (Zwijndrecht, Belgium), 617P21-Hardener for 617H37 PEDILEN (Pedilen) from Otto Bock Scandinavia AB (Norrköping, Sweden), Lupranat M 20 S (Lupranat) from BASF AB (Göteborg, Sweden), and Desmodur[®] 2460M (Desmodur) from Bayer AB (Göteborg, Sweden), which were all prepared in pet. at our department. Additionally, a dilution series of 4,4'-MDA in 7 steps (0.94, 0.50, 0.16, 0.050, 0.016, 0.0050, and 0.0016%, w/w) was prepared in pet. at our laboratory by diluting a pet. test preparation of 0.94% (w/w) 4,4'-MDA obtained from Chemotechnique

Diagnostics (Vellinge, Sweden). The dilution steps of 4,4'-MDA were prepared to be equimolar to the dilution steps of 4,4'-MDI apart from the highest concentration of 4,4'-MDA, which should have been 1.6%. However, because of Swedish work regulations, it was not allowed to be higher than 0.94%. Patch testing with dilution series was performed to (i) exclude that late patch test reactions were as a result of active sensitization because it has been shown that reactions to low concentrations appear later than reactions to high concentrations and (ii) interpret and possibly exclude cross-reactivity because an individual who has been primarily sensitized to a substance is generally supposed to react stronger (i.e. reacts to lower concentrations) to this substance than to a cross-reacting substance. Pet. was used as vehicle. Normally, it is recommended to avoid diluting in pet. because it is difficult to control that the allergen is evenly distributed in the preparation. However, in this case it was necessary to use pet. because isocyanates can react with other solvents that are normally used.

Patch test technique

The patch test technique with Finn Chambers[®] (8 mm Ø) (Epitest Ltd Oy, Tuusula, Finland) on Scanpor[®] (Norgeplaster A/S, Vennesla, Norway) tape was used. All patch test preparations were applied by the same laboratory technician in amounts of approximately 20 mg as recommended by Bruze et al. (14). The patch tests were left on the back for 48 hr and read for the first time after an additional 48 hr, i.e. on D4. Thereafter, patch tests were read twice a week for up to 4 weeks. Tables 3–4 show on which days (after application) the tests were read. Patch test reactions were scored according to ICDRG criteria (15). Additional gradings were used, strong + and ++ reactions were graded +(+) and ++(+), respectively.

Controls

20 controls among patch-tested consecutive dermatitis patients from the Department of Occupational and Environmental Dermatology, Malmö University Hospital, were tested with Pedilen 2% pet (w/w), Lupranat 2% pet (w/w), and Desmodur 0.63% pet (w/w). The patch tests were read on D4 and D7.

Ethics

The study was approved by the ethics committee of the Faculty of Medicine, Lund University, and conducted in accordance with the ethical standards specified in the Declaration of Helsinki and

Table 1. History of positive patch test reactions in the 4 patients with known contact allergies to 4,4'-MDI and/or MDI-containing work products*. For comparative reason, patch test reactions shown in the present study are presented until day (D) 14

Patient no.	Test preparation (%, w/w, in petrolatum)	Previous investigation			Retest		Present study			
		D3	D7	D10	D3	D7	D3	D7	D10	D14
1	4,4'-MDI 2.0	–	–	++	–	–	–	(+)	(+)	+
	4,4'-MDA 0.5	–	–	+	–	–	–	(+)	++	++
	Isocyanate lacquer 2.0	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Desmodur 2.0	NT	NT	NT	–	–	+	+	+(+)	+
	Pedilen 2.0	NT	NT	NT	–	–	–	(+)	++	+
	Pedilen 0.63	NT	NT	NT	–	–	–	–	–	(+)
2	4,4'-MDI 2.0	–	–		–	–	++	(+)	(+)	(+)
	4,4'-MDA 0.5	–	+++		–	–	++	+++	++	++
	Isocyanate lacquer 2.0	–	++		NT	NT	NT	NT	NT	NT
	Desmodur 2.0	NT	NT		–	–	+	+++	++	+
	Desmodur 0.63	NT	NT		–	–	+	++	++	+
	Pedilen 2.0	NT	NT		–	–	(+)	++	+	(+)
3	Pedilen 0.63	NT	NT		–	–	(+)	(+)	–	–
	4,4'-MDI 2.0	+	+		Not performed		–	(+)	++(+)	++
	4,4'-MDA 0.5	+	++		(+)	++	+++	++		
	Isocyanate lacquer 2.0	++	++		NT	NT	NT	NT		
	Desmodur 2.0	NT	NT		++(+)	++(+)	++	++(+)		
	Desmodur 0.63	NT	NT		–	++	++(+)	+		
4	Pedilen 2.0	NT	NT		–	++	++	++(+)		
	Pedilen 0.63	NT	NT		–	–	(+)	+		
	4,4'-MDI 2.0	–	–		+++		(+)	(+)	(+)	(+)
	4,4'-MDA 0.5†	+	++		++		++(+)	++(+)	++	++
	Desmodur 1.0	?	?		NT		NT	NT	NT	NT
	Desmodur 1.0 retested	+++			NT		NT	NT	NT	NT
	Desmodur 2.0	NT	NT		+++		++(+)	+	+	++(+)
	Desmodur 0.63	NT	NT		++		++(+)	+	+	+
	Pedilen 2.0	NT	NT		++		++(+)	(+)	+	(+)
	Pedilen 0.63	NT	NT		++		(+)	(+)	–	–

4,4'-MDI, diphenylmethane-4,4'-diisocyanate; 4,4'-MDA, 4,4'-diaminodiphenylmethane; NT, not tested.

*Patients 1–3 are further described in reference 3.

†In the initial investigation, the patient was tested with 4,4'-MDA in 0.25% petrolatum.

the ICH guidelines on Good Clinical Practice. All patients gave informed written consent to participate in the study.

Chemical analysis of the products containing MDI

The 3 products labelled to contain either PMDI or monomeric MDI isomers were characterized using liquid chromatography mass spectrometry and quantified with a chemiluminescent nitrogen detector as previously described by Frick-Engfeldt et al. (2) (Table 2).

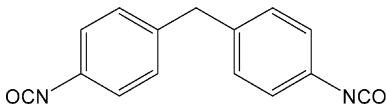
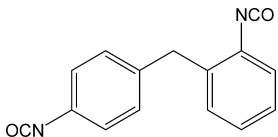
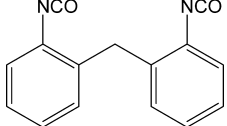
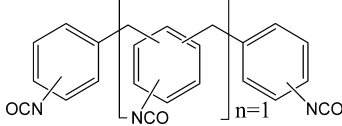
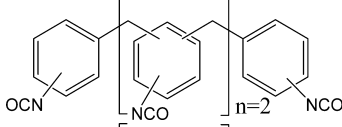
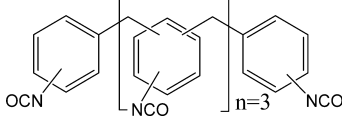
Results

In Tables 3 and 4, the patch test reactions to 4,4'-MDI, 4,4'-MDA, and the MDI products are shown. Figure 1 shows the total number of positive test reactions (including all dilutions) to the MDI-containing products and to 4,4'-MDI. Figure 2 shows the accumulated number of patients positive to each MDI-containing product as well as 4,4'-MDI and 4,4'-MDA. Desmodur detected the highest number of positive tests of all isocyanates tested followed by Pedilen, 4,4'-MDI,

and Lupranat. In Figs 3 and 4, the outcome of readings on D4, D7, and beyond D7 are illustrated for all the investigated substances. All 4 patients with a previously known contact allergy to 4,4'-MDI and/or MDI-containing work products were positive to Desmodur within ordinary readings (i.e. on D4 and/or D7). 3 were positive to Pedilen, 2 to Lupranat, and 1 to 4,4'-MDI with readings on D4 and D7. With readings after D7, Pedilen would have picked up all 4 patients, 4,4'-MDI would have picked up 3, and Lupranat 2. Within ordinary readings, 2 of the 8 patients with no previously known contact with MDI-related products reacted to Desmodur, 2 to Lupranat, 1 to Pedilen, and 1 to 4,4'-MDI, respectively. With readings after D7, Desmodur picked up 4 positive patients, Pedilen 3, 4,4'-MDI 3, and Lupranat 3 patients. Furthermore, 3 of 12 patients tested with 0.5% 4,4'-MDA (the concentration used in commercial preparations) would have been missed if readings had only occurred on D4 (Figs 3 and 4).

Desmodur, Pedilen, and Lupranat were patch tested in 20 controls, none of which showed positive patch test reactions. The difference in positive reactions between test patients and controls was

Table 2. Content of monomeric and oligomeric diphenylmethane diisocyanate (MDI) derivatives in the 3 MDI products used in the study when analysed in May 2005

Isocyanate	Formula	Molecular weight (g/mol)	Content (%)		
			Pedilen	Lupranat	Desmodur
4,4'-MDI		250	34.6	25.8	41.5
2,4'-MDI		250	0.7	3.3	48.6
2,2'-MDI		250	Not found	Not found	Not found
3-ring oligomer		381	22.6	15.6	Not found
4-ring oligomer		512	8.8	6.6	Not found
5-ring oligomer		643	4	4.1	Not found
Untraced			29.3	44.6	9.9

statistically significant ($P < 0.05$) for each comparison made when analysed with Fisher's exact test.

Discussion

Comparison of test reactivity of 4,4'-MDI and MDI products

A previous study has shown that a preparation containing the PMDI product Pedilen could be considered stable for more than a year when stored in a freezer as opposed to preparations of 4,4'-MDI (2). Thus, preparations of PMDI would be more suitable to use for diagnosing contact allergy to MDI substances than preparations of 4,4'-MDI, provided that products containing PMDI detect at least as many positive reactions as 4,4'-MDI does. 1 of the aims with the present study was to investigate whether patients with a previously known contact allergy to 4,4'-MDI also reacted to PMDIs. Therefore, the test reactivity of 4,4'-MDI, currently used in the isocyanate series, was compared with the 2 PMDIs, Pedilen and Lupranat. A MDI-containing product, Desmodur,

was also included. However, Desmodur is not technically a PMDI because it does not contain polymeric oligomers but just the monomeric isomers 2,4'-MDI and 4,4'-MDI in approximately 40% each (Table 2).

4 of the patients who participated in the study had a previously recorded contact allergy to 4,4'-MDI (Table 1). In the present study, only 1 of these would have been picked up within ordinary readings (i.e. on D3/4 and/or D7) by patch testing with 4,4'-MDI (Table 3). On the contrary, Desmodur would have picked up all 4 within ordinary readings. The efficiency for each MDI-containing substance to detect patients with positive reactions within ordinary readings was in declining order Desmodur (4/4) > Pedilen (3/4) > Lupranat (2/4) > 4,4'-MDI (1/4). With readings after D7, the corresponding efficiency and order was Desmodur (4/4) > Pedilen (4/4) > 4,4'-MDI (3/4) > Lupranat (2/4) (Tables 3 and 4).

5 of the 8 patients without any previously recorded exposure or contact allergy to 4,4'-MDI reacted to 1 or more of the MDI-containing products that were tested. The pattern was about

Table 3. Patch test reactions of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) and 4,4'-diaminodiphenylmethane (4,4'-MDA)*. Patients 1–4 had a known contact allergy to 4,4'-MDI and/or MDI-containing work material

Patient no.	Concentration† (%)	Concentration‡ (%)	4,4-MDI†					4,4-MDA‡								
			D4	D7	D11	D14	D18	D21	D25	D4	D7	D11	D14	D18	D21	D25
1§	2	0.94	–	(+)	(+)	+	–	–	–	(+)	++	++(+)	++(+)	++(+)	+	–
	0.63	0.5	–	–	–	(+)	–	–	–	–	(+)	++	++	++	–	–
	0.2	0.16	–	–	–	–	–	–	–	–	–	++	++	+	–	–
	0.063	0.05	–	–	–	–	–	–	–	–	–	++	–	–	–	–
	0.02	0.016	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	2	0.94	++(+)	(+)	(+)	(+)	(+)	+	–	++(+)	++	++(+)	++	++(+)	+	–
	0.63	0.5	(+)	(+)	(+)	–	–	–	–	++	++	++	++	++	–	–
	0.2	0.16	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.063	0.05	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.02	0.016	–	–	–	–	–	–	–	++	++	++	++	++	–	–
3	2	0.94	–	++(+)	++(+)	++	++(+)	+	–	(+)	++	++	++	++	–	–
	0.63	0.5	–	(+)	++	++(+)	++	+	–	(+)	++	++	++	++	–	–
	0.2	0.16	–	–	(+)	++	++	–	–	–	++	++	++	++	–	–
	0.063	0.05	–	–	–	++	++	–	–	–	++	++	++	++	–	–
	0.02	0.016	–	–	–	++	++	–	–	–	++	++	++	++	–	–
4	2	0.94	(+)	(+)	(+)	(+)	–	–	–	++(+)	++	++(+)	++	++	–	–
	0.63	0.5	(+)	(+)	–	–	–	–	–	++	++	++	++	++	–	–
	0.2	0.16	–	–	–	–	–	–	–	–	++	++	++	++	–	–
	0.063	0.05	–	–	–	–	–	–	–	–	++	++	++	++	–	–
	0.02	0.016	–	–	–	–	–	–	–	–	++	++	++	++	–	–
5	2	0.94	–	–	–	–	–	–	–	–	++	++	++	++	–	–
	0.63	0.5	–	–	–	–	–	–	–	–	++	++	++	++	–	–
	0.2	0.16	–	–	–	–	–	–	–	–	++	++	++	++	–	–
	0.063	0.05	–	–	–	–	–	–	–	–	++	++	++	++	–	–
	0.02	0.016	–	–	–	–	–	–	–	–	++	++	++	++	–	–
6	2	0.94	–	–	–	–	–	–	–	Not read D14 to D25	++	++	++	++	–	–
	0.63	0.5	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.2	0.16	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.063	0.05	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.02	0.016	–	–	–	–	–	–	–	++	++	++	++	++	–	–
7	2	0.94	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.63	0.5	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.2	0.16	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.063	0.05	–	–	–	–	–	–	–	++	++	++	++	++	–	–
	0.02	0.016	–	–	–	–	–	–	–	++	++	++	++	++	–	–

Table 3. Continued

Patient no.	Concentration† (%)	Concentration‡ (%)	4,4-MDI†					4,4-MDA‡								
			D4	D7	D11	D14	D18	D21	D25	D4	D7	D11	D14	D18	D21	D25
8	2	0.94	–	(+)	(+)	–	–	Not read	++	++(+)	++(+)	+	+	+	+	Not read
	0.63	0.5	–	–	–	–	–	–	++	++	++	++(+)	++(+)	++(+)	++(+)	–
	0.2	0.16	–	–	–	–	–	–	++	++	++	++(+)	++(+)	++(+)	++(+)	–
	0.063	0.05	–	–	–	–	–	–	++	++	++	++(+)	++(+)	++(+)	++(+)	–
	0.02	0.016	–	–	–	–	–	–	++	++	++	++	++	++	++	–
9	2	0.94	++	+	–	–	–	–	+	++	++	–	–	–	–	–
	0.63	0.5	+	+	–	–	–	–	++	++	++	++	++	++	++	–
	0.2	0.16	(+)	(+)	–	–	–	–	++	++	++	++	++	++	++	–
	0.063	0.05	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.02	0.016	–	–	–	–	–	–	++	++	++	++	++	++	++	–
10	0.0063	0.005	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.002	0.0016	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	2	0.94	–	–	–	–	Not read	–	+	++	++	–	Not read	–	–	–
	0.63	0.5	–	–	–	–	–	–	+	++	++	–	–	–	–	–
	2	0.94	–	–	(+)	–	–	–	++	++	++	++	++	++	++	–
11	0.63	0.5	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.2	0.16	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.063	0.05	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.02	0.016	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.0063	0.005	–	–	–	–	–	–	++	++	++	++	++	++	++	–
12	2	0.94	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.63	0.5	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.2	0.16	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.063	0.05	–	–	–	–	–	–	++	++	++	++	++	++	++	–
	0.02	0.016	–	–	–	–	–	–	++	++	++	++	++	++	++	–

*The dilution steps of 4,4'-MDI and 4,4'-MDA that were negative have not been reported.

†All preparations of 4,4'-MDI listed in the table were prepared and diluted in petrolatum at our laboratory.

‡The preparations of 4,4'-MDA listed in the table derives from a preparation purchased from Chemotechnique Diagnostics (Vellinge, Sweden) consisting of 0.94% 4,4'-MDA in petrolatum, which was diluted in petrolatum to obtain other concentrations.

§Patient no. 1 was read on day (D) 10 and not D11.

||Patient no. 8 was read on D3 and not on D4.

Table 4. Patch test reactions to the diphenylmethane diisocyanate (MDI) products*. Patients 1–4 had a known contact allergy to diphenylmethane-4,4'-diisocyanate (4,4'-MDI) and/or MDI products

Patient no.	Concentration (%)	Pedilen					Desmodur					Lupranat				
		D4	D7	D11	D14	D18	D21	D25	D4	D7	D11	D14	D18	D21	D25	D25
1†	2	-	(+)	++	+	-	-	-	+	+	++(+)	+	-	-	-	(+)
	0.63	-	-	-	(+)	-	-	-	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	2	++	+	(+)	(+)	(+)	(+)	-	++	++	+	+	+	-	-	-
	0.63	(+)	(+)	-	-	-	-	-	++	++	+	+	+	-	-	-
	0.2	-	-	-	-	-	-	-	(+)	(+)	(+)	-	-	-	-	-
	0.063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	2	-	++	++	++(+)	+	(+)	-	++(+)	++(+)	++	++	+	(+)	-	-
	0.63	-	-	(+)	+	(+)	-	-	-	++	++(+)	+	+	-	-	-
	0.2	-	-	-	-	-	-	-	-	-	+	(+)	-	-	-	-
	0.063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	2	++(+)	(+)	+	(+)	-	-	-	++(+)	+	+	+	+	+	-	-
	0.63	(+)	(+)	-	-	-	-	-	++(+)	+	+	+	+	-	-	-
	0.2	-	-	-	-	-	-	-	++(+)	+	+	+	-	-	-	-
	0.063	-	-	-	-	-	-	-	++(+)	+	+	+	-	-	-	-
5	2	++	++(+)	++(+)	Not read	Not read	D14 to D25	-	+++	+++	+++	+++	+++	+++	+++	Not read D14 to D25
	0.63	++	++	++(+)	++(+)	++	++(+)	-	-	(+)	-	-	-	-	-	-
	0.2	+	++(+)	++	++	++	++	-	-	(+)	-	-	-	-	-	-
	0.063	-	(+)	(+)	-	(+)	(+)	-	-	(+)	-	-	-	-	-	-
6	2	-	-	+	-	-	(+)	+	-	-	-	-	(+)	-	-	-
	0.63	-	-	-	-	-	-	(+)	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	2	-	(+)	+	++	++(+)	+++	++(+)	-	-	(+)	++	++(+)	++	++	++
	0.63	-	-	-	(+)	+	++	++(+)	-	-	-	-	(+)	++	++	++
	0.2	-	-	-	-	-	-	+	-	-	-	-	++(+)	++	++	++
	0.063	-	-	-	-	-	-	-	-	-	-	-	++(+)	++	++	++
8‡	2	-	(+)	(+)	(+)	(+)	-	Not read	-	(+)	+	+	+	+	+	Not read
	0.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	2	-	-	-	-	-	-	-	(+)	+	-	+	-	-	-	-
	0.63	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.063	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10–12	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		All dilutions steps were negative							All dilutions steps were negative							
									All dilutions steps were negative							

*The dilution steps that were negative have not been reported.

†Patient no. 1 was read on day (D) 10 and not on D11.

‡Patient no. 8 was read on D3 and not on D4.

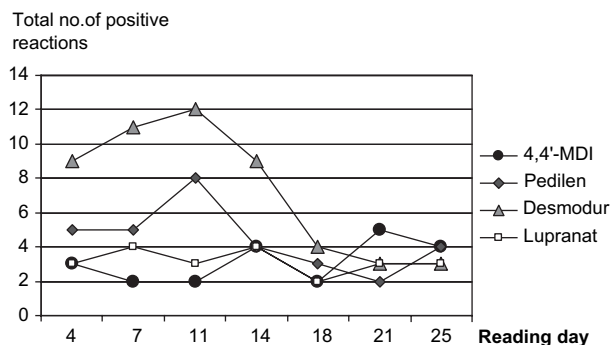


Fig. 1. Total number of positive patch test reactions for each studied diphenylmethane diisocyanate (MDI) substance (including all dilution steps).

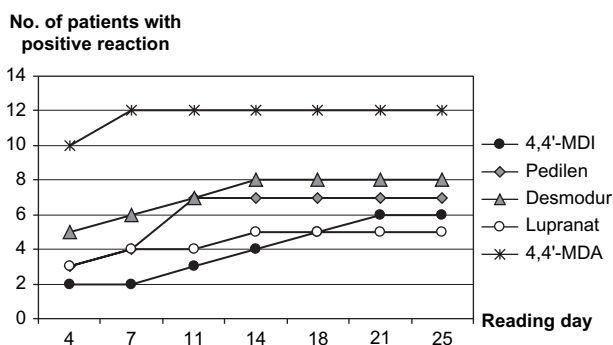


Fig. 2. The accumulated number of patients patch test positive to each studied diphenylmethane diisocyanate (MDI) substance. 4,4'-MDA, 4,4'-diaminodiphenylmethane.

the same among these patients, i.e. Desmodur detected the highest number of positive patients. The efficiency to pick up patients with positive reactions within ordinary readings (D3/4 and/or D7) was in declining order Desmodur (2/8) $\geq$ Lupranat (2/8) $>$ 4,4'-MDI (1/8) $\geq$ Pedilen (1/8). With readings after D7, the corresponding efficiency and order was Desmodur (4/8) $>$ Pedilen (3/8) $\geq$ 4,4'-MDI (3/8) $\geq$ Lupranat (3/8) (Tables 3 and 4).

The present study shows that patch testing with PMDIs and/or a product with a high 2,4'-MDI content generally detects more patients than does patch testing with pure 4,4'-MDI. The stability of Desmodur and Lupranat has not been investigated. However, the composition of Lupranat resembles that of Pedilen, which has been shown to be stable (2) and it can be assumed that they have about the same stability. According to Desmodur's information sheet, do products with a high 2,4'-MDI content have a longer pot life than products with a high 4,4'-MDI content because 2,4'-MDI is less reactive and dimerizes slower than 4,4'-MDI. As a consequence, Desmodur ought to be more stable than pure 4,4'-MDI.

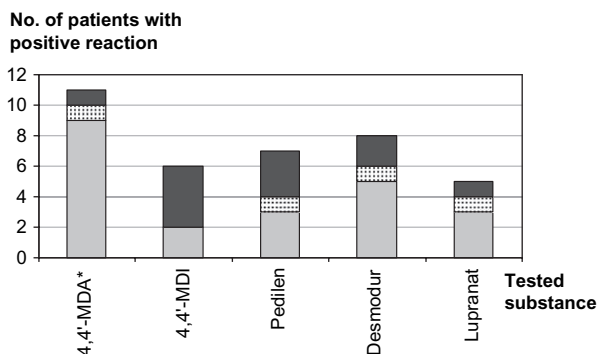


Fig. 3. Number of patients presenting positive reactions on day (D) 4 ■; number of patients showing negative reactions on D4, but positive on D7 ▨. Number of patients showing negative reactions on D4 and D7, but positive on D11 and after ▤. *4,4'-MDA in 0.5% (w/w) petrolatum. 4,4'-MDI, diphenylmethane-4,4'-diisocyanate; 4,4'-MDA, 4,4'-diaminodiphenylmethane.

Additionally, Lupranat and Desmodur are liquids in room temperature just as Pedilen is. Thus, they are easy to disperse evenly in pet., which gives homogenous preparations as opposed to preparations of the solid 4,4'-MDI. These advantageous features probably make it better to patch test with Lupranat than with 4,4'-MDI even though Lupranat yielded less positive reactions than did 4,4'-MDI (Figs 2 and 3). However, to optimize a patch test preparation for the diagnosing of contact allergy to MDI, it is advisable to choose a PMDI with a monomer content of at least the same size as Pedilen. Presently, we recommend that patch test preparations of PMDIs with a monomer content of at least 35% are used in the diagnosis of contact allergy to MDI. The recommended test concentration is 2% in pet, which corresponds to a patch test concentration of approximately 0.7% of the monomer.

Concurrent reactions to 4,4'-MDI and 4,4'-MDA

All patients included in the study had a previously known contact allergy to 4,4'-MDA. Patients 1–4 had shown positive reactions to 0.5% 4,4'-MDA in pet. when patch tested with the isocyanate series. Patients 5–12 had shown positive reactions to 4,4'-MDA when patch tested with a standard series supplemented with 4,4'-MDI and 4,4'-MDA. These 2 substances were included in our standard series in January 2002 in the concentrations 2.0% (w/w) and 0.5% (w/w), respectively. However, 10 months later, after having tested approximately 560 patients, the concentration of 4,4'-MDA was lowered to 0.25% after having sensitized 2 patients (among other patient 5). Except for patient 5, everyone recruited on the basis of positive reactions to 4,4'-MDA in the standard series

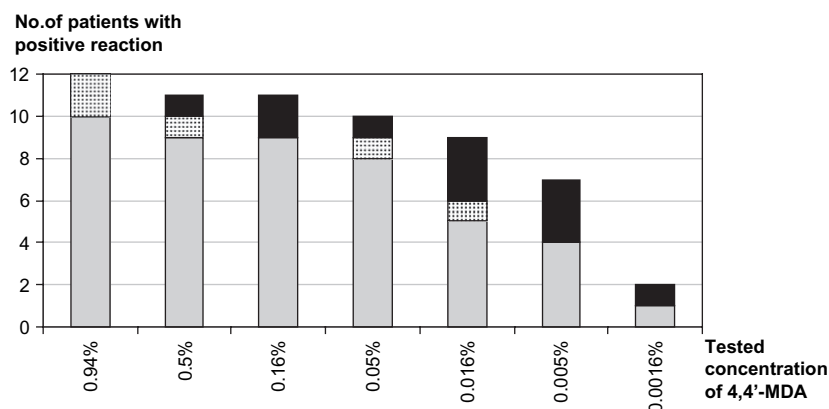


Fig. 4. The total number of patients showing positive reactions within ordinary readings and the total number of patients showing late reactions (after day [D] 7) for each of the dilutions of 4,4'-MDA). Number of patients showing positive reactions on D4 ■; Number of patients showing negative reactions on D4, but positive on D7 ▨; number of patients showing negative reactions on D4 and D7, but positive on D11 and after ▤. 4,4'-MDA, 4,4'-diaminodiphenylmethane.

had also reacted to other *p*-amino compounds such as *p*-phenylenediamine, some disperse dyes etc. None of patients 5–12 reacted to 4,4'-MDI when patch tested with the supplemented standard series. However, low concentration of allergen and/or late-appearing reactions might have caused false-negative results (1, 3).

To cause sensitization, a compound has to penetrate the skin and react with Langerhans cell surface proteins to form new chemical structures, which are considered foreign (16). Some molecules need to undergo metabolic processes and/or react with permeants on the skin surface (e.g. oxidation, complexation etc.) to gain reactive, and thereby allergenic, properties (16, 17). Amines, such as 4,4'-MDA are examples of substances that need to undergo metabolism to be able to react with proteins in the skin. Several metabolic pathways, such as acetylation and oxidation by the cytochrome P450 system, have been proposed for 4,4'-MDA (18–20). The skin is equipped with all these enzymatic systems capable of catalysing the metabolism of 4,4'-MDA encountered through the epidermis (21). 4,4'-MDI, on the other hand, possesses reactive properties and thus should not need to undergo metabolic processes to be able to act as a hapten.

Concomitant reactions to 4,4'-MDI and 4,4'-MDA have often been reported and 3 explanations have been given: (i) both 4,4'-MDI and 4,4'-MDA are primary sensitizer (3), (ii) they cross-react (6, 7, 9), and (iii) 4,4'-MDI is merely a prohaptent, which is transformed to the hapten 4,4'-MDA upon reaction with water encountered in or on the skin (Fig. 5) (5, 6, 8). There are applications within the plastics industry where MDA-based products are used as hardeners and catalysts and thus exposure to both MDI-containing as well as to MDA-containing products is possible (22). However, we found no evidence in the present study that supports explanation (i) because the investigated patients did not report exposure to

both substances. Furthermore, it is unlikely that the concurrent reaction is because of MDA contamination of 4,4'-MDI and/or PMDI products as, when 4,4'-MDI is in excess, it reacts immediately with any 4,4'-MDA formed, resulting in the formation of urea compounds (5). Neither do the results support explanation (ii) because if cross-sensitivity was the cause of concurrent reactions, the 4 patients (patients 1–4) exposed to as well as allergic to 4,4'-MDI would not have been expected to react in a higher and stronger degree to 4,4'-MDA as is the case in this study. Explanation (iii) is probably the most quoted in literature. However, according to the results obtained in this patch test study also, this explanation seems improbable. Because if it were true that positive patch test reaction to 4,4'-MDI is not directed to 4,4'-MDI itself but to 4,4'-MDA formed when 4,4'-MDI reacts with water on or in the skin, one would expect that the positive reactions to 4,4'-MDI and 4,4'-MDA would appear simultaneously because the reaction of an isocyanate with water is very rapid (Fig. 6). However, in the majority of patients with concurrent reactions, positive reactions to 4,4'-MDI appeared later than positive reactions to 4,4'-MDA (Table 3). This suggests that 4,4'-MDI follows another path than the fast reaction with water into 4,4'-MDA. Instead, another explanation could be that the amine is merely an intermediate, consumed in the same rate as it is formed in a series of fast polymerization reactions that follows when 4,4'-MDI encounters water and that ultimately leads

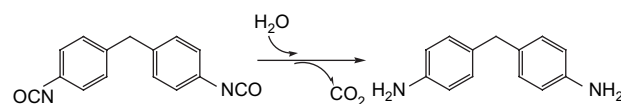


Fig. 5. Conversion of diphenylmethane-4,4'-diisocyanate into its corresponding amine 4,4'-diaminodiphenylmethane with subsequent formation of carbon dioxide upon reaction with water.

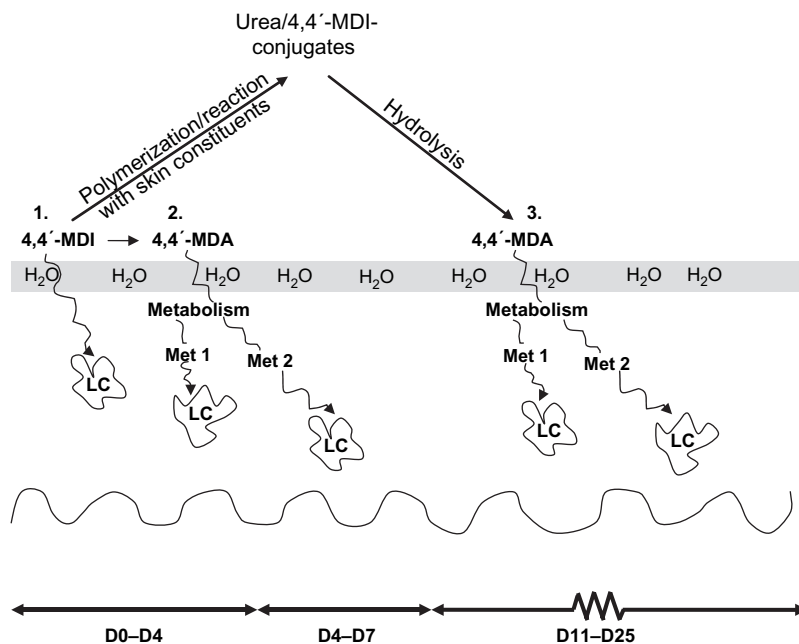


Fig. 6. 3 possible pathways for 4,4'-MDI are illustrated. Path 1 shows direct penetration of 4,4'-MDI, which reacts with Langerhans cells (LC), leading to positive reactions within D7. Path 2 illustrates the conversion of 4,4'-MDI into 4,4'-MDA upon reaction with water, which is a fast reaction that would lead to positive reactions to 4,4'-MDI within D7. Path 3 depicts a slow process in which 4,4'-MDI either polymerizes into ureas upon reaction with water or reacts with constituents of the skin. The polyurea and the MDI conjugates with skin constituents can thereafter be hydrolysed to 4,4'-MDA. If this were the case, positive reactions to 4,4'-MDI would appear after D7. It is also illustrated that 4,4'-MDA can metabolize into at least 2 (but presumably several) different haptens that can react with LC. The rate of metabolite (met) formation may differ, leading to positive reactions on different reading days. 4,4'-MDI, diphenylmethane-4,4'-diisocyanate; 4,4'-MDA, 4,4'-diaminodiphenylmethane.

to the formation of ureas and oligoureas (Fig. 6) (23). Additionally, 4,4'-MDI might react with constituents in and on the skin. The ureas as well as the conjugates formed with skin constituents can subsequently be hydrolysed into, e.g. 4,4'-MDA (Fig. 6). This is a slow process, which could explain why the reactions to 4,4'-MDI appear later than the reactions to 4,4'-MDA. The pathway proposed above is complex and the rate of both polymerization and hydrolysis is presumably dependent upon several individual factors such as, e.g. condition of the skin (with regard to pH, humidity, and individual constituents like lipids, proteins etc.) and temperature. That could explain why not all patients sensitive to 4,4'-MDA reacted to 4,4'-MDI.

Once 4,4'-MDA has entered the skin, it needs to be metabolized in order to act as a hapten (Fig. 6). There is more than 1 possible 4,4'-MDA metabolite that is able to bind to proteins within the skin. Thus, this enables more than 1 possible pattern of reactivity for 4,4'-MDA with regard to cross-reactivity as well as the course and development of positive patch test reactions because the rate of formation might differ for different metabolites. Thus, in this study, 2 different patterns of patch test behaviour can be distinguished for 4,4'-MDA.

1 pattern is of a 'crescendo type', in which the positive reactions to the highest concentrations appear early, while positive reactions to the lower concentrations appear late (11). This pattern is seen for patients 1, 2, 3, and 5 (Table 3). The other patients exhibit a pattern of reactivity where all positive reactions appeared at the first reading and were of about the same magnitude regardless of the concentration, and thereafter decreased in strength (Table 3). The occurrence of 2 different patterns of patch test response to 4,4'-MDA supports the proposal that several metabolic pathways are possible for 4,4'-MDA.

The present study shows that 4,4'-MDA tested in 0.5% pet. is a good marker for 4,4'-MDI. An advantage with testing with 4,4'-MDA is that it picks up all patients positive to 4,4'-MDI with readings on D4 and D7 as opposed to the preparation of 4,4'-MDI that only picked up 1 of 4 allergic patients with readings on D4 and D7. Because 0.5% 4,4'-MDA has sensitized patients, we recommend testing in 0.25% pet. However, there is a risk of false-negative patch test reactions when a concentration of 0.25% is used (Fig. 4). Still, if patch testing with 0.25% is negative but there are strong clinical indications that 4,4'-MDA causes the patients' problems, the patch test

concentration can be increased to 0.5% without any risk of irritation.

Development and course of the positive patch test reactions

In our experience, patch test reactions to 4,4'-MDA, 4,4'-MDI, and/or MDI products appear late, i.e. after D7 (Table 2) (3). This was also confirmed in the present study, which showed that the number of patients that would have been missed with readings on only D4 were 4/6 (67%) for 4,4'-MDI, 4/7 (57%) for Pedilen, 3/8 (38%) for Desmodur, and 3/5 (60%) for Lupranat (Figs 2 and 3). With readings on both D4 and D7, the corresponding figures were the same for 4,4'-MDI but 3/7 (43%) for Pedilen, 2/8 (25%) for Desmodur, and 1/5 (20%) for Lupranat. All 12 patients reacted within ordinary readings when tested with 0.94% 4,4'-MDA. However, when tested with the concentration found in commercially available patch test preparations, i.e. 0.5%, 2/11 (18.2%) would have been missed with only 1 reading on D4 and 1/11 (9.1%) would have been missed with readings on both D4 and D7 (Fig. 4). 1 patient (i.e. no. 10) would not have been detected at all if only patch tested with 0.5% 4,4'-MDA.

The fact that 2 persons showed positive reactions to 4,4'-MDA after the ordinary reading on D4 could possibly be attributed to the fact that the rate of metabolism into a reactive hapten varies from individual to individual (Fig. 6). As for the overrepresentation of late reactions to 4,4'-MDI and MDI products in patients with an already known contact allergy to the same (patients 1–4), this could perhaps be explained as above, namely that the MDI substances first polymerize into ureas and/or react with constituents in the skin into conjugates, which then are hydrolyzed into amines (Fig. 6). Because this proposal is not yet confirmed, active sensitization in the case of 3 patients (nos 6–8) with late positive reactions to 4,4'-MDI and/or MDI products, and without any previously reported contact with the same, cannot be completely ruled out. However, with regard to the late positive reactions in those patients with an already confirmed contact allergy to MDI (patients 1–4), we find active sensitization highly unlikely.

Active sensitization, 1 of the most serious adverse reactions when patch testing, is traditionally defined as a negative patch test reaction followed by a flare-up reaction after D10 to D20, and with a positive reaction on D2 to D4 at retest (24). A positive reaction on the second patch test occasion could also represent an increase in the level of sensitivity, but if patch testing on this test occasion is done with the substance diluted 10–100× as compared with the original test concentration,

and the test turns out positive, active sensitization must be considered likely (25). However, it has been shown that patch test reactivity to nickel sulfate in nickel-allergic women can vary 250× from one time to another (26) and other sensitizers might very well exhibit the same pattern.

In fact, a significant individual variation in test response has been seen for 4,4'-MDI in earlier investigations and where the same patient has exhibited both early, late, and no reactions at all to 4,4'-MDI and/or MDI-containing work products when tested on different occasions. In Table 1, the variation in test response for patients 1–3 is illustrated. They were all referred to us after an outbreak of work-related skin lesions among workers at a company producing flooring laminate boards, after the introduction of a water-repellent lacquer containing 4,4'-MDI and 2,4'-MDI in approximately 20% of each (3). Patient no. 1 was negative on D3 and D7 but showed a positive reaction on D10 on the test site of 4,4'-MDI. Because the majority of her colleagues showed their positive reactions on D7, it was assumed that the positive reactions on D10 in her case was rather a sign that positive reactions to 4,4'-MDI appear late than it was a sign of active sensitization (3). This was later supported in a retest where she was negative. Patient no. 2, on the other hand, was positive to the MDI-based lacquer within ordinary readings but negative to 4,4'-MDI in the isocyanates series when earlier investigated (3). Upon retesting with 4,4'-MDI, Pedilen and Desmodur, he was negative on the ordinary readings but contacted us about D11 after the patch testing on account of fresh reactions. When read on D14, he showed positive reactions to 4,4'-MDI and Desmodur (Table 1). In the present study he showed positive reactions to 4,4'-MDI and all the MDI products already on D3 (Table 1). Patient no. 3 had reacted on both D3 and D7 to 4,4'-MDI as well as to the MDI-based lacquer (3) in the first investigation. However, in the present study, he showed late patch test reactions to 4,4'-MDI but positive reactions within ordinary reading to Desmodur and Pedilen (Table 1). In the current investigation, both patient nos 1 and 3 had late reactions to 4,4'-MDI but positive reactions within ordinary readings to at least 1 of the MDI products.

The above described pattern for patient nos 1 and 3 probably coincides with the fact that patch testing with Pedilen and Desmodur generally detects more patients than does patch testing with 4,4'-MDI. The reason for this is not yet known. However, because the concentration of 4,4'-MDI in the MDI products was less than half of the concentration in pure 4,4'-MDI (Table 2), the

results indicate that more allergens besides 4,4'-MDI might be present in the MDI-containing products. For example, it seems possible that also 2,4'-MDI is a contact allergen because Desmodur detected the highest number of positive patients. However, another explanation to the higher number of positive reactions to Desmodur and the PMDIs could be that the physical state of the liquid products enhances the penetration and, thus the bioavailability of 4,4'-MDI in the skin, making the MDI products more reactive than the preparations of pure 4,4'-MDI, which is in a solid state. Another explanation could be that the rate of polymerization to ureas and/or reactions with constituents in the skin with subsequent hydrolysis to amine is faster in the more complex MDI prod-

ucts than in pure 4,4'-MDI. Possibly, this could also explain the individual variation between test occasions because both polymerization and hydrolysis are dependent upon factors that can vary from test occasion to test occasion leading to different response. Furthermore, it might explain why not all patients exhibit a crescendo type of reaction pattern that is seen in patient 7 (Fig. 7) and that has earlier been described for substances with patch test reactions appearing late, such as gold sodium thiosulfate and methacrylates (11, 13).

Conclusions

The present study shows that patch testing with MDI products detect at least as many patients

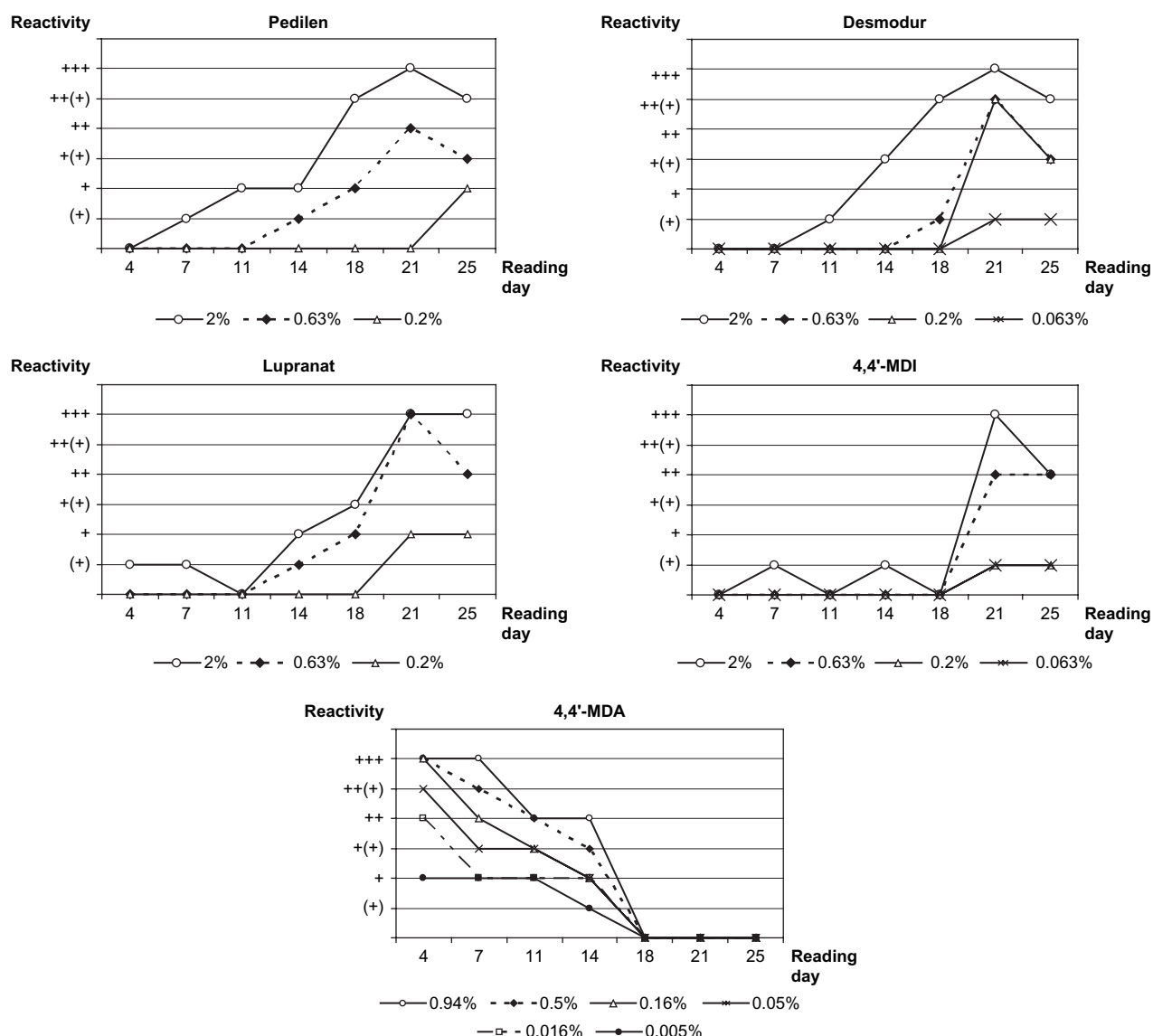


Fig. 7. Time-course of epicutaneous reactions to dilution series of each studied diphenylmethane diisocyanate (MDI) substance as well as 4,4'-diaminodiphenylmethane (4,4'-MDA) in 1 patient (no. 7). 4,4'-MDI, diphenylmethane-4,4'-diisocyanate; 4,4'-MDA, 4,4'-diaminodiphenylmethane.

as 4,4'-MDI. Hence, patch testing with MDI products are to prefer to patch testing with pure 4,4'-MDI because they are more stable and more homogenous. Additionally, other monomers and/or oligomers besides 4,4'-MDI may cause allergy, which makes it even more justified to test with MDI products. We recommend that PMDIs with a monomer content of at least 35% is used in a concentration of 2.0% in pet. (i.e. approximately 0.7% monomer). In view of that positive reactions to 4,4'-MDI and MDI products appear late, we recommend that patch tests are read on both D3/4 and D7 and that patients should be advised to make contact with the clinic if any reactions appear after the D7 reading.

However, at many patch testing departments, the possibility of patch testing with the patients' own work material is scarce. Therefore, 4,4'-MDA tested in 0.5% pet. can be used instead because it is a good marker for 4,4'-MDI. Because 0.5% 4,4'-MDA has sensitized patients, we recommend testing in 0.25% pet. However, there is a risk of false-negative patch test reactions when a concentration of 0.25% is used. Therefore, we recommend that if patch testing with 0.25% is negative but there are strong clinical indications that 4,4'-MDA caused the patient's problem, the patch test concentration can be increased to 0.5% without any risk of irritation.

The present study also shows that concurrent reactions to 4,4'-MDI and 4,4'-MDA are unlikely to be as a result of conversion of 4,4'-MDI to 4,4'-MDA upon reaction with water. Instead, it is proposed that concomitant reactions might be explained by a complex pathway of reactions that starts when 4,4'-MDI reacts with constituents of the skin and/or water, which leads to the formation of 4,4'-MDI conjugates and ureas, which subsequently are hydrolyzed into amines, e.g. 4,4'-MDA. This slow and complex process is dependent upon a number of factors. Therefore, it might explain both why positive reactions to MDI substances appear late as well as why there are an individual variation in test response from 1 test occasion to another.

Acknowledgements

The authors thank Karin Olsson for skilful technical assistance and the Swedish Council for Working Life and Social Research and Skane County Council's Research and Development Foundation for providing funds.

References

1. Frick M, Zimerson E, Karlsson D et al. Poor correlation between stated and found concentrations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) in petrolatum patch-test preparations. *Contact Dermatitis* 2004; 51: 73–78.
2. Frick-Engfeldt M, Zimerson E, Karlsson D et al. Is it possible to improve patch-test diagnostics for isocyanates? A stability study of patch-test preparations of diphenylmethane-4,4'-diisocyanate (MDI) and polymeric diphenylmethane diisocyanate (PMDI). *Contact Dermatitis* 2007; 56: 27–34.
3. Frick M, Isaksson M, Bjorkner B et al. Occupational allergic contact dermatitis in a company manufacturing boards coated with isocyanate lacquer. *Contact Dermatitis* 2003; 48: 255–260.
4. Anonymous. TA: Concise International Chemical Assessment Document (CICAD). WHO Press, Geneva, Switzerland, 2000: 27: 25.
5. Estlander T, Kanerva L, Jolanki R. Polyurethane resins. In: *Handbook of Occupational Dermatology*, Kanerva L, Elsnér P, Wahlberg J E, Maibach H I (eds): Berlin, Heidelberg, New York, Springer, 2000: 597–601.
6. Alomar A. Contact dermatitis from a fashion watch. *Contact Dermatitis* 1986; 15: 44–45.
7. Fregert S. Allergic contact reaction to diphenyl-4,4'-diisocyanate. *Contact Dermatitis Newslett* 1967: 17.
8. Rothe A. Contact dermatitis from diisocyanates. *Contact Dermatitis* 1992; 26: 285–286.
9. Liden C. Allergic contact dermatitis from 4,4'-diisocyanatodiphenyl methane (MDI) in a molder. *Contact Dermatitis* 1980; 6: 301–302.
10. Goossens A, Detienne T, Bruze M. Occupational allergic contact dermatitis caused by isocyanates. *Contact Dermatitis* 2002; 47: 304–308.
11. Bruze M, Hedman H, Bjorkner B, Moller H. The development and course of test reactions to gold sodium thiosulfate. *Contact Dermatitis* 1995; 33: 386–391.
12. Isaksson M, Bruze M. Late patch-test reactions to budesonide need not be a sign of sensitization induced by the test procedure. *Am J Contact Dermat* 2003; 14: 154–156.
13. Isaksson M, Lindberg M, Sundberg K et al. The development and course of patch-test reactions to 2-hydroxyethyl methacrylate and ethyleneglycol dimethacrylate. *Contact Dermatitis* 2005; 53: 292–297.
14. Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007; 56: 38–42.
15. Wilkinson D S, Fregert S, Magnusson B et al. Terminology of contact dermatitis. *Acta Derm Venereol* 1970; 50: 287–292.
16. Lepoittevin J P, Berl V. Molecular basis of allergic contact dermatitis. In: *Dermatotoxicology*, Zhai H, Maibach H I (eds): Boca Raton, London, New York, Washington D.C., CRC Press, 2004: 265–285.
17. Dressler W E, Appelqvist T. Plasma/blood pharmacokinetics and metabolism after dermal exposure to para-aminophenol or para-phenylenediamine. *Food Chem Toxicol* 2006; 44: 371–379.
18. Cocker J, Nutley B P, Wilson H K. A biological monitoring assessment of exposure to methylene dianiline in manufacturers and users. *Occup Environ Med* 1994; 51: 519–522.
19. Kautiainen A, Wachtmeister C A, Ehrenberg L. Characterization of hemoglobin adducts from a 4,4'-methylenedianiline metabolite evidently produced by peroxidative oxidation in vivo. *Chem Res Toxicol* 1998; 11: 614–621.
20. Kajbaf M, Sepai O, Lamb J H, Naylor S. Identification of metabolites of 4,4'-diaminodiphenylmethane (methylene dianiline) using liquid chromatographic and mass spectrometric techniques. *J Chromatogr* 1992; 583: 63–76.
21. Westphal G A, Reich K, Schulz T G et al. N-acetyltransferase 1 and 2 polymorphisms in para-substituted arylamine-induced contact allergy. *Br J Dermatol* 2000; 142: 1121–1127.
22. Emmett E A. Allergic contact dermatitis in polyurethane plastic moulders. *J Occup Med* 1976; 18: 802–804.
23. Yakabe Y, Henderson K M, Thompson W C et al. Fate of methylenedianiline diisocyanate and toluene diisocyanate

- in aquatic environment. *Environ Sci Technol* 1999; 33: 2579–2583.
24. Cronin E. *Contact Dermatitis*. Edinburgh, London, New York, Churchill Livingstone, 1980.
25. Bruze M. Simultaneous patch test sensitization to 4 chemically unrelated compounds in a standard test series. *Contact Dermatitis* 1984; 11: 48–49.
26. Hindsen M, Bruze M, Christensen O B. Individual variation in nickel patch test reactivity. *Am J Contact Dermat* 1999; 10: 62–67.

Address:

Professor Malin Frick-Engfeldt
Department of Occupational and Environmental Dermatology
Malmö University Hospital
Lund University
S-205 02 Malmö
Sweden
Tel: +46 40 33 78 24
Fax: +46 40 33 62 13
e-mail: malin.frick@med.lu.se