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Assessment of dermal uptake of diphenylmethane-4,4'-diisocyanate using tape stripping and biological monitoring

Background: Very little is known about the dermal uptake of isocyanates, and dermal exposure to isocyanates has been discussed as a factor involved in the induction of respiratory diseases. **Objectives:** To investigate the dermal uptake of diphenylmethane-4,4'-diisocyanate (4,4'-MDI). **Materials & Methods:** Four volunteers were dermally exposed to 10, 25, 49 and 50 mg 4,4'-MDI, respectively, for eight hours. The exposed areas were tape stripped. Urine and blood were biologically monitored for 48 hours. Tape strips, plasma, and urine were analysed by liquid chromatography-mass spectrometry. **Results:** In total, 35-70% of the applied dose of 4,4'-MDI was absorbed by the skin. Very low fractions of applied dose were found in the tape strips. The 4,4'-MDA concentration in plasma and urine was low, but peaked in urine at 10-14 hours and plasma at 8-32 hours after exposure. **Conclusions:** 4,4'-MDI is readily absorbed by human skin. Only small fractions of 4,4'-MDI remain as such in the superficial skin layers. The amounts found in blood and urine were only small fractions of the total applied doses which indicates that very small amounts of 4,4'-MDI penetrate the skin and reach the blood stream. The dermal uptake and distribution of 4,4'-MDI is much slower compared to that associated with airway uptake. Our data strongly indicate that formation of 4,4'-MDA from 4,4'-MDI upon reacting with water in the skin can only occur to a very limited extent.

Key words: diphenylmethane-4,4'-diisocyanate, 4,4'-MDI, diphenylmethane-4,4'-diamine, 4,4'-MDA, contact dermatitis, dermal absorption, tape stripping

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Isocyanates are mainly used in the manufacturing of polyurethane polymers for the production of plastics, rubbers, coatings, and foams. Polyurethanes are formed as a result of a reaction between polyols and isocyanates. Isocyanates can react with hydroxyl, amino, and thiol groups of important biomolecules, such as proteins and DNA. This contributes to the toxic effects of isocyanates. One of the most used isocyanates industrially is diphenylmethane diisocyanate (MDI) which mainly consists of a mixture of the three isomers: 2,2'-MDI, 2,4'-MDI, and 4,4'-MDI. Industrial samples may contain impurities such as dimers, trimers, oligomers, and polymerization products. The isomer 4,4'-MDI usually has the highest concentration in industrial MDI mixtures and is available commercially in pure form and therefore used in many studies regarding MDI toxicity.

The main exposure routes for isocyanates are inhalation and skin contact. There are several reports of occupational asthma caused by exposure to isocyanates [1-3].

Interestingly, 4,4'-MDI has been shown to cause respiratory hypersensitivity following dermal exposure in animals [4]. Other studies and current research supports the conclusion that occupational skin exposure leading to sensitization can cause the development of occupational asthma upon airway

challenge [3, 5]. 4,4'-MDI is classified as a respiratory as well as a cutaneous sensitizer, according to The Swedish Chemicals Agency's classification and labelling regulations [6].

In dermatology departments, patch test preparations of 0.5% w/w 4,4'-MDI in petrolatum are routinely used to detect MDI contact allergy. Almost all patients showing positive patch test reactions to 4,4'-MDI show positive test reactions also to the corresponding amine diphenylmethane-4,4'-diamine (4,4'-MDA) [7]. The background for these two reactions is not known. An isocyanate group that reacts with water liberates carbon dioxide and is converted into an amino group. If this occurs for the isocyanate groups of 4,4'-MDI, it is transformed into 4,4'-MDA. The chemical structures of 4,4'-MDI and 4,4'-MDA are shown in figure 1.

In rats, it has been shown that dermal absorption is below 1% of the applied dose of MDI [8]. However, another rat study [9] has shown that dermal absorption is possible, as 20-30% of the applied dose was faecally excreted and 1% of the applied dose was found in urine. Interestingly, in a study performed using *in vitro* guinea pig skin, more than 80% of the applied dose of MDI disappeared from the skin surface by diffusion [10]. However, there are no human

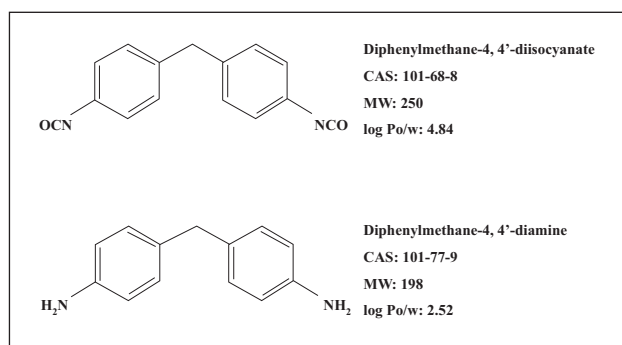


Figure 1. The chemical structure of diphenylmethane-4,4'-diisocyanate and diphenylmethane-4,4'-diamine.

experimental studies that have shown data of skin absorption of MDI, and it is thus important to investigate dermal uptake of 4,4'-MDI in humans. In this experimental study, the objective was to investigate the dermal uptake of 4,4'-MDI from exposed skin areas in volunteers.

Within the area of contact dermatitis, it is important for clinicians to know, and for experts to understand, what is happening upon skin exposure to a potential sensitizer with regards to penetration, absorption, and metabolic events. This can help predict the possibility of, for example, systemic contact dermatitis and simultaneous reactions to chemically related substances, as well as other systemic toxic effects, for example, cancer caused by 4,4'-MDA. This study provides information on such factors concerning skin exposure to 4,4'-MDI.

Materials and methods

Volunteers

The volunteers included three females: Volunteers 1, 2 and 3, who were 22, 38, and 47 years old, respectively, and one male, Volunteer 4, 54 years old. Volunteers 1 and 2 were exposed to 4,4'-MDI in 2010 [11], and Volunteers 3 and 4 in 2012. Volunteers 1 and 2 were not known to have contact allergy to 4,4'-MDI or 4,4'-MDA, while Volunteers 3 and 4 had been diagnosed with contact allergy to 4,4'-MDI prior to the study.

Ethics

This study was approved by the Research Ethics Committee of Umeå University, Sweden (Dnr 09-214M). The volunteers gave their written informed consent to participate in the study.

Chemicals and materials

Chemicals and materials used in this study were: 4,4'-MDI, 4,4'-MDA, pentafluoropropionic acid anhydride, toluene (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany), sodium hydroxide (Merck, Darmstadt, Germany), petrolatum USP/NF Kval E (Apoteket Production & Laboratories, Gothenburg, Sweden), dibutylamine (DBA) (Acros Organics, Geel, Belgium), methylene chloride (Mallinckrodt Baker B.V., Deventer, The Netherlands),



Figure 2. Dermal exposure. Application of diphenylmethane-4,4'-diisocyanate in petrolatum on filter paper covered with aluminium foil and later secured with scanpor tape.

acetonitrile multisolvent grade (Scharlau Chemie SA, Barcelona, Spain), deionised water (18.2 MΩ), MILLI-Q® system ZFMQ23004 (Millipore SA, Molsheim, France), formic acid analytical reagent grade (Riedel-deHaën, Seelze, Germany), 4,4'-MDI reaction product with dibutylamine (4,4'-MDI-DBA), 4,4'-MDI reaction product with deuterated dibutylamine as an internal standard (4,4'-MDI-DBA-d₉) (IFKAN AB, Hässleholm, Sweden), tape for tape stripping (Fixomull, BSN medical GmbH & Co., Hamburg, Germany), filter paper Quality 1F (Munktel Filter AB, Falun, Sweden), Scanpor® tape (Norgesplaster A/S, Vennesla, Norway), and aluminium foil (Duni AB, Malmö, Sweden).

Dermal exposure

Dermal exposure was performed with a 2.0% w/w 4,4'-MDI petrolatum preparation made at the Department of Occupational and Environmental Dermatology, Malmö. The 4,4'-MDI preparation was applied to the skin of the upper arms of the volunteers using filter paper evenly covered with the preparation and placing it on the outer aspect of the upper arms. The filter paper was covered with aluminium foil and then secured with Scanpor tape (figure 2). The 4,4'-MDI surface concentration was approximately 0.8 mg/cm² for all volunteers. Different doses were obtained by altering the area of exposure, *i.e.* the size of the filter papers used for application. Volunteers 1 and 2 were exposed on one arm

Table 1. Exposure doses and areas per volunteer.

	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4
Exposed skin area (cm ²)	12.5	31	62	62
Total amount 2% 4,4-MDI (g)	0.50	1.25	2.4	2.5
Total dose 4,4-MDI (mg)	10	25	49	50

using filter papers of size 12.5 and 31 cm², respectively. Volunteers 3 and 4 were exposed by applying duplicate, 31-cm² filter papers, one on each arm. The total amount of 4,4'-MDI petrolatum preparation for each subject was 10 mg, 25 mg, 49 mg, and 50 mg, respectively (*table 1*). After eight hours of exposure, the aluminium foil backing and filter paper were removed and the excess petrolatum preparation on the skin was wiped off using two to three compresses. The removed filter paper with its aluminium foil backing and the contaminated compresses from the exposed volunteers were placed in glass flasks, one for each volunteer, containing 60 mL 0.10 M DBA in methylene chloride and toluene, 50/50 v/v. The glass flasks were sent for chemical analysis of 4,4'-MDI to the Department of Occupational and Environmental Medicine in Örebro, Sweden.

Tape stripping

After removal of the filter paper and wiping off the excess petrolatum preparation, 10 cm² of the exposed area was tape stripped using a set of 20 tape strips (2.5 × 4 cm). This was performed on both arms of Volunteers 3 and 4, one set on the right arm and one set on the left. Tape stripping was not performed for Volunteers 1 and 2. The tape stripping technique has been described in detail elsewhere [12]. The set of tape strips from the right arm was intended for measurement of 4,4'-MDI levels. Each of these tape strips was placed in a glass test tube containing 10 ml 0.01 M DBA in methylene chloride and toluene 50/50 v/v, and closed with a screw-tight lid. The samples were stored refrigerated until sent for analysis.

Urine and plasma samples

The volunteers in this study contributed plasma and urine samples just before dermal exposure and full urine collections during 48 hours from the start of the exposure. The volunteers were handed urine flasks labelled with time and date for each collection period, according to the daily scheme (*table 2*). The urine samples were stored refrigerated during the entire collection time. Five plasma samples were drawn for each volunteer and sent together with the urine samples for chemical analysis at the Department of Occupational and Environmental Medicine in Lund, Sweden.

Analysis of 4,4'-MDI in the excess petrolatum preparation and tape strips

The samples of the recovered 4,4'-MDI from the skin and the tape strip set on the right arm of Volunteers 3 and 4 were analysed using liquid chromatography-mass spec-

Table 2. Daily sampling scheme for urine and plasma for all volunteers.

	Time*	Sample type
Day 1	07:00-08:00	Urine and plasma before exposure
	08:00	Start of dermal exposure
	12:00	Urine
	16:00	Urine and plasma
	16:00	End of dermal exposure
	20:00	Urine
	22:00-23:00	Urine
	23:00-08:00	Urine and plasma [‡]
Day 2	12:00	Urine
	16:00	Urine and plasma
	20:00	Urine
	22:00-23:00	Urine
Day 3	23:00-08:00	Urine and plasma [‡]

[‡]The plasma samples were drawn at 08:00.

*The actual sampling time was reported by the volunteers.

trometry (LC-MS). 4,4'-MDI was derivatized and measured in the form of its stable reaction product with DBA. The method is described elsewhere [11]. Unfortunately, one of four glass flasks containing the samples of excess petrolatum preparations of Volunteers 3 or 4 was crushed during transportation to the Department of Occupational and Environmental Medicine in Örebro, which resulted in the labels of the other three flasks being destroyed by the solvent.

Analysis of urine and plasma samples for systemically distributed 4,4'-MDI

The dermal uptake of 4,4'-MDI was determined by measurement of 4,4'-MDA in hydrolysed urine and plasma. The 4,4'-MDA levels in the hydrolysed urine and plasma samples were analysed according to Sennbro *et al.* 2003 [13]. In principle, the biological samples were hydrolysed for 24 hours in 0.3 M sodium hydroxide (NaOH) in order to release the 4,4'-MDI-corresponding amine, 4,4'-MDA, which was extracted with toluene. After derivatization with pentafluoropropionic acid anhydride, the derivatives were quantified by gas chromatography and mass spectrometry. This method detects 4,4'-MDI that has reacted with the

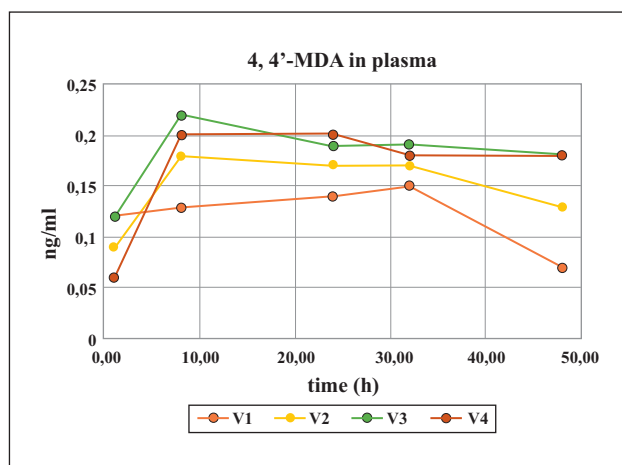


Figure 3. Diphenylmethane-4,4'-diamine concentrations in plasma. Dermal exposure doses were 10 mg (V1), 25 mg (V2), 49 mg (V3), and 50 mg (V4) diphenylmethane-4,4'-diisocyanate. V: volunteer.

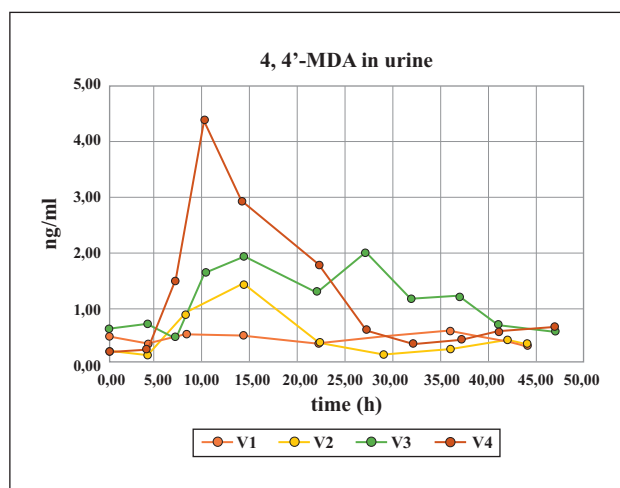


Figure 4. Diphenylmethane-4,4'-diamine concentrations in urine. Dermal exposure doses were 10 mg (V1), 25 mg (V2), 49 mg (V3), and 50 mg (V4) diphenylmethane-4,4'-diisocyanate. V: volunteer.

cell components, but also 4,4'-MDA if present in the sample. The limit of detection was 0.05 ng/ml for 4,4'-MDA in hydrolysed urine and plasma [13]. The chemical analysis was performed at the Department of Occupational and Environmental Medicine, Lund.

Results

The amounts of unreacted and unabsorbed 4,4'-MDI in the recovered excess 4,4'-MDI petrolatum preparation were 7.0 and 17.5 mg 4,4'-MDI for Volunteers 1 and 2, respectively, which represents 70% of the applied dose for both volunteers. Three out of the four samples from Volunteers 3 and 4 contained 5.2, 9.0, and 11.3 mg 4,4'-MDI. Considering all possible combinations of these unmarked samples, the average value of unabsorbed 4,4'-MDI in the three analysed samples was calculated as $35 \pm 4\%$ of the applied dose.

In hydrolysed plasma, the concentration of 4,4'-MDA did not rise above the baseline concentration in Volunteer 1, but maximum concentration was reached after approximately eight hours of exposure at 0.18 ng/mL, 0.22 ng/mL, and 0.20 ng/mL in Volunteers 2, 3, and 4, respectively. In hydrolysed urine, the concentration of 4,4'-MDA did not increase in Volunteer 1, but reached maximum concentration after 10-14 hours exposure at 1.4 ng/ml, 2.0 ng/mL, and 4.3 ng/mL in Volunteers 2, 3, and 4, respectively. The results are presented in *figures 3, 4*.

The total amount of 4,4'-MDI found in the skin layers, removed by tape strips, was 21.14 μg and 6.64 μg for Volunteers 3 and 4, respectively. Data for each tape strip layer is provided in *figure 5*.

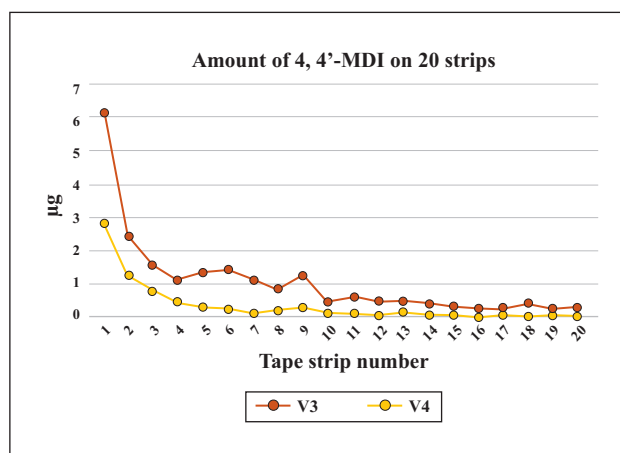


Figure 5. Amounts of diphenylmethane-4,4'-diisocyanate on tape strips. Dermal exposure dose for the tape-stripped areas (10 cm^2) was 8 mg diphenylmethane-4,4'-diisocyanate for V3 and V4. V: volunteer.

metabolites of absorbed foreign substances are eliminated in the urine and faeces. It has previously been shown that occupational airway exposure to 4,4'-MDI can be traced and monitored by analysing 4,4'-MDA as a biomarker for this exposure in hydrolysed plasma and urine in animals [14, 15]. In hydrolysed blood and urine samples from MDI airway-exposed individuals, MDA has been identified as a metabolite or biomarker [16, 17]. This has been utilised to trace the absorbed amount of 4,4'-MDI in blood and urine samples [13]. In the present study, we used similar methods to detect dermal uptake of 4,4'-MDI by analysis of 4,4'-MDA in hydrolysed blood and urine samples. We also used tape stripping [12] of the exposed skin areas to detect 4,4'-MDI remaining in the superficial skin layers.

The physical properties, $\log P_{o/w}$, and molecular weight of 4,4'-MDI are favourable for skin penetration (*figure 1*). Our data show that a substantial proportion (mean value: 47%) of the 4,4'-MDI dose was absorbed by the skin. Although

Discussion

Foreign substances absorbed by the human body are transported and distributed by blood flow. They can be transformed by metabolism mainly in the liver. Eventually,

problems occurred with the samples regarding Volunteers 3 and 4, we believe that we could make a good estimation of the absorbed amounts. For clinical patch testing, many allergens are tested in petrolatum preparations and similar proportions of the tested substances are absorbed by the skin [18]. These results are clearly in line with the physical properties of 4,4'-MDI and indicate that 4,4'-MDI readily penetrates the skin.

However, in the present study, the detected amounts of 4,4'-MDA in hydrolysed blood and urine of the volunteers were very low and represented a very small fraction of the applied 4,4'-MDI dose. We estimate, based on an assumption of a plasma volume of approximately 5 litres, that the sum of the amounts in plasma and urine is in the range of 0.01-0.2% of the applied 4,4'-MDI dose for Volunteers 2, 3, and 4. For Volunteer 1, no such calculations could be performed due to blood and urine concentrations below the detection limits. The non-absorbed amounts of 4,4'-MDI cannot account for this result. We conclude that the explanation may be based on an alternative event that takes place in 4,4'-MDI-exposed skin, in addition to diffusion-based penetration as the predominate skin penetration mechanism for many other non-polar substances. The most plausible explanation is that 4,4'-MDI reacts and binds chemically to skin constituents. This explanation is supported by earlier findings that suggest 4,4'-MDI undergoes specific reactions to the site of first contact and forms adducts, conjugates, and/or polyureas [19].

Biological monitoring of exposure to airborne 4,4'-MDI has been studied in workers exposed to airborne 4,4'-MDI in an industrial MDI product [20]. The study showed that eight hours of exposure to a median air concentration of 0.2 $\mu\text{g}/\text{m}^3$ 4,4'-MDI (range: 0.03-7.8 $\mu\text{g}/\text{m}^3$) resulted in 4,4'-MDA concentrations with a median of 2 ng/mL in hydrolysed urine and 0.7 ng/mL in hydrolysed plasma. Approximately 10 hours after the volunteers had started the dermal application of 4,4'-MDI in the present study, we found that the highest concentrations of 4,4'-MDA were 4.32 ng/mL in hydrolysed urine and 0.22 ng/mL in hydrolysed plasma (figures 3, 4). In a Finnish study, workers exposed to airborne 4,4'-MDI at 0.66 $\mu\text{g}/\text{m}^3$ showed hydrolysed urinary 4,4'-MDA levels of 0.12-0.2 nmol/mmol creatinine [21]. In the present study, the corresponding levels of 4,4'-MDA were 0.11-0.25 nmol/mmol creatinine. Thus, eight hours of skin exposure to 25-50 mg 4,4'-MDI gave similar blood and urine values as air exposure to 2-7 μg 4,4'-MDA over eight hours, based on an inhaled air volume of 10 m^3 per day. This indicates that the distribution and elimination after dermal uptake of 4,4'-MDI are much slower processes compared to similar events during airway uptake.

The very low recovery of 4,4'-MDI in blood and urine could possibly be explained by accumulation of 4,4'-MDI in the upper skin layers. However, analysis of the tape strips showed that only 0.1-0.3% of the total applied dose of 4,4'-MDI could be retrieved from Volunteers 3 and 4, respectively (figure 5), showing that accumulation of 4,4'-MDI, as such, in the upper layers of the stratum corneum is very low.

In figure 4, an increased pattern of urinary metabolites following an increased applied dermal dose, ranging from 25-50 mg, is presented. The peak in urinary metabolites appears after 10-14 hours and declines to approximately

starting values, 48 hours after the start of the exposure. In an earlier study, following exposure to 4,4'-MDI during handling of orthopaedic casting products, no urinary metabolites could be shown [22]. This was probably due to much lower skin exposure compared to our study.

Theoretically, 4,4'-MDI may react with water in the skin forming 4,4'-MDA which then penetrates the skin. This theory is supported by the low amounts of 4,4'-MDI found in the tape strips. However, if 4,4'-MDA is formed in the skin, it would be expected to easily penetrate the skin since it has been shown that 4,4'-MDA easily penetrates human skin [23, 24]. If this scenario applies, we would expect high 4,4'-MDA levels in the urine. However, we have found only traces, indicating that only a very small fraction of 4,4'-MDI could have reacted, according to the suggested mechanism. The most plausible explanation for the low recovery is that 4,4'-MDI reacts with proteins and other cell components, and possibly polymerizes as a result of hydrolysis of some of its isocyanate groups. This would mean that 4,4'-MDI remains bound in the upper layers of the skin and results in low concentrations of 4,4'-MDA from hydrolysed plasma and urine, as we found. Patch testing in individuals allergic to 4,4'-MDI has shown that positive test reactions to 4,4'-MDI may appear after two to three weeks while a contact allergy reaction usually appears after one to three days [25]. The authors hypothesize that the unusually late reactions are due to the binding of 4,4'-MDI to cell constituents and a subsequent slow release of 4,4'-MDA as hydrolysis of the formed conjugates occurs. The positive patch test reactions are then caused by 4,4'-MDA allergy. The low recovery of 4,4'-MDI in the present study supports our suspicion that a large proportion of the skin-absorbed 4,4'-MDI rapidly reacts with cell constituents in the upper skin layers. Polymerization in the upper skin layers is also a possible contributing mechanism.

The volunteers were exposed to up to 50 mg 4,4'-MDI dermally and the approximate order of absorption was half of the applied amount. This absorbed amount is probably released as 4,4'-MDA by spontaneous or enzymatic hydrolysis during weeks or months, systemically distributed and finally eliminated. The very low levels of 4,4'-MDA in hydrolysed plasma and urine samples indicate that only a small fraction of 4,4'-MDI penetrates the skin in an unchanged form (figures 3, 4).

Conclusions

4,4'-MDI is readily absorbed by human skin. Only small fractions of 4,4'-MDI remain as such in the superficial skin layers. Our results indicate that a large proportion of 4,4'-MDI reacts rapidly with cell constituents. A small proportion of the 4,4'-MDI dose seems to penetrate the skin in an unchanged form. Our data strongly indicate that formation of 4,4'-MDA from 4,4'-MDI upon reacting with water can occur only to a very limited extent.

The dermal uptake and distribution of 4,4'-MDI are much slower processes compared to those during airway uptake. The distribution of 4,4'-MDI from the skin and the subsequent elimination are slow processes. ■

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