

Cutaneous sensitization to some polyisocyanate prepolymers in guinea pigs

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Isocyanates are used extensively in the polyurethane industry. Pulmonary and dermal sensitization resulting from exposure to diisocyanates has frequently been reported, but the potential effects of polyisocyanates on health are less well known. Thus, since 1978, occupational exposure limits have been established for diisocyanates only. Nevertheless, respiratory diseases and dermatitis have been reported in the polyurethane industry after accidental isocyanate contact during spills or splashes. The aim of this experimental work was to assess the dermal hypersensitivity of guinea pigs to some polyisocyanate prepolymers by means of a well-conducted standard predictive Buehler test. Our results showed that dicyclohexylmethane 4,4'-diisocyanate (HMDI), toluylene 2,4-diisocyanate (TDI), TDI adduct triol, TDI isocyanurate, 1,6-hexamethylene diisocyanate (HDI), HDI isocyanurate, HDI biuret and isophorone diisocyanate (IPDI) induced dermal sensitization while IPDI isocyanurate did not. In conclusion, the dermal hypersensitivity of guinea pigs to some polyisocyanates was similar to those of their corresponding monomers except for IPDI isocyanurate, suggesting that the results from diisocyanate monomers could not be a valuable approach for the detection of the sensitization potency of the corresponding prepolymers.

Key words: guinea pigs; Buehler test; polyisocyanate prepolymers; delayed skin hypersensitivity.
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Isocyanates are widely used in both the production of polymeric materials and the synthesis of other chemicals such as pesticides of the carbamate family. They contain one or several NCO groups and many commercial uses stem from both di- and polyisocyanates, because they react with di- and polyfunctional hydroxylated polyesters or polyethers to form polyurethanes. These compounds are used as rigid or flexible foams, surface coatings, fibers, adhesives, sealants and elastomers (1, 2). Numerous clinical reports have mentioned the development of cutaneous and pulmonary sensitivity after accidental isocyanate exposure during industrial spills or splashes (2–4). Such accidents frequently involve isocyanate contact with the hands, face and sometimes the whole body, and thus these compounds may induce contact dermatitis (1, 5, 6–8). In 1978, the National Institute for Occupational Safety and Health (NIOSH) established occupational exposure limits for all diisocyanates (2), but not for polyisocyanates. Furthermore, a permissible exposure limit (PEL) of 1 mg/m³ has been established for HDI polyisocyanates in the State of Oregon (USA) (9) and in France (10). Few data are avail-

able regarding the asthma and contact dermatitis acquired from occupational contact with polyisocyanates chemicals (11–13). In these 2 latter clinical studies, the authors showed, by specific inhalation challenges, that polyurethane painters with occupational asthma presented asthmatic reactions when exposed to non-volatile polyisocyanate prepolymer aerosols. No experimental studies have been conducted on animals to predict the potential cutaneous sensitization induced by polyisocyanates.

The purpose of the present study was to assess the dermal hypersensitivity of guinea pigs to some monomers and their corresponding polyisocyanate prepolymers. On the basis of conventional methods recommended by the European Community for general classification and labelling requirements for dangerous substances and preparations (14), the Buehler test (15, 16) was chosen to predict the delayed contact hypersensitivity induced by these compounds. The Buehler test involves only topical applications and does not require intradermal injections as in the guinea pig maximization test of Magnusson and Kligman

(17), which cannot be used due to the high viscosity of these compounds.

Materials and Methods

Chemicals

The samples were purchased from isocyanate manufacturers (Bayer, Hüls, Rhône-Poulenc) or from Merck with the highest purity grade obtainable. The purities were provided by the manufacturer except for the Tolonate HDT and Tolonate HDB which were specially purified for this experiment by Rhone-Poulenc (France):

- dicyclohexylmethane 4,4'-diisocyanate, "hydrogenated MDI" (HMDI, Desmodur W, Bayer); no. CAS 5124-30-1; NCO content >31.8%; purity >99.5%;

- toluylene 2,4-diisocyanate (TDI, Merck Schuchardt); no. CAS 584-84-9; product for synthesis; toluylene 2,4 (80.0%), toluylene 2,6 (20%);

- toluylene diisocyanate adduct triol, 75% in ethyle acetate (Desmodur L 75, Bayer); no. CAS 1431-54-5; NCO content: 13%; purity >99.5%;

- toluylene diisocyanate isocyanurate, 51% in ethyle acetate (Desmodur IL 51, Bayer); no. CAS 26603-40-7; NCO content: 8%; purity >99.5%.

- 1,6-hexamethylene diisocyanate (HDI, Merck Schuchardt); no. CAS 822-06-0; purity >99.0%;

- 1,6-hexamethylene diisocyanate isocyanurate (Tolonate HDT, Rhône Poulenc); no. CAS 3779-63-3; NCO content: 22%; purity: residual HDI <0.02 %;

- 1,6-hexamethylene diisocyanate biuret (Tolonate HDB, Rhône Poulenc); no. CAS 4035-89-6; NCO content: 22%; purity: residual HDI <0.02%;

- isophorone diisocyanate (IPDI, Vestanat IPDI, Hüls); no. CAS 4098-71-9; NCO content: 37%; purity >99%.

- isophorone diisocyanate isocyanurate (Desmodur Z, Bayer); no. CAS 67873-91-0; NCO content: 16.9%; purity: residual monomer < limit of detection.

Positive reference substances and dilution compounds

Neomycin sulfate (CAS no. 1405-10-3) purchased from Centravet (France) was selected in accordance with the European Community Directive (14). HMDI was selected as a positive reference substance for the family of isocyanates according to Karol & Magreni (18). All the compounds were diluted (w/v) in pet. (codex) except for the adduct triol and isocyanurate of TDI originally diluted by the manufacturer in ethyl acetate (w/v).

Animals

Germ-free albino female Dunkin-Hartley guinea pigs, weighing on average 350 g at the beginning of the experiment, were purchased from Charles River (France). For each series, a control group of 10 animals and a treated group of 20 animals were housed separately in stainless-steel cages and identified by an ear tag. Sterile food pellets (UAR 114, France) and filtered tap water were supplied *ad libitum*. The environment was controlled at a temperature of $18^{\circ}\pm 1^{\circ}\text{C}$, a relative humidity of $50\pm 5\%$, ventilation of 15 cycles/h, and a 12-h light-dark cycle. 10 sets of 30 guinea pigs were tested in this experiment.

Buehler test

The Buehler test carried out for this study is a method registered in the Official Journal of the European Community (14). This method is preceded by a preliminary study to determine the test substance concentrations suitable for induction and challenge. The concentration used for induction should produce no systemic toxicity and a mild to moderate dermal response, unless the test substance is not dermally irritating at 100%. The test substance concentration used for challenge should produce no systemic toxicity and dermal negative responses unless the test substance is not dermally irritating at 100%.

The principle of the test method (16) consists of an induction exposure with epidermal occlusive applications: after a rest of 14 days during which an immune response may develop, the guinea pigs are exposed to a challenge dose. Approximately 30 h after application of the challenge patch, skin reactions are observed and recorded according to the Magnusson/Kligman grading scale for the evaluation of challenge patch test reactions (17):

0=no visible change

1=discrete or patchy erythema

2=moderate and confluent erythema

3=intense erythema and swelling

Evaluation of the sensitizing potential of substances is based only on the number of guinea pigs showing positive sensitization (only guinea pigs with a score of 2 and/or 3 were considered as positive in this experiment) and not on the reaction intensity (14). The extent and degree of skin reaction to the challenge exposure were compared with that exhibited by control animals which only received the vehicle during induction.

Histopathological examination was carried out to clarify doubtful reactions, and the results were interpreted as positive only if they were confirmed as sensitization reactions of experimental eczema.

The sensitivity and reliability of the test were assessed by the use of a reference substance (neomycin sulfate) known to have moderate skin sensitization properties (14).

Results

Petrolatum and ethyl acetate vehicles did not induce irritation and/or sensitization (19, 20). The concentrations of substances inducing sensitization and challenge as well as the sensitization rate and grade are indicated in Table 1. The positive macroscopic reactions were confirmed by a microscopic investigation showing an experimental eczema characterized by parakeratosis, hyperacanthosis with spongiosis, vesicles and exudate accompanied by mononuclear infiltrate in the dermis.

The results, summarized in Table 1, led to the following conclusions.

(i) All controls challenged with the test compounds and/or the reference substance did not show macroscopic positive reaction (notations varied between 0 and 1), and these negative results were confirmed by microscopic examination.

(ii) HDI, IPDI, TDI adduct triol and HDI biuret induced grade IV (strong) skin sensitization.

(iii) TDI, TDI isocyanurate, HMDI and HDI isocyanurate induced grade V (very strong) skin sensitization.

(iv) No skin sensitization was observed after induction and challenge with IPDI isocyanurate confirmed by microscopic examination.

Discussion

The cutaneous sensitization induced by diisocyanate monomers is well known (1, 6), but there were no experimental data available about polyisocyanate cutaneous sensitization. The aim of our study was therefore to assay the ability of polyisocyanate prepolymers to induce skin sensitization, com-

pared with skin sensitization of their respective monomers: HDI with HDI isocyanurate and HDI biuret, TDI with TDI isocyanurate and TDI adduct triol, IPDI with IPDI isocyanurate. This experiment has shown that, except for IPDI isocyanurate, polyisocyanate prepolymers are able to induce skin sensitization in guinea pigs. TDI adduct triol and isocyanurate, as well as HDI biuret and isocyanurate, show a similar sensitization grade as HMDI, considered to be a very strong sensitizer among the diisocyanate family (18).

Vandenplas et al. (13) cited HDI isocyanurate and HDI biuret as respiratory allergens having the same effects as monomeric HDI, and this experiment showed that they are skin sensitizers. In the same way, TDI isocyanurate and TDI adduct triol can cause occupational asthma (12) as well as monomeric TDI, and are also shown here as skin sensitizers. Furthermore, 2 experiments (HDI biuret and isocyanurate) were performed with samples obtained by laboratory redistillation from technical products to obtain a very low content of residual monomer (less than 0.02%). These experiments support the hypothesis that skin sensitization is induced by the prepolymer itself and not by the presence of residual monomer. This experimental result is in agreement with the studies of Vandenplas et al. (12, 13), which concluded that isocyanate prepolymers should definitively be considered as a potential cause of asthma. These 2 studies, performed with a specific inhalation challenge test protocol on polyurethane spray painters with occupational asthma, showed that some of their subjects had a positive reaction to the prepolymer and not to the corresponding monomer, to which they had probably not been exposed to or not exposed to at a level sufficient for sensitization.

Regarding IPDI prepolymer, these studies should also be considered in parallel with our experimental finding about the lack of sensitization potency of IPDI isocyanurate. Indeed, Vandenplas et al. (13) have noticed that a subject exposed to a

Table 1. Experimental results

Substances	Induction conc. % (w/v) (0.5 ml)	Challenge conc. % (w/v) (0.5 ml)	Sensitization rate sum	Sensitization grade
neomycin sulfate	50	10	10/19	III
HMDI (Desmodur W)	2	0.5	19/20	V
TDI	5	1	18/20	V
TDI adduct triol (Desmodur L 75)	30	5	15/20	IV
TDI isocyanurate (Desmodur IL 51)	50	10	18/20	V
HDI	1	0.1	14/20	IV
HDI isocyanurate (Tolonate HDT)	100	50	19/20	V
HDI biuret (Tolonate HDB)	50	30	14/20	IV
IPDI (Vestanat IPDI)	5	1	16/20	IV
IPDI isocyanurate (Desmodur Z)	10	5	0/20	—

paint hardener containing a mixture of HDI and IPDI prepolymers developed a positive reaction during the challenges with pure HDI prepolymer, but showed no bronchial response to the IPDI prepolymer.

In conclusion, this is the 1st experimental study conducted on guinea pigs to predict the potential cutaneous sensitization of some polyisocyanates. Our results show that the sensitizing prepolymers and their corresponding monomers have a similar notation on the Magnusson scale, and that these prepolymers should be tested separately due to the negative result on IPDI isocyanurate.

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