

Occupational Asthma Caused by Isocyanates

Patterns of Asthmatic Reactions to Increasing Day-to-Day Doses

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Inhalation challenges to isocyanates are conducted in specialized centers to confirm occupational asthma. The pattern of asthmatic reactions due to consecutively increasing daily doses of isocyanates is unknown. We conducted a study involving 24 subjects who had undergone specific inhalation challenges to isocyanates (toluene diisocyanate [TDI], $n = 8$; hexamethylene diisocyanate [HDI], $n = 10$; and methylene diisocyanate [MDI], $n = 6$) on three or more consecutive days. Challenge tests were given through a closed-circuit apparatus ($n = 12$) or in small cubicles ($n = 12$), allowing assessment of the total inhaled dose (concentration \times duration). The pattern of asthmatic reactions was described. In addition, dose-response curves were analyzed and tested for their linear and quadratic trends. Four patterns of response were observed: (1) linear ($n = 10$); (2) minimal effect followed by a brisk change ($n = 7$); (3) significant change followed by tachyphylaxis or a plateau ($n = 4$); (4) biphasic (i.e., significant change followed by a reduction in the effect and significant change on the last day of exposure [$n = 3$]). Subjects with a linear dose-response pattern had been exposed to isocyanates at work for a significantly shorter interval (7.2 ± 6.7 yr) than subjects with a nonlinear pattern (20.0 ± 13.1 yr). An analysis of variance covering a 3-d period for all subjects showed a significant linear model for the response ($p < 0.0001$); there was no quadratic trend. However, when the analysis was done on subjects with four or more days of challenge ($n = 10$), we found both linear and quadratic significant components. This analysis shows that the most common pattern of asthmatic reactions to inhaled isocyanates generated on consecutive days is linear; however, other patterns are also observed. In some individuals, particularly those in whom more days of challenge are required, we observed in addition to a strong linear component a quadratic component manifested by a brisk change on the last day of exposure. Malo J-L, Ghezzo H, Élie R. Occupational asthma caused by isocyanates: patterns of asthmatic reactions to increasing day-to-day doses.

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Isocyanates are a common cause of occupational asthma (1). In high-risk workplaces, it has been estimated that 5 to 10% of exposed workers are affected. Various objective means have been proposed to confirm the diagnosis. Among these, exposure to isocyanates in the laboratory is probably the single most satisfactory diagnostic tool (2).

Laboratory exposure to low-molecular-weight agents including isocyanates is generally conducted in a progressive day-to-day way. For the sake of biologic security, the concentration has to satisfy exposure standards (3), the highest concentration of isocyanate being set at levels < 20 ppb. We showed that the dose (i.e., concentration \times duration of exposure) is the key feature that determines the asthmatic reaction

to isocyanates, and not the concentration or the duration of exposure per se (4). The dose is increased until a significant asthmatic reaction is elicited or no reaction can be documented after a reasonable duration of exposure, with the maximum duration generally being set at 2 h in order to exclude asthma. The dose has to be increased from day to day, since these agents generally cause nonimmediate or atypical asthmatic reactions (5). Therefore, contrary to high-molecular-weight agents, which cause first an immediate asthmatic reaction that may sometimes be followed by a nonimmediate reaction, and for which the dose is increased progressively on the same day, challenges with increasing doses of isocyanates have to be given on different, and most often consecutive, days.

The pattern of reaction elicited by day-to-day exposure to isocyanates has not to our knowledge been characterized. It can be postulated that the reaction has a linear pattern. However, the immunologic mechanism for the reaction is generally unknown for most low-molecular-weight agents. Moreover, the metabolism of elimination of isocyanates is not well characterized, and accumulation can be hypothesized. Therefore, a nonlinear pattern of reaction is also possible, as either a priming effect of consecutive day-to-day exposure or, alternatively, a plateau or tachyphylactic effect can be hypothesized.

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We therefore examined the pattern of the day-to-day response to increasing doses of isocyanates administered to patients with isocyanate-induced occupational asthma.

METHODS

Subjects

Twenty-four subjects (20 men and four women) aged 28 to 65 yr were included in this study. The inclusion criteria were as follows: (1) three or more consecutive days of challenges with isocyanates; and (2) a sustained decline in FEV₁ that should have reached 15% or more. At the time of diagnosis and specific inhalation challenges, the subjects had been exposed to isocyanates for a mean of 15 yr (range: 0.5 to 42 yr) and had been symptomatic for a mean duration of 4 yr (range: 0.2 to 10 yr). Sixteen subjects were atopic (i.e., showed at least one immediate skin reaction to a battery of 15 common inhalants by the skin prick method).

Challenge Tests

Challenge tests were done as previously described (2) in small cubicles (n = 12) or with a closed-circuit apparatus (n = 12) that we have developed (6). Concentrations of isocyanates were monitored either with an MDA apparatus (MDA Scientific Inc., Glenview, IL) or GMD instrument (GMD Systems Inc., Pittsburgh, PA). These instruments were calibrated by means of comparison with high-performance liquid chromatography (HPLC) measurements. Calibration curves showed that the assessments of concentration made with HPLC and with these instruments had a linear relationship. FEV₁ was monitored before exposure, and then at 10-min intervals in the first hour, 30-min intervals in the second hour, and hourly for 7 to 8 h after exposure. The standardized protocol used is exposure of subjects for not more than 1 to 4 min on the first day (depending on baseline non-specific bronchial responsiveness to methacholine), 5 to 30 min on the second day, and 2 h on the third day, unless a decrease in FEV₁ of 20% or more is observed immediately or 10 min after stopping exposure. In this instance, the exposure is stopped (2). When more days are required to elicit a reaction, the duration of exposure is still 2 h, but the concentration of isocyanate is increased; the total dose is therefore higher. Although the total exposure period on each study day is as described earlier, FEV₁ is monitored serially at split intervals (e.g., after one breath and 15 s on the first study day, and after each 10-min exposure when the total exposure is 30 min). The concentration of isocyanate has to be less than 20 ppb during all exposure periods.

Eight subjects reacted to toluene diisocyanate (TDI), 10 to hexamethylene diisocyanate (HDI), and six to diphenylmethane diisocyanate (MDI). Fourteen subjects were exposed on three consecutive days, seven on four consecutive days, and three on five consecutive days. Seven subjects demonstrated an isolated immediate reaction (maximum fall of FEV₁ in the first 60 min after ending exposure); the remaining majority of subjects had an isolated late or an atypical reaction.

Analysis

Doses were calculated as the product of the mean concentration obtained every 2 min with the MDA apparatus multiplied by the duration of exposure. They were expressed on a noncumulative and cumulative basis (Day 1 + Day 2 + Day 3, etc.), and were log-transformed for analysis.

We first examined and described individual curves relating doses and responses (maximum declines in FEV₁ recorded during the day). The area under the curve was not examined because of the impossibility of standardizing this area for both immediate and nonimmediate reactions. Four patterns of response were identified: (1) a linear response, defined as a sustained and progressive decline in FEV₁ from the first day on; (2) nonlinear responses of three types, consisting respectively of little change in FEV₁ followed by a brisk change on the last day or last two days of the challenge; a significant change in FEV₁ followed by a reduction in the effect on the last day or last two days of exposure; (4) a biphasic response, defined as a significant change on the first day of exposure followed by a reduction on the next day(s) and a recurrence of the significant change on the last day of exposure.

Curves were also analyzed in terms of their linear and quadratic components, using analysis of variance (ANOVA), with the analysis

done separately for all subjects and for subjects who had been exposed to isocyanates for three days or more (n = 24), for subjects who had been exposed for four days or more (n = 10), and for those (n = 14) who had been exposed for only 3 d.

An unpaired *t* test was used for comparisons of between-group differences, and a value of *p* < 0.05 was considered significant.

RESULTS

As set by the protocol of exposure, the inhaled dose of isocyanate increased progressively and linearly as expressed on a log scale from 184 ppb-min (ln = 5.2) to 414 ppb-min (ln = 6.02) and to 1,003 ppb-min (ln = 6.9). In the subgroup of 10 subjects for whom at least 4 d of exposure was necessary, this increase was from 306 ppb-min (ln = 5.7) to 404 ppb-min (ln = 6.0), 1,097 ppb-min (ln = 7.0), and 1,858 ppb-min (ln = 7.5).

Figures 1 to 3 illustrate the individual curves according to the four types of reactions. The linear pattern was the most common type of reaction (Figure 1). Seven subjects showed an abrupt decrease in FEV₁ on the last day of exposure (Figure 2). In four subjects (*upper part* of Figure 3) the dose that caused a significant change in FEV₁ on the last day of exposure was less than the dose administered on the preceding day. In three subjects (*lower part* of Figure 3) there was a significant decline in FEV₁ on the first day of exposure, followed by a reduced response on subsequent days and a significant decline on the last day of exposure. Subjects with a linear pattern of response tended to have a significantly lower provocative concentration of methacholine producing a 20% decline in FEV₁ (PC₂₀) (mean: 2.2 mg/ml) than those with a nonlinear pattern (mean: 14.9 mg/ml), although this difference was not significant. Subjects in the former group had also been exposed to isocyanates at work for a shorter period (7.2 ± 6.7 yr)

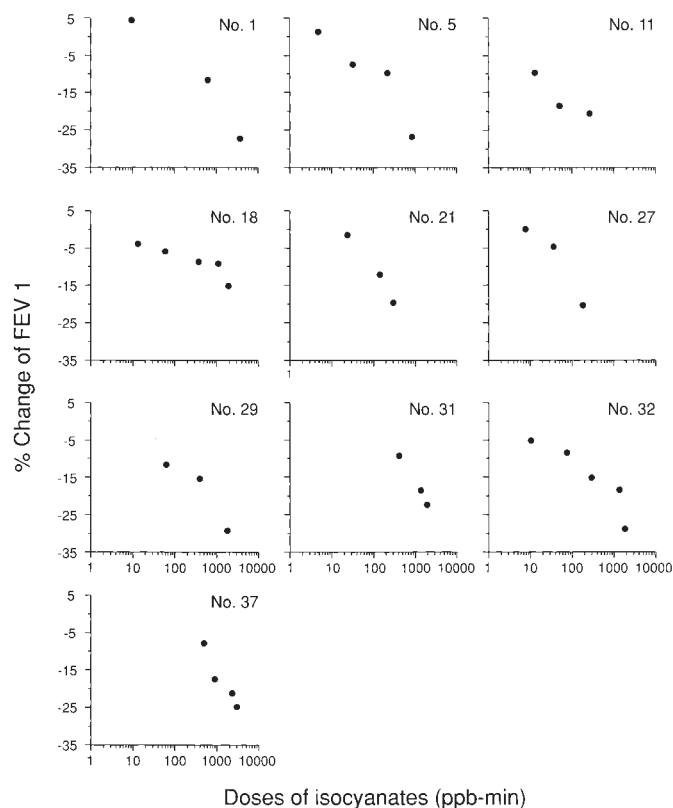


Figure 1. Individual dose-response curves for subjects with an apparent linear model.

than subjects with a nonlinear pattern (20.0 ± 13.1 yr) ($t = 3.1$, $p < 0.005$). However, they had not been away from exposure for a longer interval (mean: 11 wk; range: 0 to 68 wk) than subjects with other patterns (mean: 3.3 wk; range: 0 to 16 wk).

Curves were also subjected to ANOVA. The results are shown in Figure 4, differentiating all curves from those obtained for subjects who were exposed for four consecutive days or more and from those of subjects exposed for only 3 d. A strong linear component was demonstrated in all instances. However, curves for subjects who needed to be exposed for four consecutive days or more in order to demonstrate a significant response also showed a significant quadratic component, whereas this was only marginally significant in those who were exposed for only 3 d. Therefore, in subjects who needed to be exposed for 4 d or more to demonstrate a significant reaction, FEV₁ decreased slowly, though significantly, in the first days of exposure, but more so on the last day of exposure.

Results were unchanged by examining the dose on a cumulative basis.

DISCUSSION

We found that the most common day-to-day pattern of reaction to increasing doses of isocyanates was linear. However, other patterns were also observed, as follows: (1) a minimal effect followed by a brisk change; (2) significant change followed by a plateau; (3) a biphasic type of response (i.e., change followed by a reduction in effect and change on the last day of exposure). Analysis of the curves showed a significant linear component; however, in a subgroup of subjects who needed to be exposed for 4 d or more to demonstrate a reaction, a significant quadratic response was also documented, therefore showing a brisk increase in specific reactivity on the last day of exposure.

The shape of the dose-response curve for increasing day-to-day doses of inhaled isocyanates has not to our knowledge

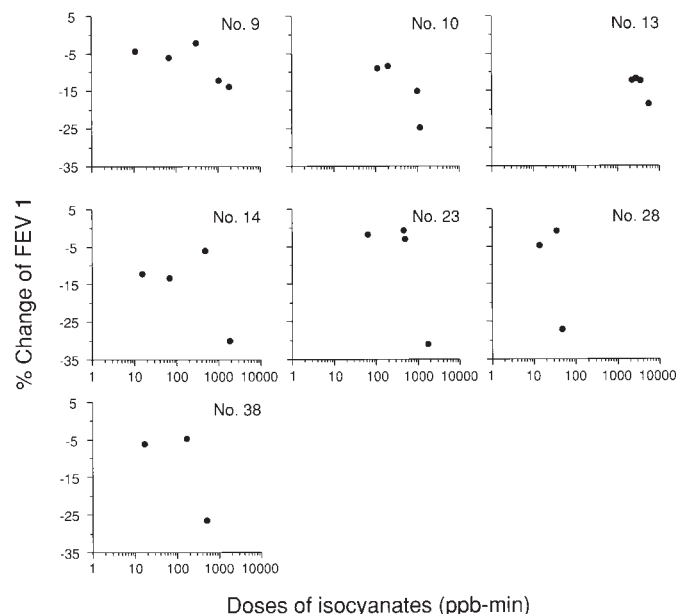


Figure 2. Individual dose-response curves for subjects who showed a minimal effect followed by a brisk change. Subjects 10 and 13 were exposed to higher doses on the first day of exposure (see METHODS) because they had been exposed to lower doses without reaction 1 mo earlier.

been described. Whereas the linear pattern of reaction can be explained in the same manner as for high-molecular-weight occupational agents that cause asthma by an IgE-mediated mechanism, the other patterns are certainly more difficult to interpret. From the strict pharmacologic point of view, isocyanates accumulate in the body and can be detected as protein-adducts and amines in urine, plasma, and erythrocytes (7, 8). It is conceivable that from one subject to the next, the rate of production and excretion of protein-adducts and amines is different. Brorson and coworkers found that the half-time for the concentration of amines in urine was 1.2 h in subjects exposed for 8 h (7). However, workers chronically exposed to isocyanates show a biphasic elimination, with the second phase, having a half life of 21 d, probably being related to release of amines in the urine from TDI adducts in the body (9). These compounds can therefore accumulate to a greater degree in some subjects than in others. Therefore, specific reactivity to isocyanates can be enhanced or reduced on the last day of exposure, depending on the bodily accumulation of isocyanates. Furthermore, the rate of excretion of isocyanates is not linear (7). In this regard, it is interesting to note that subjects who needed to be exposed for four days or more (the "slow reactors" in this study) showed a small increase in response on the first days of exposure, followed by a brisk change on the last day of exposure. It is therefore tempting to suggest that the rate of elimination of isocyanates can be slowed on the last day of exposure, which corresponded to the maximum dose that was inhaled. It would be interesting to explore this further. In our study, we assumed that the inhaled dose was entirely retained. However, by assessing exhaled concentrations of isocyanates, we recently documented that the retained dose

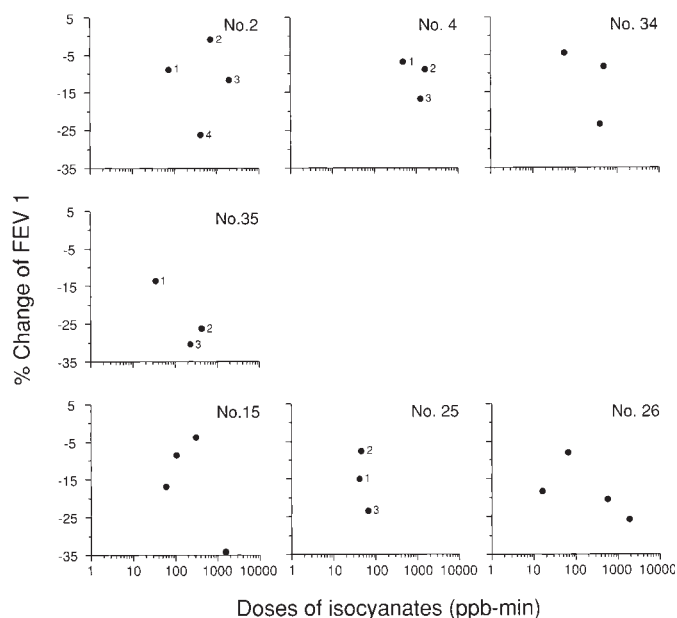


Figure 3. Individual dose-response curves for subjects who showed: (1) a significant change followed by a plateau (*upper and middle panels*); and (2) a biphasic type of response (i.e., a change followed by a reduction in effect and then a change on the last day of exposure) (*lower panel*). For some subjects in whom the dose on the last day of exposure was lower than on the previous day, the sequence of days of exposure is shown by number; otherwise, points of increasing doses correspond to consecutive days. Subject 35 had shown a $> 20\%$ decrease in FEV₁ on Day 2. However, we wanted to confirm the reaction on a third day (Day 3).

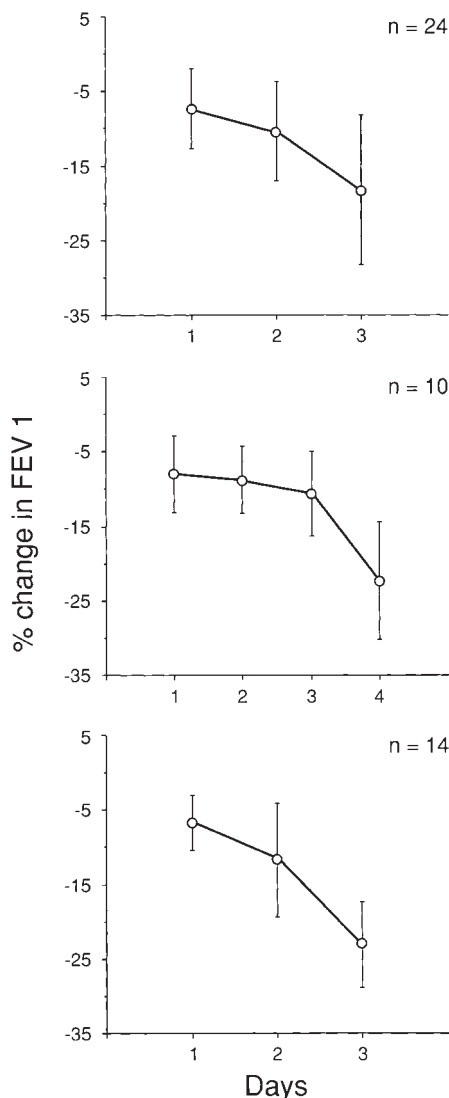


Figure 4. Mean \pm SD changes in FEV₁ according to days of exposure in the 24 study subjects (*upper panel*) (linear: $F_{1,69} = 31.2$ [$p < 0.001$]; quadratic: $F_{1,69} = 1.8$ [NS]); in those who had four days or more of exposure (*middle panel*) (linear: $F_{1,36} = 29.0$ [$p < 0.001$]; quadratic: $F_{1,36} = 8.5$ [$p = 0.006$]; cubic: $F_{1,36} = 1.2$ [NS]); and in those who had only 3 d of exposure (*lower panel*) (linear: $F_{1,41} = 55.6$ [$p < 0.001$]; quadratic: $F_{1,41} = 3.5$ [$p = 0.06$]). Significant linear components are seen in all instances, with a significant quadratic component for subjects who had four days or more of exposure.

is half the dose that is apparently inhaled (J. L. Malo and colleagues, in preparation). It would also be valuable to assess isocyanate adducts serially after inhalation challenges in order to determine whether elimination follows a linear or a nonlinear pattern. A nonlinear response to an inhaled bronchoconstrictor agent would not be unique to isocyanates. Indeed, although a linear pattern is generally observed on exposure to pharmacologic bronchoconstrictors such as histamine or methacholine (10), other patterns can also be observed, particularly plateaus in the response (11, 12), which have been documented in less responsive individuals.

The various patterns of reaction to isocyanates can also be explained from an immunologic viewpoint. The mechanism of isocyanate-induced occupational asthma is still open to discus-

sion. Whereas the role of specific IgG antibodies is clear in hypersensitivity pneumonitis caused by isocyanate (13), the role of these antibodies in isocyanate-induced asthma is less consistent, although common (14). Specific IgE antibodies are more rarely and inconsistently found, although they seem to be specific for such asthma (15). An immunologic role of isocyanates is highly probable since, as for high-molecular-weight agents, a latency period (i.e., a period of asymptomatic exposure) is necessary before the onset of symptoms. This latency period is shorter for isocyanates and for high-molecular-weight, protein-derived agents (16). If the reaction is immunologic, it is conceivable that the reaction could be linearly related to the dose of isocyanate in some subjects, could develop slowly and be followed by a brisk onset corresponding to a "priming" effect in others, or could develop rapidly and then "plateau" in others. A tachyphylactic effect is conceivable in less reactive subjects, which was the case in our study, and in those not exposed to isocyanates for a longer interval, which could not have been shown in our study, since our subjects had been exposed for too short an interval (mean of 6.5 wk since their last exposure). A longer exposure is necessary in the latter instance, causing a slowly developing reaction on the first days of exposure and, once the "priming" effect is reached, an expected brisk reaction on the last day of exposure. The analysis in our study revealed such a pattern in subjects who needed to be exposed for four days or more. In these subjects, assuming that both IgE and IgG antibodies were involved, it can be postulated that for the first days of exposure, all IgE molecules were saturated and there was no room for binding of additional allergenic epitopes. The priming effect could be a combination of: (1) replacement of bound and saturated IgE molecules with unbound IgE molecules (with newly available antigen-combining sites) on mast cell surfaces; and (2) loss of competitive effects of IgG over a period of days.

This study has practical implications. Although the pattern of reaction found in the study has been described for pharmacologic agents causing bronchoconstriction, such as histamine or methacholine, it has rarely been characterized for "sensitizing" agents administered either in a progressive way on a single day of exposure (as is generally done for high-molecular-weight agents) or, as was the case in this study, on a day-to-day progression (as is generally done for low-molecular-weight agents). The dose-response pattern was described as generally linear in the case of two chemical agents, sodium isononyl oxybenzene sulphonate (SINOS) (17) and tetrachlorophthalic anhydride (TCPA) (18). Our study shows that a subject who exhibits a slowly developing reaction from one day to the next may well be at risk of showing a brisk, more intense reaction on the last day of exposure. On the other hand, a subject who shows a rapidly developing day-to-day reaction is expected to react according to a linear pattern on the last day of exposure. Also, subjects exposed to isocyanates for shorter periods at work are more likely to react rapidly and with a linear pattern.

This study retained only subjects who needed three days or more to react to isocyanates. It is to be noted that the maximum reaction documented in any of the 24 subjects retained for analysis was less than a 35% decrease in FEV₁. Therefore, dose-response curves for isocyanates can be generated in a progressive and safe way.

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