

## Sensory Irritation by Toluene Diisocyanate in Single and Repeated Exposures

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Sensory Irritation by Toluene Diisocyanate in Single and Repeated Exposures. SANGHA, G., AND ALARIE, Y. (1979). *Toxicol. Appl. Pharmacol.* 50, 533-547. The effects of single and repeated exposures to toluene diisocyanate (TDI) at concentrations ranging from 0.007 to 2 ppm were investigated using an animal model to detect the level of sensory irritation caused by this chemical. Time-response relationships showed the slow development of the response with exposure duration and a very slow recovery following 3 hr of exposure. Concentration-response relationships also showed that the level of response was not only dependent upon exposure concentration, but also on duration of exposure and that TDI is the most potent sensory irritant tested so far. Repeated exposures to TDI at or above 0.023 ppm resulted in cumulative effects. On the basis of a prior prediction proposed from the results obtained with this animal model, it is suggested that the Threshold Limit Value (TLV) for industrial exposure be reduced to 0.006 ppm as a time weighted average (TWA) and no exposure above 0.02 ppm be permitted, even for short exposures.

It has been postulated that pulmonary reactions due to inhalation of toluene diisocyanate (TDI) by industrial workers would occur via two mechanisms, generally referred to as immunological and pharmacological. The first mechanism would involve the production of tolyl-reactive IgE antibodies in a fraction of the exposed workers. Such workers would then experience asthma-like attacks upon exposure to very low levels of TDI. The second mechanism would involve interactions of TDI with bronchial epithelium and interference with cholinergic or adrenergic systems in such a manner as to enhance the response upon reexposure to TDI, and result in a cumulative effect. Epidemiological studies by Peters *et al.* (1968, 1970) and Wegman *et al.* (1977) point in the direction of such an effect as measured by

decrement in pulmonary function.

Despite efforts to identify tolyl-reactive IgE antibodies in workers with known bronchial hypersensitivity reactions to TDI (Weill *et al.*, 1975; Butcher *et al.*, 1976, 1977; Fink and Schlueter, 1978) and induce this phenomenon in animals, only recently have such antibodies been detected in workers reactive to TDI, and tolyl-specific antibodies been induced in animals with aerosol exposures (Karol *et al.*, 1978a,b). Thus, the existence of an immunological hypersensitivity mechanism has been established and the presence of an immunological mechanism confirmed. The second mechanism, pharmacological hypersusceptibility, has not been studied in humans or animals, but suggested from results of *in vitro* studies (Davies *et al.*, 1977; Van Ert and Battigelli, 1975).

In previous studies in mice, it has been demonstrated that TDI is a very potent

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sensory irritant (Barrow *et al.*, 1978) capable of stimulating the cholinergic trigeminal nerve endings in the nasal mucosa in a manner similar to that for a wide variety of airborne chemicals (Alarie, 1973a). Studies with other sensory irritants such as formaldehyde or acrolein clearly demonstrated that with daily repeated exposures, a definite increase in the sensory irritation response could be observed, without any known immunological interaction (Kane and Alarie, 1977) and thus belonging to a pharmacological hyper-reactivity mechanism. Similar studies are presented in this report to investigate the possibility of a pharmacological hyper-reactivity mechanism with repeated exposures to TDI.

## METHODS

### *Animals*

Specific pathogen-free male Swiss-Webster mice, 24–27 g in body wt, from Hilltop Labs were used.

### *Exposure Conditions and Measurement of the Response*

An all glass chamber was used for this study as described by Barrow *et al.*, 1977. Briefly, groups of four mice were exposed by restraining them in body plethysmographs for measurement of respiratory rate while the head of each animal protruded into the exposure chamber. The average respiratory rate of each group was obtained prior to, during, and following exposures as previously described (Kane and Alarie, 1977). The level of response, calculated as percentage change in respiratory rate from control level, and reflecting the level of sensory irritation of the upper respiratory tract (Alarie, 1966, 1973a), was plotted versus the logarithm of exposure concentration to obtain concentration–response relationships.

TDI atmospheres were obtained by bubbling filtered and dried air through an impinger containing 2,4-toluene diisocyanate supplied by Mobay Chemical Corporation as 99.7+ % pure (Möndur TDS). By varying the airflow rate through the impinger or the airflow rate through the exposure chamber (but never less than 20 liters/min), a concentration range of 0.007 to 3 ppm was generated with excellent reproducibility, as given below. It was ascertained that TDI vapor only, free of TDI aerosol, was produced by this generation system since passing the output of the impinger through a Millipore-type HA filter resulted in less than 10% difference in concentration compared with the output concentration without the filter.

Analytical determinations of chamber atmospheres for TDI were conducted according to Marcali (1957) with modifications according to NIOSH (1977). For high concentrations, repeated determinations were made during the exposure period, while for the lowest concentrations fewer samples were taken due to the limit of detection, as reported below.

### *Exposure Groups*

*Series I.* This series consisted of exposing groups of four mice to varied TDI concentrations in order to obtain levels of responses of approximately 25–30, 40–50, and 70–80% decrease in respiratory rate by the end of either 30, 60, or 180 min respectively, and to observe the rate of recovery from these levels of responses. Time–response relationships were obtained for each exposure period with data points fitted by a piece-wise linear regression method adapted from Bogartz (1968). The method searches for the fit that minimizes the sum of the mean squared errors for a series of data points to define segments in the series.

*Series II.* In this series of experiments, groups of four mice were exposed for a period of 240 min. The response was measured at 10, 30, 60, 120, 180, and 240 min. The percentage decrease observed at each time interval was then plotted versus the logarithm of the exposure concentration and the data analyzed by least squares regression analysis to calculate the RD50, concentration associated with a 50% decrease in respiratory rate, as previously described (Kane and Alarie, 1977). TDI concentrations in the exposure chamber for this series were measured at 15, 30, 60, 120, and 180 min.

*Series III.* To assess the reproducibility of the response, five sets of experiments were conducted. Each set consisted of five groups of four mice with each group being exposed to one concentration of TDI for a duration of 30 min and the level of response being measured at 10 and 30 min. Five exposure concentrations completed one set. Concentration–response relationships were established for each set and RD50 values calculated as in Series II.

*Series IV.* The possibility of development of pharmacological hyperreactivity with repeated exposures to TDI vapor was studied by exposing groups of four mice for a period of 3 hr each day for 5 consecutive days at the following concentrations: 0.007, 0.0016, 0.0032, 0.012, and 0.018 ppm, all below the current Threshold Limit Value (TLV 0.02 ppm) for industrial exposures and 0.023, 0.078, 0.301, 0.505, 0.82, and 1.18 ppm, all above the current TLV. Prior to each daily exposure to the intended concentration, a group of four mice, never previously exposed, was exposed for 30 min and their level of response was observed at that concentration to be within the expected range (expected from the results obtained in Series I, II, and III above) so that any

lower or higher level of response in the repeated exposure groups could be attributed to the effect of such repeated exposures with confidence.

For one exposure concentration, 0.0016 ppm, a second group was exposed and given two 5 consecutive day periods of exposure separated by 2 days without exposure.

*Series V.* In order to ascertain that the decrease in respiratory rate was due to the action of TDI on the receptors in the upper respiratory tract and not because of an action on the pulmonary system or systemic effects, groups of mice were exposed via tracheal cannulation, as previously described (Kane and Alarie, 1977). The exposure concentrations selected were those resulting in 50% decrease in respiratory rate in normal control mice, RD50, and 2 and 5 × RD50 for 30-min exposure periods.

*Series VI.* This series was undertaken to investigate if 14 days after exposures to high concentrations of TDI the response upon reexposure would be similar to that of control mice.

*Series VII.* As the effect of TDI on the nasal trigeminal nerve endings was found to be cumulative with repeated exposures (see results) histopathological evaluation of the nasal mucosa was undertaken. Groups of animals were exposed 3 hr/day for 3 days to TDI at 0.031 or 0.25 ppm. The following day the animals were sacrificed and each head was placed in 10% buffered aqueous formalin solution for fixation followed by decalcification in 5% nitric acid for 48 hr. Four transverse sections of the nasal area were made as previously described (Lucia *et al.*, 1977) for microscopic examination. Evaluation of each section and scoring of lesions, if any, were conducted as previously described (Lucia *et al.*, 1977).

## RESULTS

### *Onset of the Response and Recovery, Series I*

As shown in Fig. 1, the development of the response was concentration dependent and

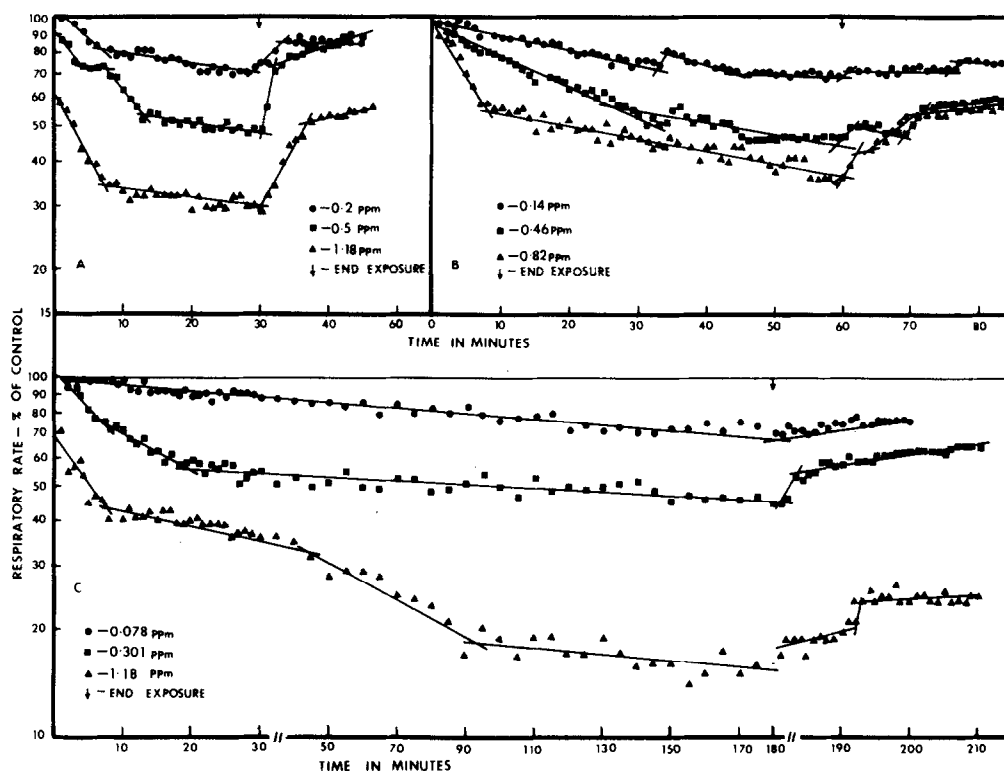


FIG. 1. Time-response curves obtained with TDI concentrations selected to obtain approximately 25–30, 40–50, and 70–80% decrease in respiratory rate by the end of 30 (A), 60 (B), or 180 (C) min of exposure. Each point represents the average respiratory rate of a group of four mice, expressed as percentage of preexposure level. The exposure concentrations are the average of three to six samples taken during each exposure with a coefficient of variation of less than 15% for each. The data points were fitted by piece-wise linear regression (see text).



FIG. 2. Concentration-response relationships obtained for various exposure concentrations of TDI with the level of response, percentage decrease in respiratory rate, measured after 10, 30, 60, 120, 180, and 240 min of exposure. Each point represents the average respiratory rate of a group of four mice, expressed as percentage of preexposure level. The exposure concentrations are the average of five samples taken during each exposure, with a coefficient of variation of less than 12% for each. The curves were obtained by linear least squares regression analysis.

very slow, taking at least 10 min to reach a first maximum even at high concentrations. Following this first 10-min period, the response continued to increase very slowly and for the next 20 to 30 min, an apparent plateau was established at high concentration. However, this apparent plateau was followed at high concentration, with a further decrease in respiratory rate between 50 and 90 min of exposure which continued up to 180 min. Thus, the level of response was not only dependent on exposure concentration, but also on duration of exposure. Similarly, recovery rate was dependent on exposure duration, being rapid with short exposures and very slow with long exposures. This pattern of response was in sharp contrast with the pattern observed for acrolein (Kane and Alarie, 1977), or for many other sensory irritants (Alarie, 1973a) where the level of response was *only* concentration dependent and recovery was always rapid, regardless of the exposure duration. Since exposure via

tracheal cannulation (Series V) failed to evoke any decrease in respiratory rate, the response seen here can be attributed to sensory irritation of the upper respiratory tract (Alarie, 1966, 1973a).

#### *Concentration Response Relationships, Series II*

Since the level of response was not only dependent on exposure concentration, but also on exposure duration, a series of concentration-response relationships was developed for various exposure durations from 10 to 240 min as shown in Fig. 2. For each, the regression analysis and RD50 values are presented in Table 1. As can be observed from both Fig. 2 and Table 1, RD50 values decreased significantly between 10 and 180 min and the level of response was exactly similar at 180 and 240 min. Plotting RD50 values for various exposure durations (Fig. 3) shows this relationship with exposure duration.

TABLE 1

CALCULATED RD50 (50% DECREASE OF RESPIRATORY RATE) AND ASSOCIATED STATISTICS FROM LEAST SQUARES LINEAR REGRESSION ANALYSIS OF THE DATA ON EXPOSURE TO TOLUENE DIISOCYANATE<sup>a</sup>

Exposure time (min)	RD50 (ppm)	95% Confidence interval	$y =$
10	0.813	0.707-0.963	$54.34 + 48.41 \log x$
30	0.498	0.406-0.635	$64.47 + 47.80 \log x$
60	0.386	0.275-0.531	$68.69 + 45.17 \log x$
120	0.249	0.146-0.359	$75.55 + 42.29 \log x$
180	0.199	0.154-0.245	$81.12 + 44.44 \log x$
240	0.199	0.154-0.245	$81.12 + 44.44 \log x$

<sup>a</sup> Values are presented in Fig. 2.  $r$  values for each regression  $> 0.94$ .

#### Reproducibility of the Response, Series III

As shown in Table 2, reproducibility of the results for concentration-response relationships was found to be excellent.

#### Hyperreactivity and Recovery with Repeated Exposures, Series IV

Exposure concentrations above TLV (0.02 ppm). At the first concentration investigated, 0.023 ppm, as shown in Fig. 4, the expected slight decrease in respiratory rate occurred by the end of the 3-hr exposure. On the second

day, this group exhibited a preexposure respiratory rate comparable to that on the first preexposure day. However, by the end of the 3-hr exposure, the decrease in respiratory rate was greater than for the first day of exposure. These animals did not fully recover and started at a lower respiratory rate on the third day of exposure and reached a further greater level of response by the end of the 3-hr exposure on that day. Exposures on the fourth and fifth day resulted in approximately the same level of response as on the third day. The group exhibited a respiratory rate comparable to control when tested on the fifth day following the last exposure. For the next highest level, 0.078 ppm, as shown in Fig. 4, the level of response at the end of 3 hr was as expected from the concentration-response relationship presented in Fig. 1. On the second day, the preexposure respiratory rate was lower than for the first day, and although the level of response was not higher at the end of 3 hr, the onset of the reaction was much faster than for the first day. Similarly, overnight recovery was incomplete and higher levels of responses were reached on the following exposure days. Five days after the last exposure, recovery to control respiratory rate was observed. For the next five levels of

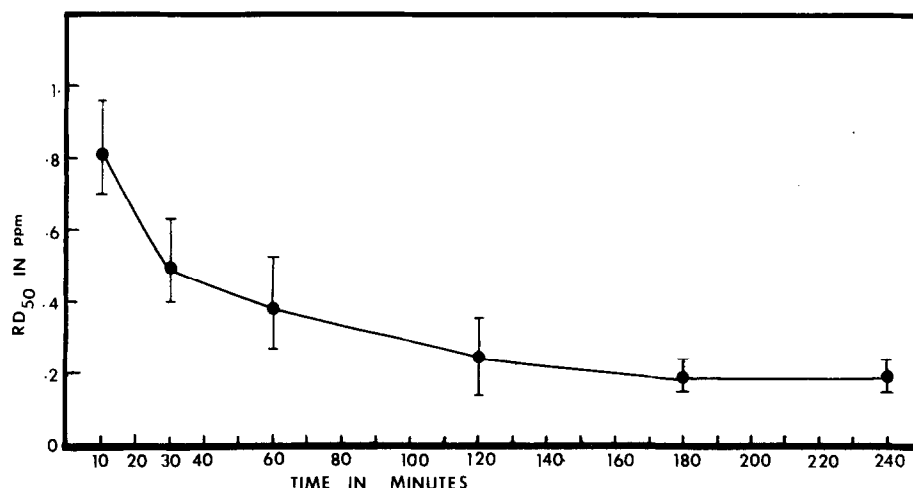


FIG. 3. Relationship between RD50 values and 95% confidence limits (from Table 1) with duration of exposure.

TABLE 2  
 REPRODUCIBILITY OF RD50 (50% DECREASE OF RESPIRATORY  
 RATE) DATA FOR 10 AND 30 MIN OF EXPOSURE TO  
 TOLUENE DIISOCYANATE

Set number <sup>a</sup>	RD50 (ppm) and 95% confidence intervals	
	Response measured after 10 min of exposure	Response measured after 30 min of exposure
1	0.790 (0.606–1.158) <sup>b</sup>	0.498 (0.406–0.635) <sup>b,c</sup>
2	0.637 (0.422–1.173)	0.508 (0.394–0.677) <sup>b,c</sup>
3	0.813 (0.707–0.963) <sup>c</sup>	0.461 (0.277–0.810)
4	0.764 (0.390–4.502)	0.455 (0.296–0.726)
5	0.938 (0.603–1.406)	0.474 (0.278–0.881)
$\bar{X} \pm SD$	0.788 $\pm$ 0.107	0.479 $\pm$ 0.24

<sup>a</sup> Each set consisted of exposures to at least five different concentrations, between 0.07 and 1.0 ppm, using new groups of four mice for each exposure. RD50 values were calculated as presented for the data in Fig. 1.

<sup>b</sup> These sets were used to compare the level of response of mice preexposed to TDI as given in Table 3 and Fig. 7.

<sup>c</sup> This set is presented in detail in Fig. 2 and Table 1 and was used in Fig. 3.

exposure, 0.301, 0.505, 0.82, and 1.18 ppm as shown in Figs. 5 and 6, a similar pattern was observed and it was unnecessary to continue exposures for 5 days at these higher concentrations to establish the fact that extremely slow recovery was a main characteristic of these exposures. At the highest concentration, 1.18 ppm, the group was re-exposed 8 days after the 3 days of exposure. The response, from preexposure level was of the same magnitude as on Day 1 of this series. To more specifically demonstrate that recovery from high exposures would be achieved within 14 days, Series VI was undertaken. A group of 32 mice were exposed to 1.2 ppm of TDI for 5 hr/day on 3 consecutive days. Fifteen days later, they were divided into eight groups of 4 mice and each group exposed at one concentration between 0.1 and 1.35 ppm. The response for both control and preexposed animals was measured at 10 and 30 min of exposure. The results in Fig. 7 and Table 3 indicate that the pre-exposed mice, following a 15-day recovery,

showed the same level of response as normal mice not preexposed to TDI.

*Exposure concentrations below TLV (<0.02 ppm).* These exposures covered the range from 0.007 to 0.018 ppm as shown in Figs. 8 and 9 and for one concentration, 0.0016 ppm, two groups were exposed with the second group exposed for two consecutive weeks. In contrast with the series of exposures performed at levels higher than 0.02 ppm, none of these exposure levels resulted in a consistent response after 3 hr on the first day, and there was no tendency toward a consistent reduction in respiratory rate with each repeated exposure (*t* test, regression analysis). There was, however, possibly a trend toward higher levels in respiratory rate in a few groups, although small, but within each set, impossible to confirm from statistical analysis (*t* test, regression analysis). Attempts to relate, for example, the preexposure respiratory rate with days of exposures failed to yield a consistent pattern as seen for the >0.02-ppm exposures. Similarly, levels of response

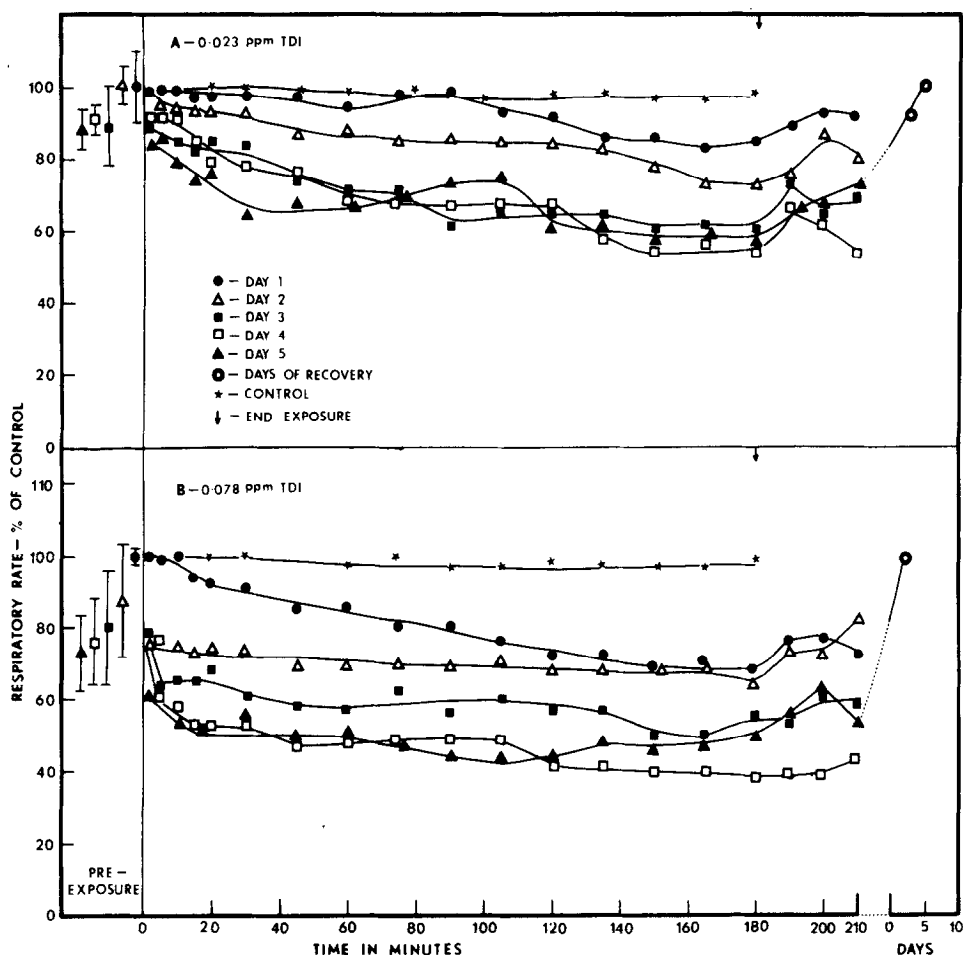


FIG. 4. Time-response relationships for repeated exposures to TDI. Each point represents the average of four animals, preexposure Day 1 being used as control and set at 100% with the vertical bars on preexposures as SD of the average of the group. For 0.023 ppm, determinations of exposure concentration were made at 30, 60, 120, and 160 min every day. All samples were within 0.022 and 0.024 ppm. For 0.078 ppm, determinations made as above were all between 0.062 and 0.083 ppm except for one determination on Day 4 at 0.1 ppm.

at 3 hr of exposure were not consistently related to exposure concentrations or cumulative days of exposure. There was no decrease in respiratory rate consistent with the hypothesis of hyperreactivity or cumulative effects for the sensory irritation response with repeated exposures as seen with concentrations  $> 0.02$  ppm.

*Histopathological evaluations.* Microscopic examination of the four transverse sections of the nasal area for the group exposed to 0.031 ppm of TDI 3 hr/day for 3 days re-

vealed no lesion and these sections were undistinguishable from those from normal control animals. For the group similarly exposed at 0.25 ppm definite histopathological changes were noted. The damage was consistently present in the first section, external nares, with 25–50% of the mucosa involved and some extension into the submucosa. The damage was much less severe on the second and third sections representing the respiratory epithelium and absent on the fourth section representing the olfactory epithelium.

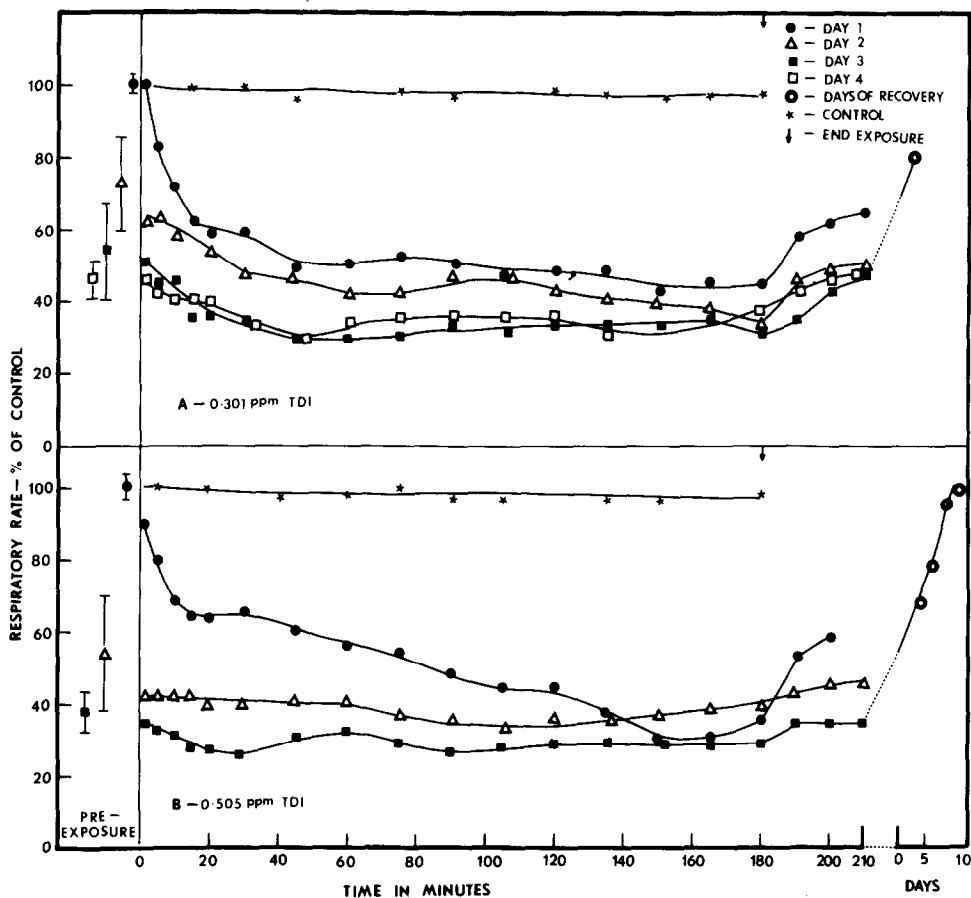


FIG. 5. Time-response relationships for repeated exposures to TDI. Each point represents the average of four animals, preexposure Day 1 being used as control and set at 100% with the vertical bars on preexposures as SD of the average of the group. For 0.301 ppm, determinations of exposure concentration were made at 30, 60, 120, and 160 min each day. All samples were between 0.298 and 0.305 ppm. For 0.505 ppm, similar determinations were made; all were between 0.463 and 0.550 ppm.

## DISCUSSION

The total production of isocyanates, including toluene diisocyanate (TDI), *p,p'*-diphenyl methane diisocyanate (MDI), and hexamethylene diisocyanate (HDI) is projected to be above 1 million metric tons by 1980, and over 100,000 workers are likely to come in contact with these isocyanates during production, formulation, industrial use, or disposal. An even larger number of workers as well as consumers are anticipated to be exposed to these isocyanates since isocyanates are the primary building blocks for polyurethanes and isocyanurates. These

are widely distributed as consumer products for insulation, coatings, paints, cushions, clothing, automobiles, and aircraft. Thus, the importance of studying the reactions to these chemicals can be seen, particularly at low concentrations, as there may be small residue of unreacted isocyanates in these products.

So far, hypersensitivity reactions due to an immunological mechanism have been clearly demonstrated by Karol *et al.* (1978a,b) for both bronchial and skin reactions in industrial workers. The results presented in this report establish that a cumulative effect

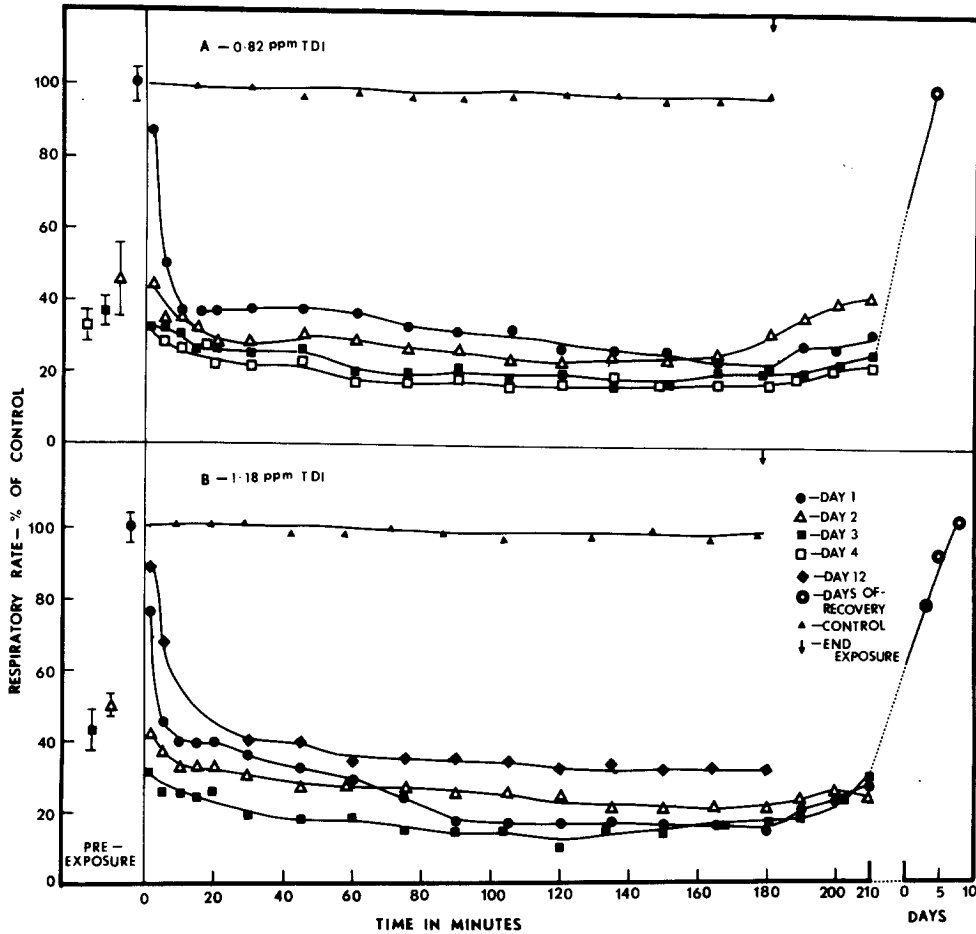


FIG. 6. Time-response relationship for repeated exposures to TDI. Each point represents the average of four animals, preexposure Day 1 being used as control and set at 100% with the vertical bars on preexposures as SD of the average of the group. For 0.82 ppm, determinations of exposure concentrations were made at 15, 30, 60, 120, and 160 min each day. All samples were between 0.788 and 0.836 ppm. For 1.18 ppm, similar determinations were made; all were between 1.160 and 1.196 ppm.

can be expected above certain levels. This cumulative effect was the result of incomplete recovery prior to a second or third exposure. These results are different from those obtained with repeated exposures to acrolein or formaldehyde (Kane and Alarie, 1977). With these two irritants, a greater response was achieved on successive days but not because of incomplete recovery. Only an increased sensitivity, i.e., hyperreactivity, was demonstrated as opposed to the results obtained with TDI.

The results obtained with TDI clearly demonstrated that the level of response was not only dependent on exposure concentration, but also on duration of exposure. Clearly, a maximum response was achieved gradually with exposure time. Similarly, recovery rate was dependent on duration of exposure. The first possibility to explain this cumulative effect and slow recovery which was observed beginning at 0.023 ppm could be that the nerve endings in the respiratory epithelium of the nasal mucosa are stimu-

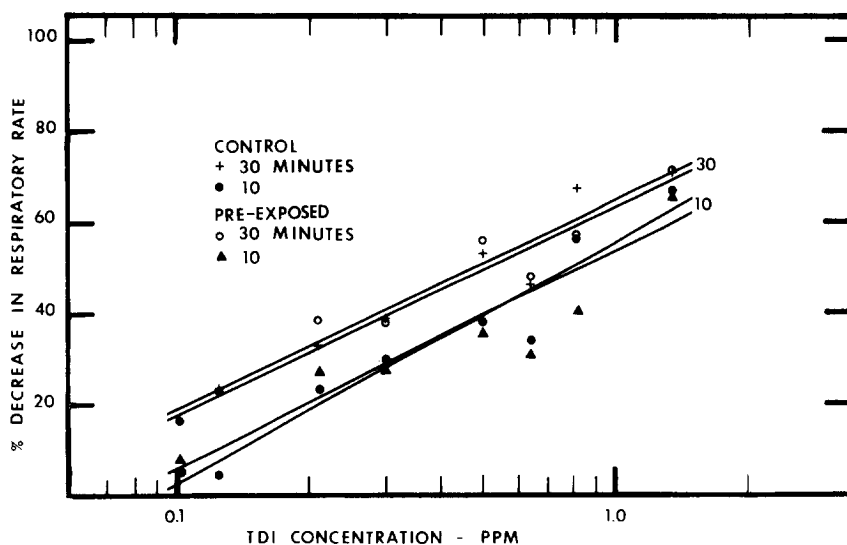


FIG. 7. Concentration-response relationships obtained for exposure to TDI with the level of response, percentage decrease in respiratory rate, measured after 10 or 30 min of exposure in control mice, or in mice previously exposed to 1.0 ppm of TDI 5 hr/day for 3 consecutive days and tested 14 days later.

TABLE 3

CALCULATED RD50 (50% DECREASE OF RESPIRATORY RATE) AND ASSOCIATED STATISTICS FROM LEAST SQUARES LINEAR REGRESSION ANALYSIS COMPARING THE RESULTS OBTAINED IN CONTROL VERSUS PRE-EXPOSED ANIMALS<sup>a</sup>

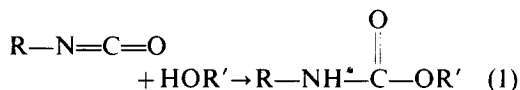
Exposure time (min)	RD50 (ppm)	95% Confidence interval	$y =$
Preexposed			
10	0.895	0.57-2.03	$52.29 + 47.14 \log x$
30	0.514	0.406-0.635	$66.87 + 44.54 \log x$
Control			
10	0.790	0.606-1.158	$55.48 + 53.57 \log x$
30	0.498	0.406-0.635	$64.47 + 47.8 \log x$

<sup>a</sup> Data presented in Fig. 7.  $r$  values for each regression  $> 0.92$ .

lated as a result of an inflammatory reaction. This can be ruled out since microscopic examination did not reveal such an occurrence at 0.031 ppm after 3 consecutive days of exposure.

A mechanism for stimulation of these nerve endings has been suggested on the

basis of correlation between the potency of sensory irritants and their reactivity with SH or NH<sub>2</sub> groups in protein (Alarie, 1973b; Tarantino and Sass, 1974). Isocyanates can react with SH, NH<sub>2</sub>, OH, and COOH groups. The reaction with OH groups results in the formation of urethanes (1) which are extremely stable. The reaction is unlikely to be reversible under biological conditions (Mukaiyama and Hoshino, 1955; Adams and Baron, 1965).



Brown and Wold (1971, 1973a) and Brown (1975) demonstrated that various mono-isocyanates were active site-specific reagents toward the OH group of serine in proteases. Such reactions resulted in irreversible inactivation of the enzymes. Similar results were obtained by Ozawa (1967) and Brown and Wold (1973b) for diisocyanates. Isocyanates react with NH<sub>2</sub> groups to form ureas (2) which are also highly stable and unlikely to dissociate under biological conditions.

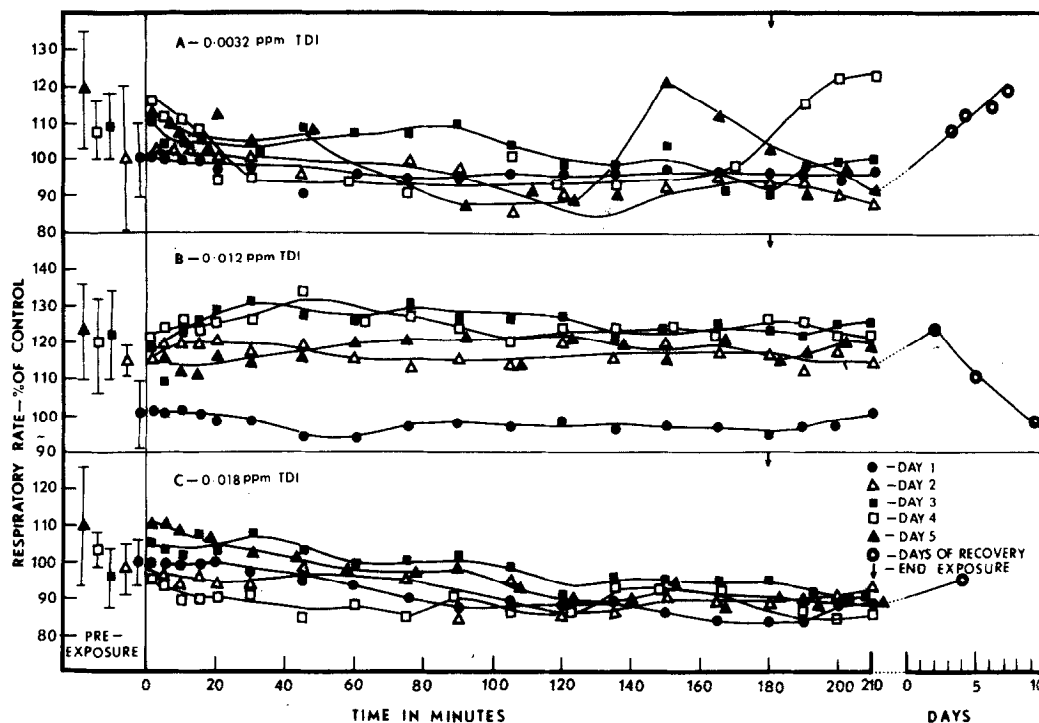
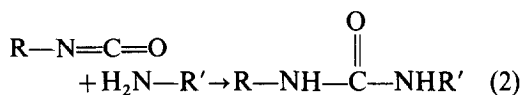
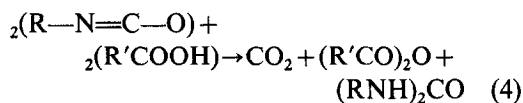
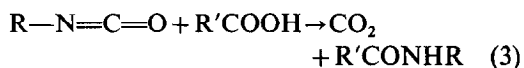


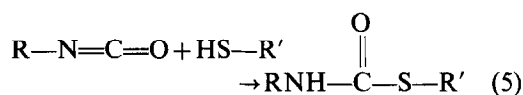
FIG. 8. Time-response relationships for repeated exposures to TDI. Each point represents the average of four animals, preexposure Day 1 being used as control and set at 100% with the vertical bars on preexposures as SD of the average of the group. For 0.0007 ppm  $\pm$  0.00006, the concentration was established each day from a single 3-hr sample. For 0.0016 ppm, samples varied from 0.0016-0.0018 ppm, measurements being made from 1-hr samples, in duplicate for each exposure.



Fraenkel-Conrat *et al.* (1945) showed that isocyanates react with  $\text{NH}_2$  groups in proteins to yield the stable ureido linkage and this reaction was used to study the tertiary structures of proteins. Ozawa (1967) used hexamethylene diisocyanate and hexamethylene diisothiocyanate, and Fasold (1965) used azophenyl-*p*-diisocyanate as bridging reagents. Schick and Singer (1961) used 2,4-toluene diisocyanate to covalently link various proteins by reaction with their  $\text{NH}_2$  groups. Isocyanates react with carboxyl groups with the formation of amides (3), acid anhydrides and ureas (4).



Such reactions have been demonstrated by Dieckmann and Breest (1906) and Fry (1953) using various isocyanates and fatty acids. Isocyanates form thiolic acid esters (5) when reacting with SH groups in proteins. These are unstable at pH at or above neutrality (Twu and Wold, 1973; W. Brown, personal communication).



Twu and Wold (1973), using butyl isocyanate as an active site-specific reactant for alcohol dehydrogenase, found inactivation of the enzyme following reaction with the SH

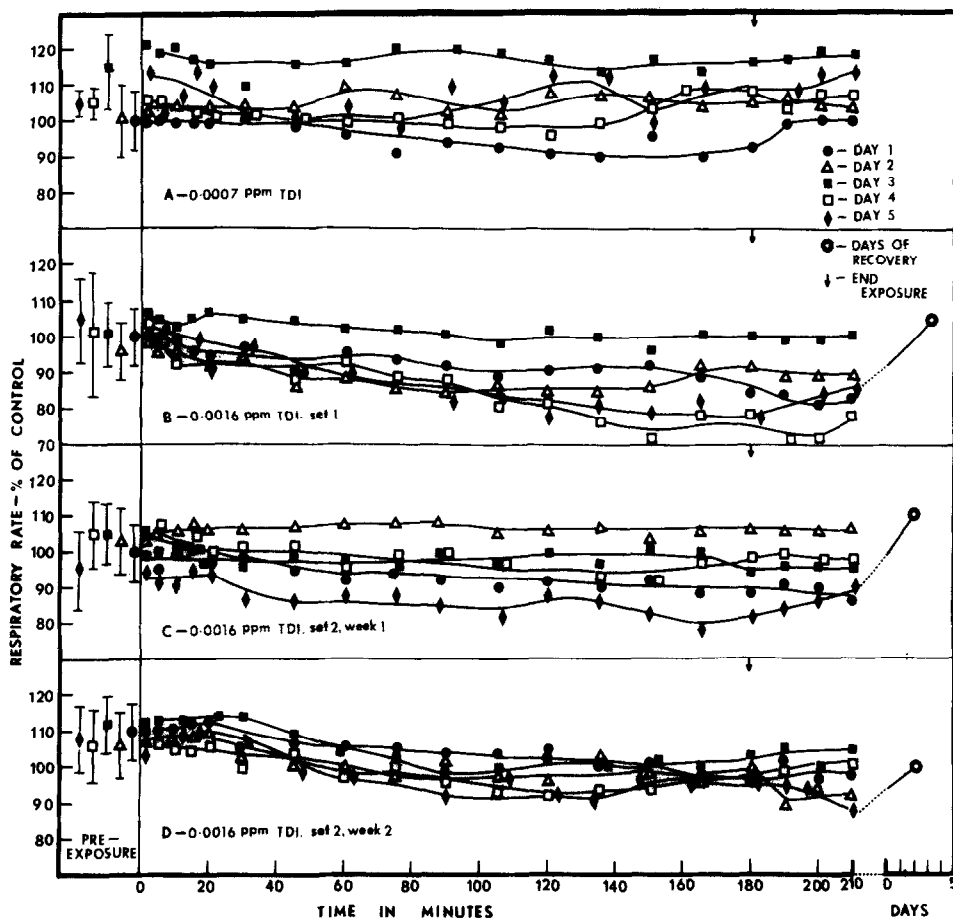
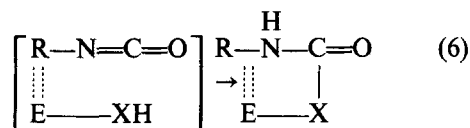
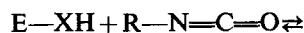


FIG. 9. Time-response relationships for repeated exposures to TDI. Each point represents the average of four animals, preexposure Day 1 being used as control and set at 100% with the vertical bars on preexposures as SD of the average of the group. For 0.0032 ppm, 30-min samples were taken during each hour of exposure each day and ranged from 0.0029 to 0.0034 ppm. For 0.012, samples were taken for a period of 20 min during each hour of exposure and ranged between 0.0113 and 0.0130 ppm. For 0.0175, hourly samples taken each day ranged between 0.0160 and 0.0186 ppm.

groups. However, this reaction took place at low pH only, and similarly for the inactivation for other dehydrogenases. From these possible reactions, it is most likely that covalent binding and slow recovery would follow reaction of isocyanates with OH or NH<sub>2</sub> groups of receptor proteins in the nasal mucosa. Brown and Wold (1971) visualized reaction of isocyanates with enzymes as a two-step process, the first one resulting in a reversible noncovalent complex. With proper alignment, the second phase occurs which

leads to covalent binding and irreversibility, as in (6).



This mechanism may explain our results, since with short duration of exposure, recovery was rapid. Under these conditions, only the first complex may have been formed.

Upon longer exposure, the slow recovery would be due to the formation of the covalent complex.

As previously described (Alarie, 1966, 1973a,b), this animal model measures the potency of airborne chemicals stimulating the cholinergic nerve endings of the trigeminal nerve located in the nasal mucosa. The demonstration that TDI stimulates these cholinergic nerve endings and that a cumulative effect occurs with repeated exposure cannot be directly extrapolated to the bronchial epithelium which is also innervated by a variety of cholinergic nerve endings. Nevertheless, the results presented certainly reinforce the suggestions of a possible pharmacological mechanism of bronchial hyperreactivity or cumulative effects with repeated exposures to TDI.

In previous reports (Barrow *et al.*, 1977; Kane *et al.*, 1979), it has been proposed that the animal model used here to determine the level of sensory irritation to airborne chemical irritants can be used to predict safe levels of exposure in industry, i.e., a Threshold Limit Value (TLV). The predictions were made as follows:

(a) At RD50, humans would report intense sensory irritation of the eyes, nose, and throat.

(b) At 0.1 RD50, humans would experience some slight discomfort and this should be the highest level (i.e., ceiling value) permitted in industry, particularly to prevent cumulative effects or the development of hypersusceptibility.

(c) At 0.01 RD50, there would be little, if any, response.

The RD50 found at maximum response time of 180 or 240 min of exposure was 0.2 ppm. Thus, if the above predictions are used for TDI, a ceiling value of 0.02 ppm should be applied for industrial exposures. The current TLV is set at 0.02 ppm with a ceiling notation for this level. However, for a TLV expressed as a time weighted average (TLV-TWA), a level between 0.002 and 0.02 should be selected in order that no exposure

above 0.02 ppm will be likely to occur. Occasional excursions above 0.02 ppm are likely to vary from plant to plant depending on engineering procedures, but certainly a TLV-TWA of 0.006 (the midpoint between 0.002 and 0.02 on logarithmic scale) would seem more appropriate than a 0.02-ppm TLV with a ceiling notation, since even in a new TDI manufacturing plant, frequent excursions above 0.02 ppm were reported by Weill *et al.* (1975). Although TDI is the most potent of the sensory irritants we have tested so far (Barrow *et al.*, 1978; Kane *et al.*, 1979), its action is very slow. Workers would be more likely to tolerate short exposures during an accidental spill than they would a spill of another sensory irritant such as acrolein, which although less potent than TDI, is much faster acting and thus has good warning properties. For this reason, we would like to stress that existing work practices and procedures be strictly followed and appropriate respiratory protection be worn by those workers who must enter areas where a spill has occurred, regardless of size, so that no exposure above 0.02 ppm will occur.

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