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Sensory Irritation, Pulmonary Irritation, and Acute Lethality of a Polymeric Isocyanate and Sensory Irritation of 2,6-Toluene Diisocyanate

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Sensory Irritation, Pulmonary Irritation, and Acute Lethality of a Polymeric Isocyanate and Sensory Irritation of 2,6-Toluene Diisocyanate. WEYEL, D. A., RODNEY, B. S., AND ALARIE, Y. (1982). *Toxicol. Appl. Pharmacol.* **64,** 423–430. The use of monomeric and polymeric isocyanates in a wide variety of industries has been increasing. Little is known about the toxicity of polymeric isocyanates and the widely used 2,6-toluene diisocyanate (TDI) isomer. The pulmonary and sensory irritation of an aliphatic polyisocyanate (DES-N) based on hexamethylene diisocyanate (HDI) was studied in Swiss–Webster male mice during aerosol exposures in the range of 25 to 131 mg/m^3. The sensory irritation of 2,6-TDI vapor was studied in the range of 0.37 to 7.6 mg/m^3 (0.05 to 1.1 ppm). The aerodynamic equivalent diameter and geometric standard deviation for the DES-N aerosol were 0.6 μm and 2.4, respectively. High-speed liquid chromatography was used to determine both free HDI in DES-N and HDI in the exposure chamber. Each exposure was for 3 hr during which the tidal volume pattern and respiratory rate of groups of four mice were recorded. Unlike the monomeric isocyanates, DES-N acted predominantly as a pulmonary irritant, evoking little sensory irritation. The concentration needed to reduce the respiratory rate 50% due to pulmonary irritation was 57.1 mg/m^3. The LC50, determined by counting the number of deaths within the 24 hr period following a 4-hr exposure, was 91.2 mg/m^3. In groups of animals killed 2 hr after the 4-hr exposure, the concentration of DES-N needed to increase lung weight by 50% was 45 mg/m^3. Based on comparisons with another pulmonary irritant, nitrogen dioxide, the maximum concentration for DES-N permitted in industry should be 1 mg/m^3 with a time-weighted average for an 8-hr period of 0.5 mg/m^3. From the concentration–response relationship due to sensory irritation for 2,6-TDI, the RD50 was determined to be 1.8 mg/m^3 (0.26 ppm) which is close to the value of 1.4 mg/m^3 (0.20 ppm) determined previously for 2,4-TDI. No pulmonary irritation was observed. For industrial applications the exposure limit for 2,4-TDI of 0.04 mg/m^3 (0.006 ppm) is also suggested as appropriate for the 2,6-TDI isomer.

In addition to the use of monomeric isocyanates, such as toluene diisocyanate (TDI) and diphenylmethane - 4,4 - diisocyanate (MDI), polymeric isocyanates, based on various monomeric isocyanates, are used in the polyurethane industry. One such polyisocyanate is the biuret structure[2] of hexamethylene diisocyanate (HDI) as shown in Fig. 1. This polyisocyanate is a major ingredient in two-component polyurethane spray paints which are used in automotive refinishing, airplane coating, maintenance coating, and many other applications where durability and chemical as well as mechanical resistance are required. With the exception of a 4-hr LC50 reported to be 425 mg/m^3 in male rats (Bunge *et al.*, 1977), nothing is known about the toxicological effects of this polyiso-

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[2] Manufactured and marketed by Mobay Chemical Corporation as Desmodur N (DES-N).

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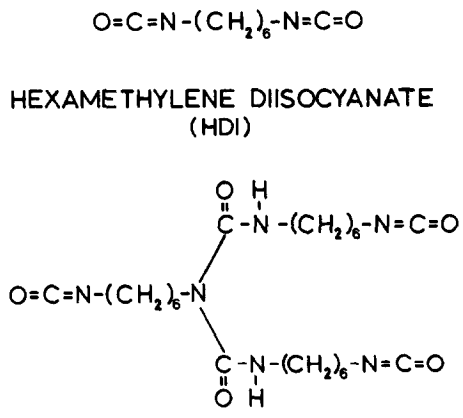

FIG. 1. Chemical structure of hexamethylene diisocyanate (HDI) and the biuret structure of HDI (DES-N).

cyanate. We are presenting in this report the results of inhalation exposures to this polyisocyanate as well as for 2,6-toluene diisocyanate obtained with methods previously used to investigate various monomeric isocyanates (Sangha and Alarie, 1979; Sangha *et al.*, 1981).

METHODS

Animals

Specific pathogen-free male Swiss-Webster mice, 24 to 27 g body wt, supplied by Hilltop Laboratories (Scottdale, Pa.) were used.

Exposure Conditions

An all-glass exposure chamber was used for this study as described by Barrow *et al.* (1977) for exposures of groups of four animals. For exposures of groups of eight animals the size of the chamber was doubled in length. Each animal was restrained in a body plethysmograph with its head protruding into the exposure chamber. The average respiratory rate of a group of four mice, or of four of the exposed eight mice, was monitored prior to, during, and following exposures as previously described (Kane and Alarie, 1977). The airflow in the chamber was maintained at 20 liters/min for all exposures.

Generation of Vapor and Aerosol

The biuret structure of hexamethylene diisocyanate (DES-N) was a commercial sample. When manufactured, this polyisocyanate contains approximately 0.7% free hexamethylene diisocyanate (HDI) which may increase upon storage to about 1.6% (Mobay Chemical Corporation, 1976). Analysis of this sample by the method described by Dunlap *et al.* (1976) revealed 1.13% free HDI. To generate various airborne concentrations of DES-N, weighted amounts of DES-N, which is a viscous liquid with a very low vapor pressure (2×10^{-5} Torr) at room temperature, were dissolved in reagent grade, water-free acetone to make final solutions ranging from 0.5 to 3.3%, w/v. These solutions were fed with a syringe pump at 0.22 ml/min into a Pitt No. 1 glass aerosol generator. This generator has been previously described (Wong and Alarie, 1982). The output of the generator, containing both acetone vapor and DES-N aerosol, was mixed with the air entering the exposure chamber. The DES-N chamber concentrations were determined gravimetrically by drawing known amounts of chamber air through preweighted membrane filters, 0.4-μm pore size from Millipore. The particle size distribution of the DES-N aerosol was determined with a cascade impactor (DC1-5) and an Andersen Mini Impactor. The aerodynamic equivalent diameter by weight was found to be 0.6 μm with a geometric standard deviation of 2.4. The acetone concentration in the chamber was determined with detector tubes (National Draeger CH22901) and a Miran Model I infrared analyzer. All exposures were conducted with acetone concentrations of 2800 to 3000 ppm. The free HDI concentration in the chamber was monitored by drawing air from the exposure chamber through an impinger containing a nitroreagent solution with subsequent analysis by liquid chromatography (Dunlap *et al.*, 1976). The various 2,6-TDI concentrations were generated by bubbling various amounts of dried and filtered air through an impinger containing 10 ml of 99.1% pure 2,6-toluene diisocyanate provided by Mobay Chemical Corporation. The concentrations of 2,6-TDI in the exposure chamber were measured by the method described by Marcali (1957) as modified by NIOSH (1977) and using 2,6-toluene diamine for the preparation of the standard solutions.

Exposure Groups

Series I. This series consisted of exposing groups of four mice to varied DES-N aerosol concentrations to measure the extent of respiratory rate depression at each exposure concentration. Each exposure was for 3 hr. Time-response relationships and concentration-response relationships were obtained as previously de-

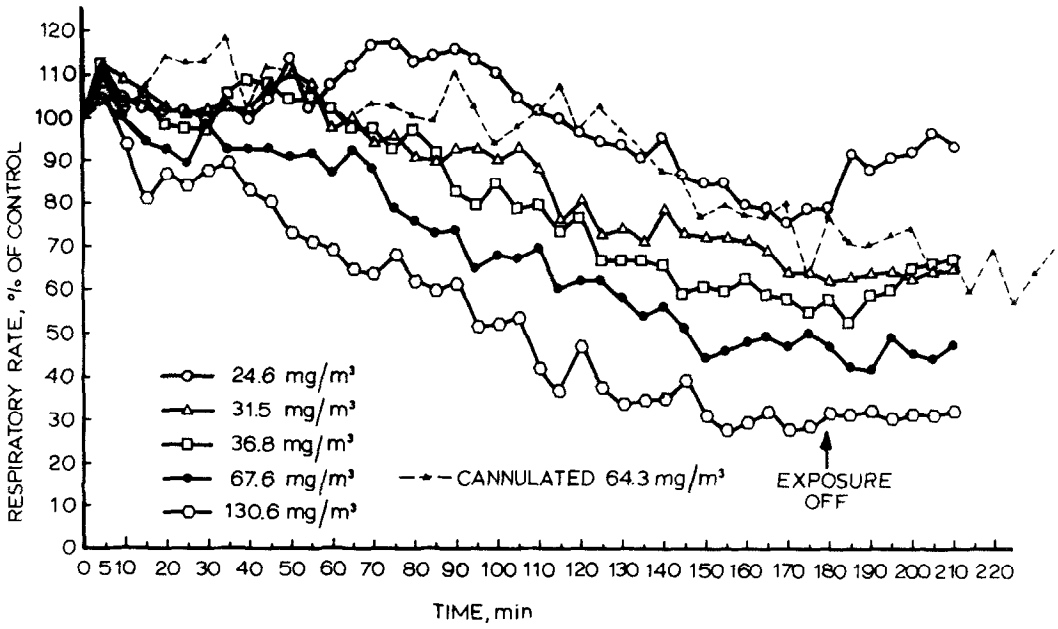

FIG. 2. Time–response relationship obtained in normal mice or mice exposed via tracheal cannulation to various concentrations of DES-N. Each point represents the average of four animals.

scribed with other isocyanates (Sangha and Alarie, 1979; Sangha *et al.*, 1981).

Series II. This series consisted of exposing groups of eight mice to varied DES-N aerosol concentrations for 4 hr. Four of the eight mice were monitored for respiratory rate. Two hours following exposure all animals were killed by cervical dislocation. The lungs were removed from the thorax, blotted, and weighed; the trachea, esophagus, and heart were also removed.

Series III. This series consisted of exposing groups of eight mice to varied DES-N aerosol concentrations for 4 hr. All animals were observed for a period of 24 hr following exposure with dead animals counted during that period for determination of the LC50. Survivors were killed by cervical dislocation at 24 hr postexposure to obtain lung weights as above.

Series IV. This series consisted of exposing groups of four mice to varied 2,6-TDI vapor concentrations to measure the extent of respiratory rate depression at each exposure concentration. Each exposure was for 3 hr. Time–response relationships and concentration–response relationships were obtained as previously described with other isocyanates (Sangha and Alarie, 1979; Sangha *et al.*, 1981).

Series V. To ascertain the area of the respiratory tract affected by DES-N aerosol, groups were exposed similar to Series I, but via tracheal cannulation as previously performed for 2,4-toluene diisocyanate (Sangha and Alarie, 1979).

Series VI. This series consisted of exposing three groups of four mice for 3 hr to acetone alone. The acetone concentration in the exposure chamber was the same as during the exposures to DES-N. This series served as a control for the exposures of DES-N dissolved in acetone. No change in respiratory pattern and frequency was observed in this series and no lethality occurred during or following exposure.

RESULTS

At exposure concentrations of 25 to 131 mg/m^3 of DES-N, normal mice showed a progressive decrease in respiratory rate during the 3-hr exposure as shown in Fig. 2. For the three highest exposure concentrations a plateau response was observed during the last 30 min of exposure. Recovery was very slow or nil at these high concentrations during the 30 min of postexposure observation. Plotting the decrease in respiratory rate obtained at the end of exposure versus the logarithm of the exposure concentration yielded the concentration–response relationship shown in Fig. 3. The decrease in

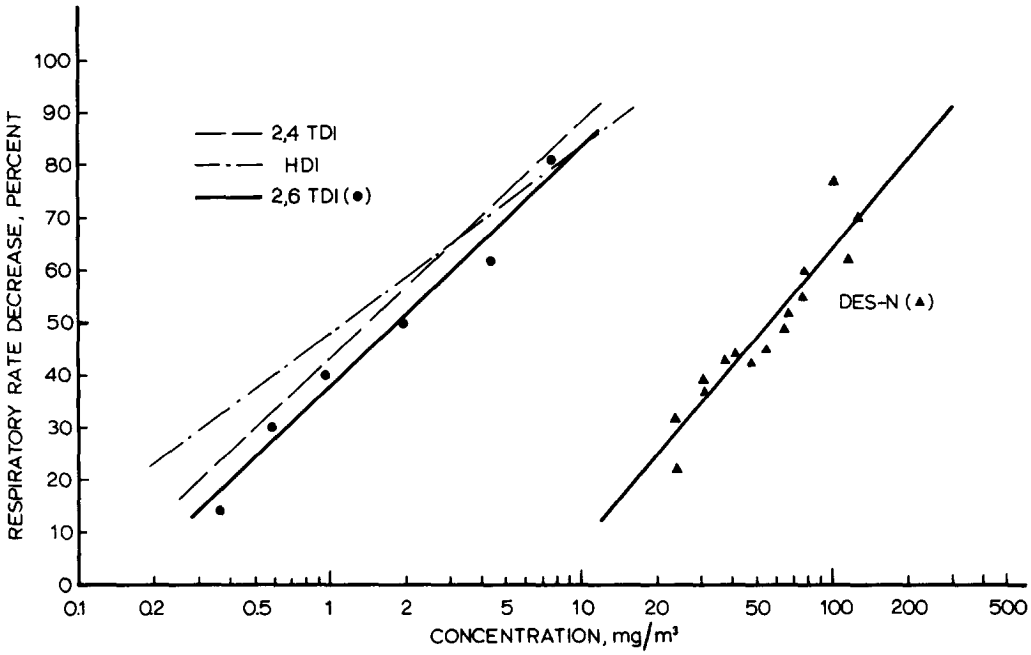

FIG. 3. Concentration–response relationships with various exposure concentrations of 2,6-TDI and DES-N. Each point represents the decrease in respiratory rate, the average of four mice observed following 3 hr of exposure expressed as percentage of pre-exposure level. The concentration–response relationships for HDI and 2,4-TDI (Sangha and Alarie, 1979; Sangha *et al.*, 1981) are shown for comparison. Curves were fitted by linear least-squares analysis. To convert mg/m^3 of HDI and TDI to ppm multiply each value by 24.4 and divide by the molecular weight values of HDI and TDI, which are 168 and 172, respectively.

respiratory rate observed with DES-N was not obtained with the characteristic pattern described for the action of sensory irritants on the upper respiratory tract (Alarie, 1966, 1981a) which was previously shown to occur with a variety of aromatic and aliphatic mono-and diisocyanates (Sangha and Alarie, 1979; Sangha *et al.*, 1981). Instead, we observed a decrease in respiratory rate with a pattern due to sensory irritation only at the beginning of each exposure. This pattern changed to the pattern observed with pulmonary irritation (Alarie, 1981a). With pulmonary irritation, the decrease in respiratory rate is due to a pause between each breath (Alarie, 1981a). Thus the final effect of DES-N in decreasing the respiratory rate was mainly due to its action on the lower airways rather than on the upper respiratory tract. This conclusion was confirmed by the results of exposures of mice via tracheal cannula as also shown in Fig. 2. This group showed no decrease in respiratory rate at the beginning of exposure. The effect began to occur following approximately 60 to 120 min of exposure, and with a respiratory pattern entirely due to pulmonary irritation. Thus, DES-N has an effect on respiratory rate due to irritation of both the upper and lower respiratory tract unlike the action of toluene diisocyanate which failed to elicit the effect in mice breathing via tracheal cannula (Sangha and Alarie, 1979).

The results obtained with Series II and III are presented in Fig. 4. It is apparent that lung weight increased with exposure concentrations at 2 hr following exposure, and that lung weights remained elevated at 24

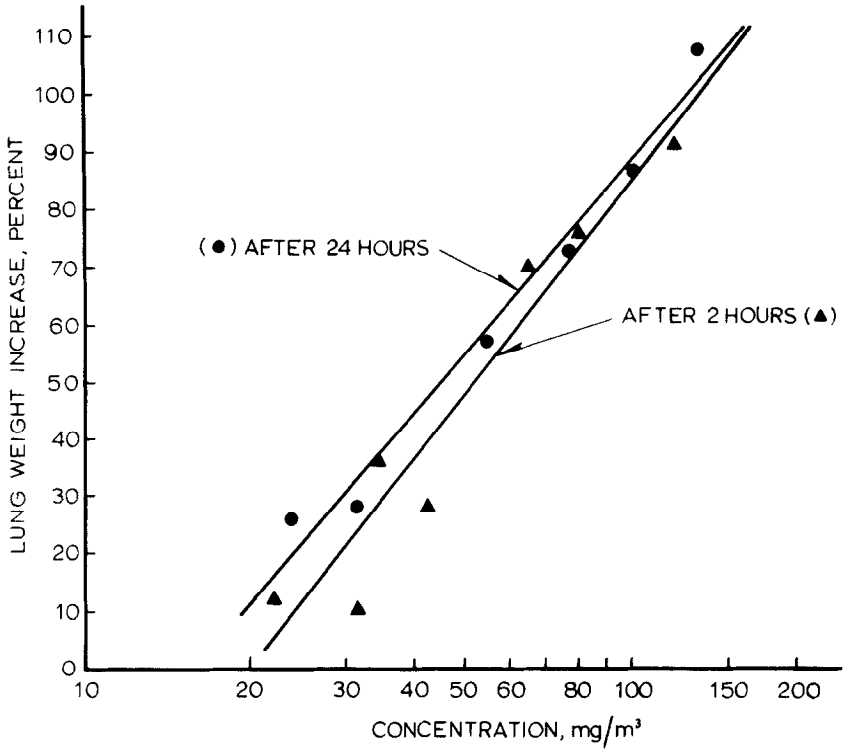

FIG. 4. Concentration–response relationships obtained for lung weight increases following exposure to various concentrations of DES-N. Each point represents the average of eight mice killed 2 hr after a 4-hr exposure or the average of the survivors of a group of eight mice 24 hr after a 4-hr exposure to DES-N. The data are given as the percentage increase from lung weight of a group of eight mice of comparable body weight used as control. Curves were fitted by linear least-squares analysis.

hr following exposure. The results for mortality within 24 hr following exposure are presented in Fig. 5.

Free HDI concentrations were determined during exposures to DES-N since analysis of the DES-N sample used, revealed the presence of 1.13 ± 0.06% (average ± SD of three analyses) free HDI. All chamber sample analyses revealed the presence of HDI. A typical chromatogram is shown in Fig. 6. In addition to the HDI and DES-N peaks, another peak which could be due to a dimer was consistently observed. Two small peaks, possibly due to higher molecular structures of HDI, appeared also on the chromatogram. However, only the HDI and DES-N peaks were identified and quantified. Duplicate samples obtained during the exposure range of 25 to 131 mg/m^3 of DES-N showed consistent HDI concentrations of 0.35% of the DES-N concentrations instead of 1.13% found prior to aerosolization. This decrease in HDI concentration may be due to its reaction with water vapor (Sangha *et al.*, 1981) or to other factors.

The results with exposures to 2,6-TDI were similar to the results previously obtained for 2,4-TDI (Sangha and Alarie, 1979). The decrease in respiratory rate occurred with a pattern indicating sensory irritation of the upper respiratory tract (Alarie, 1966, 1981a). By plotting the decrease in respiratory rate at the end of the 3-hr exposure versus the logarithm of the exposure concentration, a linear relationship was obtained as presented in Fig. 3.

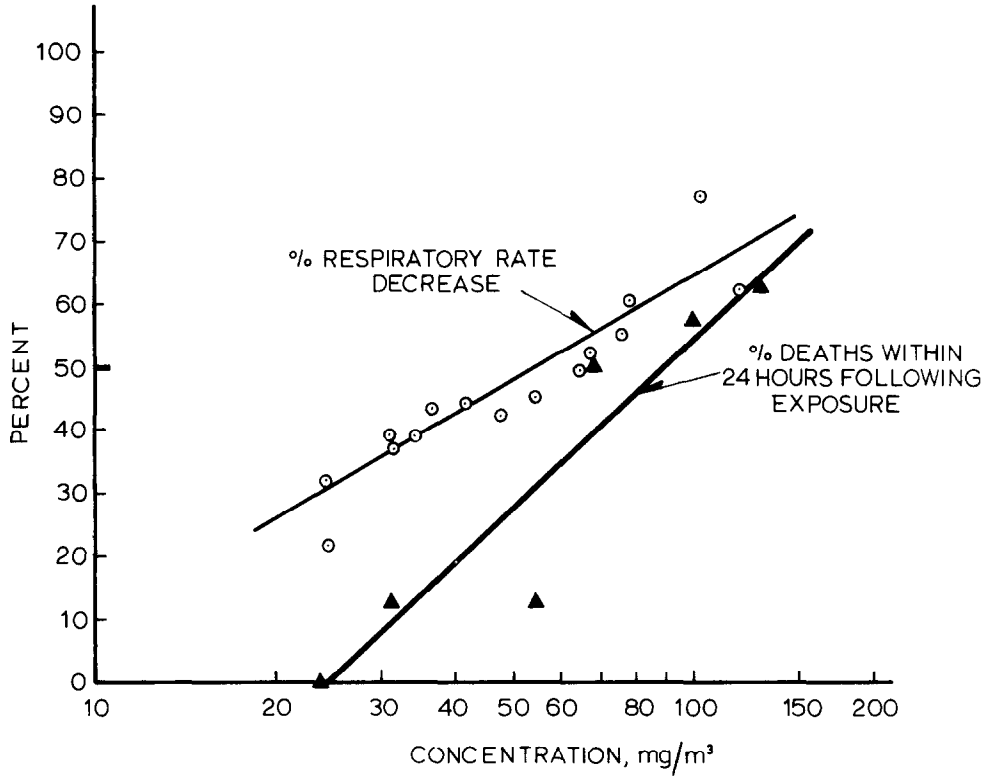

FIG. 5. Comparison of concentration–response relationships for decrease in respiratory rate (from Fig. 3) and deaths occurring within 24 hr following a 4-hr exposure to various concentrations of DES-N.

DISCUSSION

The findings that DES-N induced a decrease in respiratory rate with a pattern indicating pulmonary irritation following an initial pattern of sensory irritation were unexpected. All previously tested monomeric isocyanates failed to induce a pattern of pulmonary irritation (Sangha and Alarie, 1979; Sangha *et al.*, 1981) in normal mice. Furthermore, even in mice breathing with tracheal cannula, exposure concentrations of 2,4-toluene diisocyanate, up to five times the concentration necessary to induce a 50% decrease in respiratory rate due to sensory irritation, failed to induce a decrease in respiratory rate due to pulmonary irritation (Sangha and Alarie, 1979). Furthermore repeated daily exposures to 2,4-toluene diisocyanate at concentrations five times the concentration required to produce a 50% decrease in respiratory rate due to sensory irritation also failed to induce mortality. In contrast, as shown in Fig. 5, lethality was observed within 24 hr for single exposure to DES-N within the concentration range inducing 20 to 80% decrease in respiratory rate. The results obtained from lung weights following exposure to DES-N further confirm the pulmonary irritating nature of this polymeric isocyanate.

The presence of free HDI cannot explain the pulmonary irritating effect observed, since no such effect was found previously (Sangha *et al.*, 1981) at the concentration range presented in this study during exposure to DES-N. However, the presence of free HDI can explain the sensory irritation pattern at the beginning of exposure to DES-N. For example, from Fig. 3, the concentra-

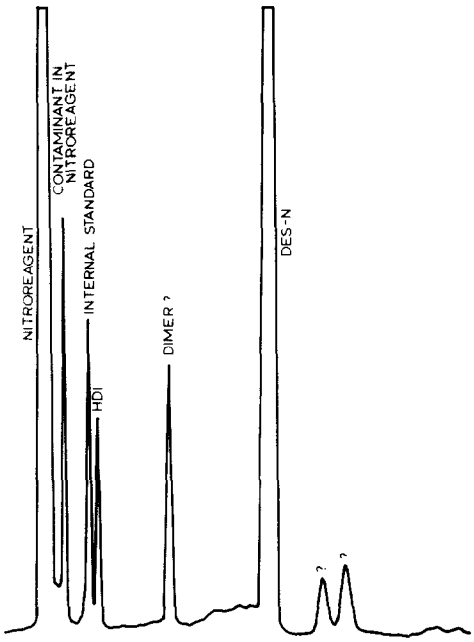

FIG. 6. Typical high-speed liquid chromatogram obtained from air samples drawn during exposure to DES-N aerosols. The peak preceding DES-N is possibly the dimer structure, while the two following peaks probably represent higher-molecular-weight structures.

tion of DES-N required to produce 50% decrease in respiratory rate is 57 mg/m^3. At this concentration, 0.2 mg/m^3 of free HDI would be present and would induce 20% decrease in respiratory rate due to sensory irritation. The presence of about 3000 ppm of acetone could also not contribute to either pulmonary or sensory irritation because no such effects were observed in Series VI.

In previous studies on monomeric isocyanates we have used the decrease in respiratory rate due to sensory irritation to propose acceptable exposure levels in industry (Sangha and Alarie, 1979; Sangha *et al.*, 1981). We cannot use this approach in proposing a TLV-TWA for DES-N since the decrease in respiratory rate was due to both sensory as well as pulmonary irritation, and the approach used for the monomeric isocyanates is valid only for sensory irritation (Alarie, 1981b). However, if we compare DES-N as a pulmonary irritant, subtracting the contribution of free HDI for sensory irritation, with nitrogen dioxide which has been evaluated in mice for pulmonary irritation (Alarie, 1981a), the potency of DES-N is approximately six times that of nitrogen dioxide. Since the TLV-TWA for NO_2 is 6 mg/m^3 and was established to prevent pulmonary irritation, a TLV-TWA for DES-N would be 1 mg/m^3. In order to prevent exposures above 1 mg/m^3 for DES-N, a value around 0.5 mg/m^3 as the TLV-TWA would be appropriate. We emphasize the preliminary nature of such a suggested level since no long-term studies have been conducted on polymeric isocyanates. Furthermore, their potential for inducing pulmonary hypersensitivity, as shown for monomeric isocyanates, (Karol *et al.*, 1981) has not been investigated. A point of practical importance is the control of both DES-N and free HDI simultaneously. Assuming a spraying operation yielding the same ratio of DES-N/HDI as found in our exposure conditions, controlling the level of DES-N at 0.6 mg/m^3 would result in proper control of HDI, since a concentration of 0.002 mg/m^3 would be predicted which is below the 0.034 mg/m^3 proposed for the TLV-TWA of HDI (Sangha *et al.*, 1981).

The threshold limit value, as the time-weighted average concentration for an 8-hr workday (TLV-TWA) adopted by the American Conference of Governmental Industrial Hygienists (ACGIH, 1980) for toluene diisocyanate is specifically for 2,4-toluene diisocyanate. However, 2,4-TDI is rarely used alone in industry. Rather, an 80/20 mixture of 2,4/2,6-TDI is used. Since the results obtained in this study with 2,6-TDI are very similar to the results obtained with 2,4-TDI (Sangha and Alarie, 1979) and similar to the results obtained with shorter exposures to a 2,4/2,6-TDI mixture (Barrow *et al.*, 1978), it appears that a TLV-TWA for different isomers of TDI and mixtures of them can be set equal to 2,4-TDI.

ACKNOWLEDGMENTS

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REFERENCES

ALARIE, Y. (1966). Irritating properties of airborne materials to the upper respiratory tract. *Arch. Environ. Health* **13,** 433–449.

ALARIE, Y. (1981a). Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions. In *Proceedings of the Inhalation Toxicology and Technology Symposium* (B. K. J. Leong, ed.), pp. 207–231. Ann Arbor Science Publ., Ann Arbor, Mich.

ALARIE, Y. (1981b). Bioassay for evaluating the potency of airborne sensory irritants and predicting acceptable levels of exposure in man. *Food. Cosmet. Toxicol.* **19,** 623–626.

American Conference of Governmental Industrial Hygienists (ACGIH) (1980). *Documentation of the Threshold Limit Values*, 4th ed. Cincinnati, Ohio.

BARROW, C. S., ALARIE, Y., WARRICK, J. C., AND STOCK, M. F. (1977). Comparison of the sensory irritation response in mice to chlorine and hydrogen chloride. *Arch. Environ. Health* **32,** 68–76.

BARROW, C. S., ALARIE, Y., AND STOCK, M. F. (1978). Sensory irritation and incapacitation evoked by thermal decomposition products of polymers and comparisons with known sensory irritants. *Arch. Environ. Health* **33,** 79–87.

BUNGE, W., EHRLICHER, H., AND KIMMERLE, G. (1977). Medical aspects of work with surface coating systems using the spraying technique. In *Special Edition Zentralblatt für Arbeitsmedizin, Arbeitsschutz und Prophylaxe*, Vol. 4. Verlag für Medizin Dr. E. Fischer, Heildelberg Pilger-Druckerei GmbH, D-6720 Speyer.

DUNLAP, K. L., SANDRIDGE, R. L., AND KELLER, J. (1976). Determination of isocyanates in working atmospheres by high speed liquid chromatography. *Anal. Chem.* **48,** 497–499.

KANE, L. E., AND ALARIE, Y. (1977). Sensory irritation to formaldehyde and acrolein during single and repeated exposures in mice. *Amer. Ind. Hyg. Assoc. J.* **38,** 509–522.

KAROL, M. H. (1981). Immunologic response of the respiratory system to industrial chemicals. In *Proceedings of the Inhalation Toxicology and Technology Symposium* (B. K. J. Leong, ed.), pp. 233–246. Ann Arbor Science Publ., Ann Arbor, Mich.

MARCALI, I. (1957). Microdeterminations of toluene diisocyanate in atmospheres. *Anal. Chem.* **29,** 552–588.

Mobay Chemical Corporation (1976). *Chemistry for Coatings*, p. 3. Pittsburgh, Pennsylvania.

NIOSH (1977). *Manual of Analytical Methods*, Vol. 1, *2,4-Toluene Diisocyanate (TDI) in Air*, 2nd ed., pp. 141.1–141.8. U.S. Dept. of Health, Education and Welfare, Washington, D.C.

SANGHA, G. K., AND ALARIE, Y. (1979). Sensory irritation by toluene diisocyante in single and repeated exposures. *Toxicol. Appl. Pharmacol.* **50,** 533–547.

SANGHA, G. K., MATIJAK, M., AND ALARIE, Y. (1981). Comparison of some mono- and diisocyanates as sensory irritants. *Toxciol. Appl. Pharmacol.* **57,** 241–246.

WONG, K. L., AND ALARIE, Y. (1982). A method for repeated evaluation of pulmonary performance in unanesthetized, unrestrained guinea pigs and its application to detect effects of sulfuric acid mist inhalation. *Toxicol. Appl. Pharmacol.* **63,** 72–90.