TOXICOLOGY AND APPLIED PHARMACOLOGY 57, 241–246 (1981)

Comparison of Some Mono- and Diisocyanates as Sensory Irritants

G. K. SANGHA, M. MATIJAK, AND Y. ALARIE

Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Received August 12, 1980; accepted October 3, 1980

Comparison of Some Mono- and Diisocyanates as Sensory Irritants. SANGHA, G. K., MATIJAK, M., AND ALARIE, Y. (1981). *Toxicol. Appl. Pharmacol.* 57, 241–246. The aromatic monoisocyanates phenylisocyanate, *o*-toluene isocyanate, and *p*-toluene isocyanate and aliphatic isocyanates 1,6-hexamethylene diisocyanates and hexylisocyanate were investigated using an animal bioassay to determine their time–response and concentration–response relationships as sensory irritants. The results were compared with those with toluene diisocyanate. The results indicated that the level of response was dependent on both duration of exposure as well as exposure concentration. The recovery rate was extremely slow following a 3-hr exposure for all isocyanates. The aromatic and aliphatic diisocyanates had comparable potency while the aromatic monoisocyanates were slightly less potent and the aliphatic monoisocyanate was found to be the least potent.

Exposure to isocyanates is known to cause irritation as well as immunologic sensitization of the respiratory tract as recently reported for toluene diisocyanate (TDI) using animal models (Sangha and Alarie, 1979, Karol *et al.*, 1980). Our previous findings showed that in an animal bioassay designed to evaluate sensory irritation of the upper respiratory tract in mice the recovery following a 3-hr exposure to TDI was extremely slow and cumulative effects could be observed at exposure levels above 0.02 ppm with daily repeated exposures (Sangha and Alarie, 1979). We attributed such findings to be probably due to covalent bonds formed by TDI with nucleophilic groups on receptor protein(s), in particular with OH and NH_2 groups, forming very stable urethanes or ureas (Sangha and Alarie, 1979). The possibility also exists that a slow recovery depends not only because such reactions occur but in addition cross-linking also occcurs because of the bifunctional nature of TDI (Shick and Singer, 1961). To elucidate this point further the present study was undertaken. Monoisocyanate analogs of TDI and also an aliphatic diisocyanate, hexamethylene diisocyanate (HDI), and its monoisocyanate analog, hexylisocyanate (HI), were investigated for comparison with the aromatic isocyanates.

METHODS

Animal model. The mice used in the study were obtained from Hilltops Laboratories, Scottsdale, Pennsylvania. They were outbred, male Swiss Webster mice, specific pathogen free and weighed between 26 and 28 g.

Isocyanates. 1,6-Hexamethylene diisocyanate (HDI) was supplied by Mobay Chemical Corporation. The monoisocyanates, *o*-toluene isocyanate (TMI), *p*-toluene isocyanate (*p*-TMI), phenyl isocyanate (Ph.I.), and hexyl isocyanate (HI) were obtained from Eastman Kodak.

Animal exposures and measurement of response. Groups of four animals were exposed to the isocya-

0041-008X/81/020241-06$02.00/0

nates in an all glass chamber (Barrow *et al.*, 1977) and their respiratory rates were recorded by plethysmography prior to, during, and following exposure as previously described for the investigation of TDI (Sangha and Alarie, 1979). The response was taken as the average percentage of change in respiratory rate of the group of four mice from their preexposure control level (Kane and Alarie, 1977; Sangha and Alarie, 1979). New mice were used for each exposure concentration.

Generation of isocyanate atmosphere. Various levels of isocyanate were generated in the exposure chamber by bubbling dried filtered air through the pure compound contained in an impinger. The impinger output was diluted with room air and entered the chamber at a flow rate at or above 20 liters/min. When using HDI the dilution air was also dried to reduce the relative humidity toward 10–20% because of the reactivity of HDI with water.

Analytical determination of chamber atmosphere. A Perkin–Elmer Model 3920 gas chromatograph with a nitrogen-phosphorus (NP) detector was used to analyze chamber atmospheres. A glass column (Supelco: 6 ft × ¼ in. o.d.) with on-column injection was cleaned and treated with dimethylchlorosilane (Supelco), and packed with 80/100 mesh OV-210 (Supelco). The column was conditioned overnight at 200°C. The following conditions were used for analysis and calibration: bead setting at 680, hydrogen and air flow at 1–3 and 100 ml/min, respectively, and helium at 60 ml/min. The injector and interface temperature were both set at 200°C. A temperature program of 90 to 200°C at a rate of 16°C/min was used to establish the temperature at which each isocyanate eluted from the column. For calibration, fresh stock solutions of each isocyanate were prepared daily in volumetric flasks flushed with dry nitrogen and toluene (Mallinckrodt, nanograde) was used as the solvent. The following temperatures were selected for subsequent calibration and analysis of each isocyanate: HDI, 180°C; HI, 100°C; *o*-TMI and *p*-TMI 110°C; and Ph.I., 95°C. Under these conditions it was possible to detect 10 ng of each isocyanate. However, there was a frequent need to recalibrate the chromatograph because of the change in the NP detector response. The reliability of this method was checked in the case of *o*-TMI and *p*-TMI. For these two isocyanates chamber atmospheric concentrations were determined simultaneously using modifications of the Gutmann *et al.* (1952) colorimetric method as described by Modesto and Pesce (1973). Less than 15% difference was observed between the two methods. For analytical determinations, samples were taken at 30, 60, 120, and 160 min of exposure and the average was taken as the exposure concentration for each experiment. Each sample was taken from the chamber via a polytetrafluoroethylene tube leading to an impinger containing toluene kept in an ice bath with a sampling rate of 1 liter/min. All samples were kept on ice until analysis, performed within 15–30 min.

Onset of response and recovery. The onset of the response and recovery rates for all the isocyanates were determined by exposing groups of four mice to each isocyanate at different concentrations in order to get approximately 30 ± 5, 50 ± 5, and 70 ± 5% decrease in respiratory rate from control level at the end of 30, 60, and 180 min of exposures. Recovery times of 10, 15, and 30 min were allowed for 30, 60, and 180 min of exposure, respectively. Time–response relationships were plotted for each exposure for each isocyanate and points were fitted by piece-wise linear regression as previously done for TDI (Sangha and Alarie, 1979).

Concentration–response relationships. Concentration–response relationships for each isocyanate were established for 10, 30, 60, 120, and 180 min by exposing groups of four mice to various concentrations (at least six) of each isocyanate. A maximum of 180 min was chosen because no significant additional increase in response was observed after 180 min of exposure with TDI (Sangha and Alarie, 1979). The maximum percentage decrease in respiratory rate at each time interval was plotted against the logarithm of exposure concentration and points were fitted by least-squares linear regression analysis to obtain an RD_{50} value, the concentration necessary to decrease the average respiratory rate of a group of four mice by 50%, as previously done with TDI (Sangha and Alarie, 1979).

RESULTS

Onset of response and recovery. As shown in Fig. 1 a general pattern emerged

FIG. 1. Time–response relationships obtained with HDI (top left), Ph.I. (top right), *p*-TMI (bottom left), and *o*-TMI (bottom right) at concentrations selected to obtain approximately 30, 50, and 70% decrease in respiratory rate by the end of 30, 60, and 180 min of exposures (A, B and C for each). Downward arrow indicate termination of exposure. Each data point represents the average respiratory rate of four mice as percentage of preexposure control condition. The data points were fitted by piece-wise linear regression analysis (see text). Three similar groups of four animals showed no significant trend in their average respiratory rate during a 3-hr exposure to room air.

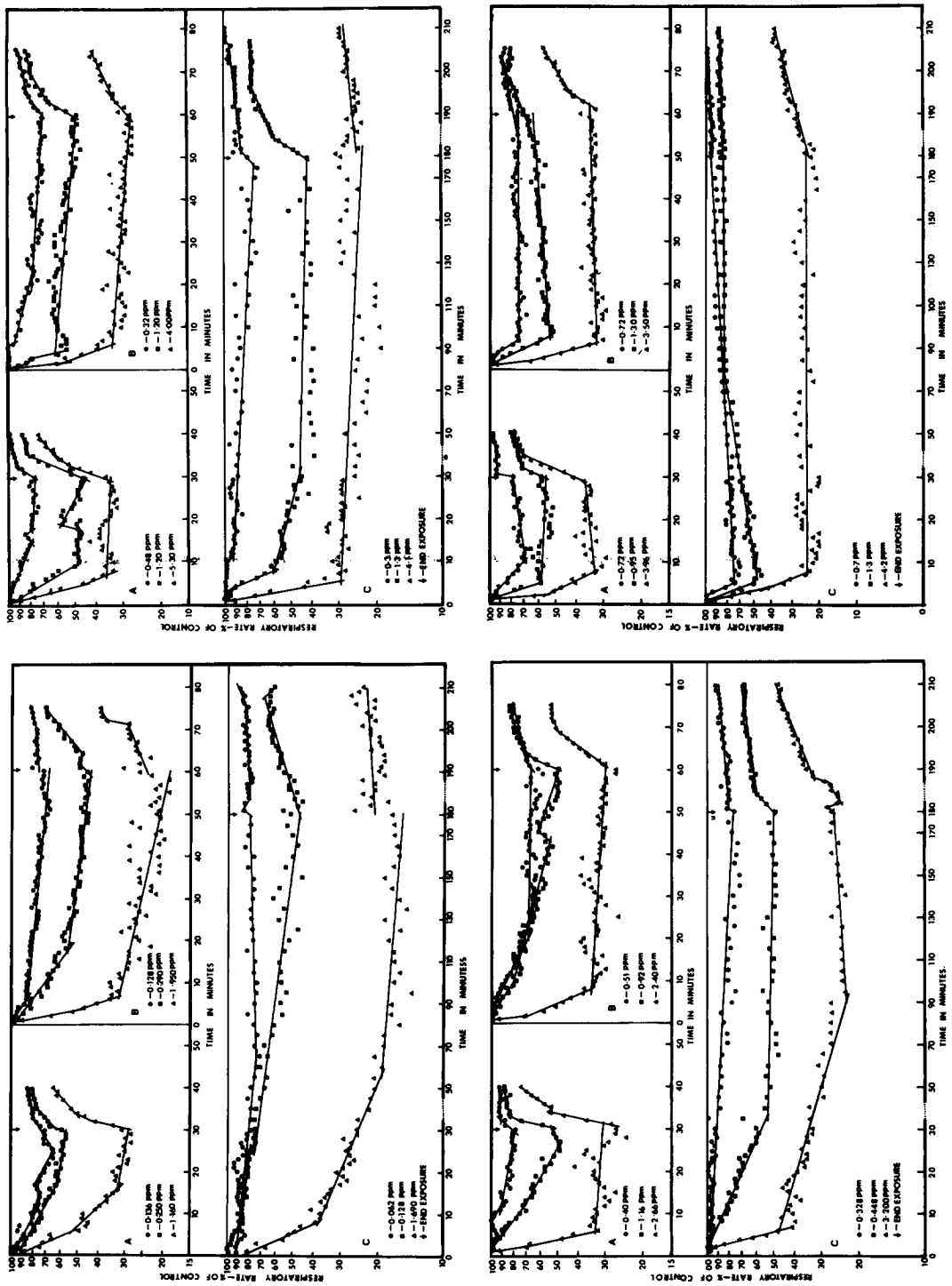
RESPIRATORY RATE—% OF CONTROL
TIME IN MINUTES
A
B
C
TIME IN MINUTES

TABLE 1

CALCULATED RD_{50} VALUES (ppm) AND 95% CONFIDENCE INTERVALS FOR VARIOUS EXPOSURE DURATION AND SUGGESTED TLV FOR INDUSTRIAL EXPOSURE

Exposure duration (min)	Isocyanates					
	HDI	HI	PhI	*o*-TMI	*p*-TMI	TDI[a]
10	0.96 (0.77–1.3)	12.6 (10.5–16)	1.8 (1–4.6)	1.51 (1.2–2.0)	1.63 (1.2–2.6)	0.81 (0.71–0.96)
30	0.35 (0.27–0.44)	11.1 (10–12.7)	1.2 (0.81–2)	1.45 (1.2–1.9)	0.90 (0.71–1.17)	0.50 (0.41–0.64)
60	0.35 (0.25–0.55)	9.2 (7.0–13)	0.90 (0.43–1.6)	—	0.84 (0.55–1.33)	0.39 (0.28–0.53)
120	0.22 (0.20–0.31)	6.2 (4.1–13)	0.82 (0.51–1.2)	—	0.69 (0.51–0.91)	0.25 (0.15–0.36)
180	0.17 (0.08–0.28)	4.8 (4.0–5.9)	0.73 (0.54–0.93)	—	0.63 (0.47–0.82)	0.20 (0.15–0.25)
240	—	—	—	—	—	0.20 (0.15–0.25)
Suggested TLV (ppm)[b]	0.005	0.15	0.02	0.02[c]	0.02	0.006

[a] From previous report (Sangha and Alarie, 1979).
[b] See text.
[c] On the basis of the results with *p*-TMI.

which was very similar to the pattern previously observed with TDI (Sangha and Alarie, 1979). The response was gradual with time, reaching a first maximum within 10 to 20 min of exposure and continuing to increase slowly reaching an apparent plateau within 180 min. Recovery was rapid with short exposures and very slow for longer exposures regardless of the level of response induced in each exposure group. The response to *o*-TMI was slightly different than from *p*-TMI or the other mono- or diisocyanates in that at low concentrations there was a fade in the response as exposure continued. However, the recovery pattern at high concentrations was similar to that observed with the other isocyanates. Although not shown in Fig. 1, the results obtained with HI were similar in every respect to those obtained with HDI.

Concentration–response relationships. Table 1 presents the RD_{50} values for each isocyanate for various durations of exposure. In each case, as seen earlier with TDI (Sangha and Alarie, 1979) the RD_{50} values decreased as the duration of exposure increased. For *o*-TMI RD_{50} values were not obtained for periods longer than 30 min because of the fade in the response for low-exposure concentrations as reported above. From these results, the diisocyanates HDI and TDI have comparable potency and the aromatic monoisocyanates were only slightly less potent than the diisocyanate TDI. However, there was a large difference between the aliphatic monoisocyanate HI and the diisocyanate.

DISCUSSION

In the present study the time–response relationship of sensory irritation was indi-

cated by the decrease in respiratory rate was found to be similar for the mono- and diisocyanates (with some variation in the case of *o*-TMI) and similar to the previous report on TDI (Sangha and Alarie, 1979). Also the potency of the aliphatic diisocyanate HDI was found to be similar to the aromatic diisocyanate TDI. It is of interest to note that the LC50 values for HDI and TDI are also very similar, 310 and 350 mg/m^3, respectively, as reported by Bunge *et al.* (1977). The main difference observed was that the mono- and diisocyanate analogs HI and HDI have a very different potency while the aromatic mono- and diisocyanates were very comparable in potency. Since both aliphatic and aromatic mono- or diisocyanates induced a similar pattern of response crosslinking of receptor protein(s) by the diisocyanates does not seem to be necessary for the very slow recovery pattern observed following long exposure.

Thus simple covalent binding would be sufficient to explain the observed slow recovery pattern although a marked decrease in potency was obvious for the aliphatic monoisocyanate. The fade in response observed with *o*-TMI at low concentrations was unexpected and was observed only for this isocyanate. However, fade in sensory irritation response has been previously observed for other sensory irritants such as sulfur dioxide (Alarie *et al.*, 1973), formaldehyde (Kane and Alarie, 1977), capsaicin (Alarie and Keller, 1973), and ammonia (Barrow *et al.*, 1978). Nevertheless, a slow recovery pattern was also observed for this isocyanate at the higher concentrations. Due to the paucity of toxicological data on these isocyanates it is impossible to set an appropriate threshold limit value (TLV) for industrial exposures. However, some tentative levels can be suggested which will at least prevent sensory irritation and cumulative effect, as previously proposed with TDI (Sangha and Alarie, 1979). The TLV of 0.006 ppm for TDI was based upon the results obtained with a variety of sensory irritants (Kane *et al.*, 1979) by dividing the RD_{50} value obtained for 180 min of exposure by a factor of 30. This empirical model was recently extended to include 23 airborne chemicals of widely different potency and structure (Alarie, 1980). Thus the suggested TLVs for these isocyanates are given in Table 1 and should serve as guidelines for industrial engineering design.

For *o*-TMI it was not possible to obtain an RD_{50} value for 180 min of exposure because of the fade in response at low concentrations and the proposed TLV is given on the basis of the results with *p*-TMI. Procedures for the safe handling of these isocyanates should be similar to those used with TDI, including complete respiratory protection in cases of spills (Alarie and Sangha, 1979).

ACKNOWLEDGMENT

This study was supported in part by the Mobay Chemical Corporation Fellowhip in Toxicology and Grant OH-00367 from the National Institute of Occupational Safety and Health.

REFERENCES

ALARIE, Y. (1980). Dose-response analysis in animal studies; prediction of human responses. In *First Annual Symposium on Environmental Epidemiology*, sponsored by the Center for Environmental Epidemiology, Graduate School of Public Health, University of Pittsburgh and the Environmental Protection Agency, April 28–30, Pittsburgh, *Environmental Health Perspectives*, in press.

ALARIE, Y., AND KELLER, L. W. (1973). Sensory irritation by capsaicin. *Environ. Physiol. Biochem.* **3**, 169–181.

ALARIE, Y., WAKISAKA, I., AND OKA, S. (1973). Sensory irritation by sulfur dioxide and chlorobenzylidene malononitrile. *Environ. Physiol. Biochem.* **3**, 53–64.

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**, 79–88.

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 fur Medizin Dr. Erwald Fischer, Heidelberg Pilge-Druckerei GmbH, D-6720 Speyer.

GUTMANN, H. R., KIELY, G. E., AND KLEIN, M. (1952). The metabolism of 2-aminofluorene in the rat. *Cancer Res.* **12,** 350–355.

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.

KANE, L. E., BARROW, C. S., AND ALARIE, Y. (1979). A short-term test to predict acceptable levels of exposure to airborne sensory irritants. *Amer. Ind. Hyg. Assoc. J.* **40,** 207–229.

KAROL, M. H., DIXON, C., BRADY, M., AND ALARIE, Y. (1980). Immunologic sensitization and pulmonary hypersensitivity by repeated inhalation of aromatic isocyanates. *Toxicol. Appl. Pharmacol.* **53,** 260–270.

MODESTO, R. R., AND PESCE, A. J. (1973). Use of tolylene diisocyanate for the preparation of a peroxidase-labelled antibody conjugate. *Biochem. Biophys. Acta* **295,** 283–295.

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

SCHICK, A. F., AND SINGER, S. G. (1961). On the formation of covalent linkages between two proteins molecules. *J. Biol. Chem.* **236,** 2477–2485.