2,4-TDI:	$CH_3C_6H_3(NCO)_2$	MW:	174.16	CAS:	584-84-9	RTECS:	CZ6300000
2,6-TDI:	$CH_3C_6H_3(NCO)_2$		174.16		91-08-7		CZ6310000
MDI:	$CH_2(C_6H_4NCO)_2$		250.26		101-68-8		NQ9350000
HDI:	$OCN(CH_2)_6NCO$		168.20		822-06-0		MO1740000
NDI:	$C_{10}H_6(NCO)_2$		210.20		3173-72-6		NQ9600000

METHOD: 5521, Issue 2 **EVALUATION: UNRATED** **Issue 1: 15 May 1989**
Issue 2: 15 August 1994

OSHA : Table 1
NIOSH: Table 1
ACGIH: Table 1

PROPERTIES: Table 1

SYNONYMS: Table 1

SAMPLING

SAMPLER: IMPINGER
(solution of 1-(2-methoxyphenyl)-piperazine in toluene)

FLOW RATE: 1 L/min

VOL-MIN: 5 L @ 35 µg TDI/m^3
-MAX: 500 L

SHIPMENT: ship in screw-cap vial
refrigerated @ 4 °C or lower

SAMPLE STABILITY: may be unstable; perform steps 7 & 8 as soon as possible

BLANKS: 2 to 10 field blanks per set

ACCURACY

RANGE STUDIED: not studied

BIAS: not known

OVERALL PRECISION ($\hat{S}_{rT}$): not known

ACCURACY: not determined

MEASUREMENT

TECHNIQUE: HPLC, ELECTROCHEMICAL AND UV DETECTION

ANALYTE: urea derivatives of isocyanates

SAMPLE PREP: acetylate excess reagent, evaporate toluene, redissolve in 5 mL CH_3OH

INJECTION VOLUME: 10 µL

MOBILE PHASE: acetonitrile (20% to 40%)/pH 6.0 methanolic buffer (80% to 60%)
1 mL/min; ambient temperature

COLUMN: Supelcosil, LC-8-DB, 3-µm particle size, 7.5 cm x 4.6-mm; 2-cm guard column, 10-µm particle size

DETECTOR: 242 nm; ECHD, + 0.80 V vs. Ag/AgCl

CALIBRATION: standard solutions of ureas in methanol

RANGE: 2,4-TDI: 0.5 to 8 µg per sample [1]
2,6-TDI: 0.7 to 10 µg per sample [1]
MDI: 0.3 to 4 µg per sample [1]
HDI: 1 to 15 µg per sample [1]
NDI: 0.2 to 13 µg per sample

ESTIMATED LOD: ca. 0.1 µg diisocyanate per sample [1]

PRECISION ($\bar{S}_r$): not determined

APPLICABILITY: The working range is from 5 µg/m^3 2,4-TDI, 7 µg/m^3 2,6-TDI, 3 µg/m^3 MDI, 1 µg/m^3 HDI, and 2 µg/m^3 NDI to more than 1 mg/m^3 for 100-L air samples. This method determines the air concentration of specific diisocyanates. The method has been applied to samples from general foaming, spray- or dip-painting industries [1].

INTERFERENCES: Any substance which elutes with the ureas and is electroactive will interfere with the analysis. Mobile phase conditions can be adjusted to separate most co-eluting peaks, however, ureas of HDI and TDI are difficult to separate.

OTHER METHODS: This method is a modification of Method MDHS 25 published by the Health and Safety Executive of Great Britain [2,3]. Method 2535 is an alternate method for TDI vapor, employing collection on glass wool impregnated with N-(4-nitrophenylmethyl)propylamine.

REAGENTS:

1. 1-(2-Methoxyphenyl)piperazine*, 98%.
2. Acetic anhydride, reagent grade.
3. Methanol, HPLC grade.
4. Acetonitrile, HPLC grade.
5. Water, deionized, distilled.
6. Sodium acetate, anhydrous
7. Acetic acid, glacial.
8. Nitrogen, 99.995%.
9. Toluene, HPLC grade.
10. Sampling medium, 1-(2-methoxyphenyl)-piperazine in toluene, 43 mg/L. (see APPENDIX A)
11. Ureas derived from the isocyanate. (See APPENDIX B).
12. Dimethyl sulfoxide, reagent grade.
13. Mobile phase, acetonitrile and buffer solution to achieve appropriate mobile phase.
14. Buffer solution. Dissolve 15 g anhydrous sodium acetate in 1 L distilled-deionized water. Add 1 L methanol. Add glacial acetic acid to bring pH to 6.0.
15. Urea calibration stock solution, 0.01 µg/µL urea in methanol.
16. Reagent calibration stock solution, 1.0 µg/µL 1-(2-methoxyphenyl)-piperazine in methanol.
17. Helium, prepurified.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: Midget impinger, 25-mL
2. Personal sampling pump, 1.0 L/min, with flexible connecting tubing free of phthalate plasticizer.
   NOTE: Avoid collection of plasticizer in the toluene during sampling. Fluran™ tubing is an acceptable, PVC tubing is not.
3. Liquid chromatograph (HPLC) with electrochemical (ECHD) detector (+0.80 V vs. Ag/AgCl), recorder, integrator and column (page 5521-1)
4. Ultrasonic water bath.
5. Vials, 4-mL glass, with screw caps and 20-mL glass, screw caps with cone-shaped polyethylene liner and shrinkable sealing bands.
6. Pasteur pipets, 7-cm glass, disposable.
7. Flasks, volumetric, glass, 10-mL
8. Syringes, sizes appropriate for preparing standard solutions.
9. Pipets, 5- and 15-mL glass, delivery, with pipet bulb.
10. Hot plate, spark free, 60 °C.
11. Evaporator, Mini-Vap, 6-port or equivalent.
12. pH meter.
13. Vacuum oven.
14. Buchner funnel, fritted glass, medium porosity, 100-mL.
15. Vacuum pump.
16. Flask, filtration, 500-mL.

SPECIAL PRECAUTIONS: Preparation of urea derivatives, samples, and standards should be done in a hood to avoid exposure to isocyanate and solvent vapors. Isocyanates are known respiratory irritants.[4] Toxicity of 1-(2-methoxyphenyl)piperazine is unknown.

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Transfer 15 mL sampling medium to an impinger.
3. Connect the assembled impinger to a sampling pump.
4. Sample 5 to 500 L of air at 1.0 L/min.
   NOTE 1: Toluene evaporates during sampling; when level of solution drops below 10 mL, restore volume to 15 mL with toluene.
   NOTE 2: The reagent in the sampling medium reacts with isocyanates to form ureas:
   $CH_3OC_6H_4NC_4H_8NH + R\text{-}N{=}C{=}O \rightarrow CH_3OC_6H_4NC_4H_8NC(=O)NHR$
5. Prepare blank samples by transferring 15 mL sampling medium to 20-mL vials.
6. Transfer the sample solution to a 20-mL vial for shipment. Rinse both impinger parts with 2 to 3 mL toluene and add rinsings to the sample. Secure vial's screw cap with sealing band. Refrigerate samples as soon as possible. If samples are to be shipped, carefully pack the vials to avoid breakage or spillage of sample.

SAMPLE PREPARATION:

7. Add 25 µL acetic anhydride to acetylate the excess 1-(2-methoxyphenyl)piperazine remaining in the sample solution, to provide for efficient chromatography.
   NOTE: The acetylation reaction is:

$$CH_3OC_6H_4NC_4H_8NH + CH_3C(=O)OC(=O)CH_3 \rightarrow CH_3OC_6H_4NC_4H_8NC(=O)CH_3 + CH_3C(=O)OH$$

8. Evaporate the acetylated sample to dryness under a gentle stream of nitrogen while warming to 60 °C on a hotplate.
9. Redissolve the residue in 5.0 mL methanol, while agitating the sample in an ultrasonic water bath for 15 min.

CALIBRATION AND QUALITY CONTROL:

10. Prepare at least six working standards containing 0.01 to 4.0 µg/mL of the appropriate urea(s) (TDIU, HDIU, and/or MDIU) and 100 µg/mL of 1-(2-methoxyphenyl)piperazine by adding aliquots of calibration stock solutions to 2 mL methanol in a 10-mL volumetric flask. Add 10 µL acetic anhydride to each standard. Mix and dilute to the mark with methanol.
    NOTE: The standard solutions need include only ureas derived from the diisocyanates expected in the air samples.
11. Analyze working standards together with samples and blanks (steps 13 through 15). Prepare a calibration graph for the urea in terms of quantity of isocyanate, M (ECHD area vs. mmol of isocyanate group per sample). Molecular weights of typical ureas are: TDIU = 558.7 g/mol; MDIU = 634.8 g/mol; HDIU = 552.7 g/mol; NDIU = 594.7 g/mol.

$$M = \frac{(C) \cdot MW_I \cdot (5)}{MW_U}, \ \mu g/sample.$$

Where: M is the quantity of isocyanate per sample (µg)
C is the concentration of urea in the standard solution (µg/mL)
5 is the liquid volume of a sample (mL)
MW_u is the molecular weight of the urea
MW_I is the molecular weight of the isocyanate

12 Prepare control samples by adding 0.1, 1.0 and 10.0 µg of urea to 15 mL sampling medium. Prepare these samples for analysis (steps 7 through 9).

MEASUREMENT:

13. Set the HPLC system according to manufacturer's recommendations and to the conditions given on page 5521-1.
14. Inject a 10-µL aliquot of the sample solution from step 10. Capacity factors for the urea derivatives are:

Isocyanate	Mobile Phase: Acetonitrile	Mobile Phase: Buffer Solution	Capacity Factor (k')[a]
2,4-TDI	30%	70%	2
MDI	35%	65%	4
2,4-TDI	40%	60%	3
HDI	40%	60%	3
NDI	60%	40%	3

[a]$k' = (t_r - t_o) / t_o$, where t_r is the retention time of the urea and t_o is the retention time of an unretained compound.

15. Measure peak area.

CALCULATIONS:

16. Read from calibration graph the quantity, M (µg per sample), of isocyanate.
17. Calculate the concentration of isocyanate, C_M (mg/m^3), in the air volume sampled, V (L):

$$C_M = \frac{M}{V}, \ mg/m^3.$$

EVALUATION OF METHOD:

The stability of 2,4-TDIU in toluene was investigated using groups of six samples stored at room temperature for up to two weeks or at 4 °C for 1 week with the following results:

Quantity (µg)	Storage Period (days)	Storage Temperature	Percent Recovery, 95% Confidence Interval
1.9	0	room	99 ± 11
3.8	0	room	98 ± 4
1.9	7	room	78 ± 7
1.9	7	4 °C	88 ± 7
3.8	7	room	67 ± 6
3.8	14	room	70 ± 11

The data demonstrate sample instability at room temperature (about 22 °C) and suggest that the samples are somewhat unstable even at 4 °C.

Estimates of the limits of quantitation (LOQs) (expressed in terms of the quantity of diisocyanate per sample) were made from the electrochemical detector calibration curves used for the analysis of field samples or control samples: 2,4-TDI, 0.5 µg [1, Sequence 6043]; 2,6-TDI, 0.7 µg [1, Sequence 6043]; MDI, 0.3 µg [1, Sequence 6019]; HDI, 1 µg [5]; NDI, 0.6 µg [1, Sequence 6878-C]. The corresponding limits of detection (LODs) were: 2,4-TDI, 0.2 µg; 2,6-TDI, 0.2 µg; MDI, 0.09 µg; HDI, 0.3 µg; NDI, 0.2 µg.

REFERENCES:

[1] NIOSH - Measurement Research Support Branch Analytical Report, Sequences #6019, 6043, 6100, 6354, and 6878-C (NIOSH, unpublished, 1988).
[2] "MDHS 25: Methods for the Determination of Hazardous Substances, Organic Isocyanates in Air. Laboratory Method Using 1-(2-Methoxyphenyl)piperazine Solution and High Performance Liquid Chromatography," Occupational Medicine and Hygiene Laboratory, Health and Safety Executive, London, U.K. (1987).
[3] Bagon, D. A., C. J. Warwick, and R. H. Brown, Am. Ind. Hyg. Assoc. J., 45, 39-43(1984).
[4] NIOSH/OSHA Occupational Health Guidelines for Chemical Hazards, U.S. Department of Health and Human Services, Publ. (NIOSH) 81-123 (1981), available as GPO Stock #17-033-00337-8 from Superintendent of Documents, Washington, D.C. 20402
[5] User check, DataChem Inc., NIOSH analytical seq. #6543 (unpublished, Feb. 24, 1989).

METHOD REVISED BY:

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TABLE 1: SYNONYMS, EXPOSURE LIMITS, AND PROPERTIES

Compounds (Synonyms)	Exposure Limits, µg/m^3*			Properties
	(OSHA)	(NIOSH)	(ACGIH)	
2,4-TDI (toluene-2,4-diisocyanate)	140 (ceiling)	lowest feasible (Ca)	36 140 STEL (Ca)	Liquid; d 1.224 g/mL @ 20 °C; BP 251 °C; VP 1.3 Pa (0.01 mm Hg) @ 20 °C; MP 19.5 -21.5 °C
2,6-TDI (toluene-2,-6-diiocyanate)	no PEL	lowest feasible (Ca)	no TLV	liquid; d 1.22 g/mL @ 20 °C; VP 1.3 Pa (0.01 mm Hg) @ 20 °C
MDI (4,4'-methylenediphenyl isocyanate; diphenylmethane-4,4'-diisocyanate; methylenebis phenyl isocyanate)	200 (ceiling)	50; 200/10 min (ceiling)	51	solid (fused); d 1.23 g/mL @ 25 °C; MP 37.2 °C; VP 0.0002 kPa (0.00014 mm Hg) @ 20 °C
HDI (hexamethylene diisocyanate)	no PEL	35 140/ 10/min (ceiling)	34	liquid; d 1.04 g/mL @ 20 °C; BP 255 °C; 0.007 kPa (0.05 mm Hg) @ 25 °C
NDI (naphthylene diisocyanate)	no PEL	40; 70/10 min	no TLV	solid flakes; MP 127 °C; VP <0.01 Pa @ 20 °C

* 1 ppm = 7100 µg/m^3 TDI; 10208 µg/m^3 MDI; 7350 µg/m^3 HDI;
Ca = Carcinogen

APPENDIX A: PURIFICATION OF 1-(2-METHOXYPHENYL)PIPERAZINE AND PREPARATION OF

SAMPLING MEDIUM

Place 25 g 1-(2-methoxyphenyl)piperazine (yellowish white solid) in a 250-mL beaker. Add approximately 125 mL pentane. Bring to a boil (CAUTION: FLAMMABLE) on a hotplate and allow to boil until all but a small amount of yellow oil is in solution. The 1-(2-methoyphenyl)piperazine will melt as it is warmed in the pentane. Decant the solution into a clean beaker, cover with a watchglass and cool in the freezer for 2 to 3 h. White, fluffy crystals will form. Filter with a Buchner funnel. Transfer the crystals to a 50-mL round bottom flask and dry briefly under vacuum to remove final traces of pentane. Store the hygroscopic crystals in an air-tight container in a refrigerator. The melting range of the crystals is 26 to 29 °C.

Prepare the sampling medium using the purified 1-(2-methoxyphenyl)piperazine. Dissolve this reagent in toluene at a concentration of 43 µg/mL.

APPENDIX B: PREPARATION OF UREA DERIVATIVE

Dissolve 0.005 mole (1 g) of 1-(2-methoxyphenyl)piperazine in 25 mL dimethyl sulfoxide. Dissolve 0.002 mole (350-500 mg) of isocyanate in 25 mL dimethyl sulfoxide. Over a period of 1-2 min, gradually add the isocyanate solution to the stirred derivatizing reagent solution. Warm the resulting solution to 60-90 °C and continue to stir for at least 30 min. Discontinue heating of the solution and add 300 mL deionized water. The urea will precipitate as a white solid. Stop stirring after addition of water. Collect the urea in a fritted-glass Buchner funnel by suction filtration. Dry the compound in a vacuum oven at 75 °C to remove water. Recrystallize until a constant melting point is obtained.

To recrystallize urea, add toluene (150 mL) to dried urea and warm mixture to 60 °C. Slowly and very carefully add just enough methanol (BP 65 °C) to completely dissolve the urea. Remove from heat and allow to cool. Collect the crystals by suction filtration and dry them in vacuum oven at 35 °C. The urea derivatives and their melting points are as follows:

Diisocyanate	Urea Derivative	MP (°C)
2,4-TDI	N,N'-bis[4-(2-methoxyphenyl)piperazine-1-carbonyl]-2,4-toluenediamine (2,4-TDIU)	212-213 (platelets)
2,6-TDI	N,N'-bis[4-(2-methoxyphenyl)piperazine-1-carbonyl]-2,6-Toluenediamine (2,6-TDIU)	231-233 (platelets)
MDI	N,N'-bis[4-(2-methoxyphenyl)piperazine-1-carbonyl]-4,4'-methylenedianiline (MDIU)	209-210 (needles)
HDI	N,N'-bis[4-(2-methoxyphenyl)piperazine-1-carbonyl]-hexamethylenediamine (HDIU)	199-200 (needles)
NDI	N,N'-bis [4-(2-methoxyphenyl)piperazine-1-carbonyl]-1,5-naphthalenediamine (NDIU)	274