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

METHOD: 5522, Issue 1 **EVALUATION: PARTIAL** **Issue 1: 15 May 1996**
Issue 2: 15 January 1998

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

PROPERTIES: Table 1

SYNONYMS: Table 1

SAMPLING

SAMPLER: IMPINGER (tryptamine/DMSO; 20 mL)

FLOW RATE: 1 to 2 L/min

VOL-MIN: 15 L @ 35 µg/m^3 TDI
-MAX: 360 L

SHIPMENT: ship in screw cap vial

SAMPLE STABILITY: at least 28 days in dark @ 25 °C [1]

BLANKS: 2 to 10 field blanks per set

BULK: isocyanate-based oligomers, 1 to 2 mL

ACCURACY

RANGE STUDIED: not studied

BIAS: not determined

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

ACCURACY: not determined

MEASUREMENT

TECHNIQUE: HPLC, FLUORESCENCE DETECTOR/ ELECTROCHEMICAL DETECTOR

ANALYTE: tryptamine derivatives of isocyanates

INJECTION VOLUME: 25 µL

MOBILE PHASE: acetonitrile (40 to 50%)/0.6% sodium acetate buffer (60 to 50%); 1 mL/min

COLUMN: 3.9-mm ID x 150 mm stainless steel packed with 10-µm µ-Bondapak C_{18}, or equivalent

DETECTOR: fluorescence: ex 275 nm; em 320 nm (electrochemical, +0.80V - confirmatory)

CALIBRATION: tryptamine derivatives in sampling medium

RANGE:
2,4-TDI: 0.3 to 14.0 µg/sample
2,6-TDI: 0.6 to 14.0 µg/sample
MDI: 1.0 to 10.0 µg/sample
HDI: 0.6 to 20.0 µg/sample [1]

ESTIMATED LOD:
2,4-TDI: 0.1 µg/sample
2,6-TDI: 0.2 µg/sample
MDI: 0.3 µg/sample
HDI: 0.2 µg/sample [1]

PRECISION ($\hat{S}_r$):
2,4-TDI: 0.059 MDI: 0.029
2,6-TDI: 0.062 HDI: 0.045 [1]

APPLICABILITY: The working range for TDI is 10 to 250 µg/m^3 for a 50-L air sample. This method determines the air concentration of monomers and estimates the concentration of oligomers of specific diisocyanates. It is applicable to vapors and aerosols, such as those produced in a spray-painting operation. The method is not applicable to mixtures of different isocyanates, nor to condensation aerosols from inefficient collection by impingers.
NOTE: This method should be used for area sampling only because of the potential exposure hazard from DMSO solutions.

INTERFERENCES: Any substance which elutes with the tryptamine derivatives and fluoresces will interfere with the analysis, e.g., some aromatic diamines. Mobile phase conditions can be adjusted to separate most co-eluting peaks.

OTHER METHODS: This method is a modification of a method developed by the Occupational Health Laboratory, Ontario Ministry of Labour [2]. NIOSH Method 5521 is an alternate method for the determination of monomers of 2,4-TDI, 2,6-TDI, MDI, and HDI.

REAGENTS:

1. Water, deionized, distilled.
2. Acetonitrile, HPLC grade.
3. Dimethyl sulfoxide (DMSO), HPLC grade.*
4. Sodium acetate trihydrate.
5. Glacial acetic acid, reagent grade.
6. Toluene, HPLC grade.
7. *n*-Propanol, reagent grade.
8. Heptane, reagent grade.
9. Buffer solution: Dissolve 20.4 g sodium acetate trihydrate in 2 L distilled-deionized water. Add glacial acetic acid to pH 5.5.
10. Mobile phase, acetonitrile and buffer solution.
11. Sampling medium, 450 µg/mL,
    (a) For sampling in ambient temperatures >60 °F: tryptamine, 99+% pure, in DMSO;
    (b) For ambient temperatures <60°F: 80/20 (v/v) DMSO/ acetonitrile containing tryptamine, 99+% pure.
    NOTE: Recrystallize tryptamine in acetonitrile before use. (Stable up to 6 mo. in the dark at ambient temperature.)
12. Tryptamine derivatives of isocyanates* (See APPENDIX).
13. Helium. prepurified.

* See SPECIAL PRECAUTIONS

EQUIPMENT:

1. Sampler: Midget impinger containing 20 mL sampling medium.
2. Personal sampling pump, 1 to 2 L/min, with flexible tubing.
3. HPLC, fluorescence detector, ex 275 nm, em 320 nm; integrator and column (page 5522-1). (Electrochemical detector for confirmation of fluorescent peaks.)
4. Vials, 20-mL, scintillation, and 40-mL opaque, with PTFE-lined caps.
5. Pipets, 20-mL.
6. Cylinders, graduated, 25-mL.
7. Syringes, 25-µL, or fixed sample loop.
8. Volumetric flasks, 10-mL.
9. Fritted glass funnel.
10. Filtering flasks.
11. Sealing bands.

SPECIAL PRECAUTIONS: Isocyanates are known respiratory irritants. Prepare standards and derivatives in a fume hood. DMSO is readily absorbed into the skin. Wear neoprene latex gloves when handling the solvent, sampling media, and field samples [3].

SAMPLING:

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Transfer 20 mL sampling medium to an impinger, and connect to sampling pump with flexible tubing.
   NOTE: Prepare field blank samples by transferring 20 mL sampling medium to 20-mL vials.
3. Sample at an accurately known flow rate between 1 and 2 L/min for a total sample size of 15 to 360 L.
4. Estimate final impinger volume to the nearest 0.5 mL. Record on sample submittal sheet.
   NOTE: DMSO is hygroscopic; therefore, final sampling volumes may be greater than 20 mL.
5. Transfer the sample solution to a 40-mL opaque vial for shipment. Secure screw cap with sealing band.
6. Obtain a bulk sample (1 to 2 mL) and the material safety data sheet for each component of the formulation.

SAMPLE PREPARATION:

7. Transfer an aliquot of each sample to a HPLC autosampler vial.

CALIBRATION AND QUALITY CONTROL:

8. Calibrate daily with at least six working standards in the range of interest.
   a. Prepare working standards containing 0.05 to 10.0 µg/mL of the appropriate monomeric tryptamine derivative in the sampling medium.

b. Analyze together with samples, controls, and blanks (steps 10 through 12).
c. Prepare calibration graph (response vs. µg per mL monomeric diisocyanate-tryptamine derivative).

9. Analyze three quality control blind spikes and three analyst spikes to ensure that the calibration graph is in control.

MEASUREMENT:

10. Set liquid chromatograph according to manufacturer's recommendations and to conditions given on page 5522-1. Inject 25-µL sample aliquot with a syringe, a fixed volume sample loop, or an autosampler.
    NOTE: Field sample run time = 60 min.
11. To investigate any intrinsic fluorescence from the bulk components, add an aliquot of the bulk sample to 100% DMSO containing no tryptamine reagent and run a chromatogram (See Figure 1).
    NOTE: If the bulk sample is not soluble in DMSO, prepare a stock solution in an alternative solvent such as dichloromethane. Add aliquots of the stock solution to DMSO.
12. Measure fluorescent response for all peaks in the chromatogram that also give an electrochemical response (See Figure 2).
    NOTE: If peak response is above the linear range of the working standards, dilute with sampling medium, reanalyze, and apply the appropriate dilution factors in the calculations.

CALCULATIONS:

13. Determine solution concentration (µg/mL) of each monomer derivative in the sample, C_s, and average media blank, W_b, from calibration graph (step 8.c.). Sum the responses of all other confirmed peaks in the chromatogram that elute later than the monomer peak. Read solution concentration, C_s (µg/mL), from calibration graph, and report as oligomer diisocyanate-tryptamine derivative.
    NOTE: Report the results for each monomer separately and the isocyanate-based oligomers as a group.
14. Using the solution volumes (mL) of the samples, V_s, and media blanks, V_b, calculate the concentration, C (mg/m^3), of each monomer and oligomer in the volume of air sampled, V (L), applying the ratio of molecular weight of diisocyanate, MW_{DI} (see Table 1), to the molecular weight of diisocyanate-tryptamine derivative, MW_{DIT} (see APPENDIX):

$$C = \frac{(C_s V_s - C_b V_b)\ (MW_{DI} / MW_{DIT})}{V},\ mg/m^3$$

NOTE: µg/mL = mg/m^3

EVALUATION OF METHOD:

During method development, the performance of the fluorescence detector was verified through the use of a second detector in series (an electrochemical detector).

Recovery studies were conducted by analyzing groups of five to six samples of each diisocyanate. Vapor spikes of 2,4-TDI, 2,6-TDI, and HDI were prepared. Because of the low vapor pressure of MDI, liquid spikes were prepared instead of vapor spikes. For 2,4-TDI, 3 concentrations ranging from 4.9 to 60 µg per sample yielded an average recovery of 90.5%. For 2,6-TDI, 3 concentrations ranging from 6.0 to 60 µg per sample yielded an average recovery of 102.8%. For HDI, 3 concentrations ranging from 5.0 to 47 µg per sample yielded an average recovery of 89.5%. For MDI, 3 concentrations ranging from 3.2 to 72 µg per sample yielded an average recovery of 96.4%. The recovery studies also were conducted in the presence of 17% water, since the DMSO solvent is known to be hygroscopic. The recoveries for 2,4-TDI, HDI, and MDI with 17% water present were measured at one concentration level with an average recovery of 94.5%.

Storage stability studies were conducted at one concentration level by spiking groups of five to six impinger samplers with each diisocyanate-tryptamine derivative and storing at room temperature in the dark. Since

the DMSO solvent is hygroscopic, additional storage studies were conducted in the presence of 17% water for each of the four diisocyanate-tryptamine derivatives. The average recovery for the four diisocyanate-tryptamine derivatives measured at one level was 95.8% for a 28-day storage period. Some of the samples were stored over a period of several months. HDI derivative had a recovery of 96.4% after 4 months. MDI derivative was stable for 5 months with a 98.9% recovery.

No interference in the method was found from acetone, methyl ethyl ketone, benzaldehyde, acetophenone, or cyclohexanone. Aromatic diamines with the same retention times as the analytes are potential interferences. Therefore, the use of an electrochemical detector is recommended for confirmation of isocyanate peaks.

Because of the high solidification point of DMSO, the addition of a reagent to lower the freezing point was necessary for sampling in environments <60ºF. An 80:20 DMSO:acetonitrile solution containing the derivatizing reagent is recommended. Recovery studies were conducted in 80:20 DMSO:acetonitrile. For each diisocyanate, liquid spikes were prepared in groups of six samples at 3 concentration levels. The average recoveries were 84% for 2,4-TDI, 104% for 2,6-TDI, 85% for HDI, and 88% for MDI. Storage stability studies of six samples at one level for each diisocyanate-tryptamine derivative yielded an average recovery for the four diisocyanate-tryptamine derivatives of 96% for 7 days and 95% for 28 days.

For more detail, see the Backup Data Report for this method [1].

REFERENCES:

[1] Key-Schwartz RJ, Tucker SP [1994]. Backup data report for isocyanates, Method 5522, NIOSH/DPSE. Unpublished report.
[2] Wu WS, Stoyanoff RE, Szklar RS, Gaind VS, Rakanovic M [1990]. Application of tryptamine as a derivatising agent for airborne isocyanate determination. Analyst *115*: 801-807.
[3] Schwope AD, Randel MA, Broome MG [1981]. Dimethyl sulfoxide permeation through glove materials. Am Ind Hyg Assoc J *42*(10): 722-725.

METHOD WRITTEN BY:

Rosa J. Key-Schwartz, Ph.D. and Samuel P. Tucker, Ph.D., NIOSH/DPSE

TABLE 1: SYNONYMS, EXPOSURE LIMITS, AND PROPERTIES

Compounds (Synonyms)	MW	Exposure Limits, µg/m³*			Properties
	(g/mol)	(OSHA)	(NIOSH)	(ACGIH)	
2,4-TDI (toluene-2,4-diisocyanate)	174.16	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 to 21.5 °C
2,6-TDI (toluene-2,-6-diiocyanate)	174.16	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)	250.26	200 (ceiling)	50; 200/10 min (ceiling)	51	solid (fused); d 1.23 g/mL @ 25 °C; MP 37.2 °C; VP 0.19 Pa (0.00014 mm Hg) @ 25°C
HDI (hexamethylene diisocyanate)	168.20	no PEL	35; 140/10 min (ceiling)	34	liquid; d 1.04 g/mL @ 20°C; BP 255 °C; 7 Pa (0.05 mm Hg) @ 25 °C

* 1 ppm = 7100 µg/m³ TDI; 10208 µg/m³ MDI; 7350 µg/m³ HDI;
Ca = Carcinogen

APPENDIX: PREPARATION OF TRYPTAMINE DERIVATIVE

Dissolve 0.00250 mole (0.41 g) of tryptamine (>99% purity) in 300 mL of toluene. Heat the solution to 80°C while stirring until much of the tryptamine is dissolved. Dissolve 0.001 mole (150-300 mg) of isocyanate in 20 mL of toluene. Add the isocyanate solution to the tryptamine solution. The tryptamine derivative will precipitate as a white gel. Collect the precipitate in a fritted-glass funnel by suction filtration. For the tryptamine derivatives of HDI and MDI, dissolve the precipitate in 100 mL (for HDI) or 450 mL (for MDI) of hot n-propanol. Filter the solution and allow it to cool. Collect the crystals. For the tryptamine derivatives of 2,4- and 2,6-TDI, dissolve the precipitate in 50 mL of hot n-propanol. Filter the solution and allow to cool. Add heptane (175 mL for 2,4-TDI and 100 mL for 2,6-TDI) and collect the precipitate in a fritted-glass funnel by suction filtration. For all derivatives, dry in a vacuum oven at 60°C to remove solvent.

TRYPTAMINE DERIVATIVES

Diisocyanate	Tryptamine Derivative	MP (°C)	MW (g/mol)
2,4-TDI	2,4-TDI-tryptamine	216-219	494.60
2,6-TDI	2,6-TDI-tryptamine	298-301	494.60
MDI	MDI-tryptamine	~270	570.70
HDI	HDI-tryptamine	201-201.5	488.64