7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring TDI and MDI, their metabolites, and other biomarkers of exposure and effect to TDI and MDI. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Some of the methods used for determining TDI and MDI in biological media are reported in Table 7-1.

Since diisocyanates react much more rapidly with the sulfhydryl, amino, and hydroxyl groups present on proteins than with water in physiological situations, they are primarily eliminated from the body as protein adducts. TDI and MDI can be effectively assayed in urine by first carrying out a strong acid extraction of the urine samples, which releases the corresponding free amine (i.e., TDA or MDA) (Rosenberg and Savolainen 1986b). The urine hydrolysate is then extracted with toluene and a perfluoroalkyl anhydride, commonly heptafluorobutyric anhydride or pentafluoropropionic anhydride, is added to produce perfluoroacylated amide derivatives that are analyzed by gas chromatography (GC)/mass spectrometry (MS) (Dalene et al. 1997; Rosenberg and Savolainen 1986b). Instead of using GC/MS, these amide derivatives may also be evaporated from the toluene solution and then dissolved in a mobile phase consisting of 0.1 M ammonium acetate in 55/20/25% acetonitrile/methanol/water for analysis by liquid chromatography (LC)/MS (Skarping et al. 1994).

Alkaline hydrolysis of diisocyanates protein adducts to amines has also been used for quantification in urine. A method involving strongly alkaline conditions to hydrolyze TDI protein adducts to toluene diamines followed by extraction with toluene and analysis using reverse phase high performance liquid chromatography (HPLC) and electrochemical detection has been described (Carbonelle et al. 1996).

Table 7-1. Analytical Methods for Determining TDI and MDI in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human urine (diisocyanate-derived amines)	Heat with sulfuric acid; adjust pH to ~9.2; cleanup on SPE silica cartridge; solvent extraction with toluene; derivatization with heptafluorobutyric anhydride	GC/MS	0.2 pmol per injection	No data	Rosenberg and Savolainen 1986a
Human urine (2,6-TDA)	Heat with sulfuric acid; solvent extraction with toluene; derivatization with heptafluorobutyric anhydride	GC/MS	2 pg for a 1 µL sample	96% (3% RSD)	Rosenberg and Savolainen 1986b
Human urine (4,4'-MDA)	Acidic hydrolysis with sulfuric acid; extraction with toluene; derivatization with pentafluoropropionic anhydride; evaporation; dissolution in 0.1 M ammonium acetate in 55/20/25% ACN/methanol/ water	LC/PSP-MS	0.1 pg/µL	No data	Skarping et al. 1994
Human urine (TDAs)	Alkaline hydrolysis with sodium hydroxide; extraction with toluene; purification with a cation-exchange column containing methanol and phosphoric acid solution	RP-HPLC/ED	0.1 µg/L (2,6-TDA); 0.15 µg/L (2,4-TDA)	87.6% (7.9% RSD) (2,6-TDA); 88.3% (5.3% RSD) (2,4-TDA)	Carbonnelle et al. 1996
Human urine, plasma (TDA, MDA)	Acidic hydrolysis with sulfuric acid; extraction with toluene; derivatization with pentafluoropropionic anhydride	GC/MS	No data	No data	Dalene et al. 1997
Human blood (MDI adduct ABP-Val-Hyd)	Blood sample centrifuged and washed with 2.5 mL of 0.9% sodium chloride solution; hydrolysis with HCl; derivatization with heptafluorobutyric anhydride	GC/MS-NCI	0.02 ng ABP-Val-Hyd/g globin	99.8% (3.0–9.3% RSD)	Gries and Leng 2013

ABP-Val-Hyd = 5-isopropyl-3-[4-(4-aminobenzyl)phenyl]hydantoin; ACN = acetonitrile; ED = electrochemical detection; GC = gas chromatography; HCl = hydrochloric acid; LC = liquid chromatography; MDA = methylene dianiline; MDI = methylenediphenyl diisocyanate; MS = mass spectrometry; NA = not applicable; NCI = negative chemical ionization; PSP = plasma spray; RP-HPLC = reverse phase high performance liquid chromatography; RSD = relative standard deviation; SPE = solid-phase extraction; TDA = toluene diamine; TDI = toluene diisocyanate

Amine hydrolysis products may also be detected in plasma. Similar to urinalysis, sample preparation involves heating the plasma with sulfuric acid to hydrolyze the amines diisocyanate-protein adducts, which are extracted with toluene and then pentafluoropropionic anhydride is added to produce perfluoroacylated amide derivatives that are analyzed by GC/MS (Dalene et al. 1997).

Gries and Leng (2013) have described a method for detecting the MDI-hemoglobin adduct ABP-Val-Hyd in human blood as a marker for MDI exposure. In this technique, a blood sample is centrifuged to separate the erythrocytes from the plasma, which are then washed with 2.5 mL of 0.9% sodium chloride solution. A globin residue is produced, which is hydrolyzed using 2 mL of 2 M hydrochloric acid and derivatization was done by adding heptafluorobutyric anhydride to produce a perfluoroacylated amide. Analysis is performed by GC and high-resolution MS with negative chemical ionization.

7.2 ENVIRONMENTAL SAMPLES

Methods of analysis are available for the determination of TDI and MDI in air. These include HPLC, GC, and spectrophotometry. The use of a bubbler that collects air through an impinger containing an absorbent solution is the most common sampling procedure (Rosenberg and Savolainen 1986b). A critical review of sampling and analysis methods for TDI and MDI in air is presented in Levine et al. (1995). A summary of analytical methods is shown in Table 7-2.

Analysis of aromatic diisocyanates was historically performed using Marcali or Ranta colorimetric methods, with the Marcali method being the method of choice. However, these methods are limited by their lack of specificity. The Marcali method uses an acidified aqueous bubbler solution to collect diisocyanates in air and convert them into their respective diamines. The diamines then couple with N-1-naphthyl ethylenediamine to produce a colored complex. The intensity of the color measured at two different wavelengths is a measure of the amount of diisocyanates in the bubble. The inability to distinguish the diisocyanates from the produced diamines is the major limitation of this method (OSHA 1980).

The first sensitive and diisocyanate-specific method of analysis is employed by OSHA as Method 18 (OSHA 1980). Diisocyanates in air are trapped in a bubbler solution consisting of a nitro reagent (0.0002 M p-nitrobenzyl-N-n-propylamine) in toluene. This amine reacts with the diisocyanates to

Table 7-2. Analytical Methods for Determining TDI and MDI in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (diisocyanates)	Air drawn through a glass tube with a glass fiber filter and 0.1 mg of 1-2PP adsorbent; extraction with 90:10 (v/v) ACN/DMSO	HPLC/UV (Method 42)	1.6 µg/m^3 (2,6-TDI); 1.3 µg/m^3 (2,4-TDI); for a 15-L sample	86.4% (1.6% RSD) (2,6-TDI); 80.3% (2.4% RSD) (2,4-TDI)	OSHA 1989a
Air (MDI)	Air drawn through a glass tube with a glass fiber filter and 1.0 mg of 1-2PP adsorbent; extraction with 90:10 (v/v) ACN/DMSO	HPLC/UV (Method 47)	0.8 µg/m^3 for a 15-L sample	94.8% (4.5% RSD)	OSHA 1989b
Air (diisocyanates)	Air drawn into a bubbler containing nitro reagent in toluene	HPLC (Method 18)	0.15 ppb (1 µg/m^3) (2,4-TDI); 0.10 ppb (1 µg/m^3) (MDI); for a 20-L sample	100% (2,4-TDI and MDI)	OSHA 1980
Air (total diisocyanates)	Air collected on fiberglass filters impregnated with nitro reagent (4-nitro-N-propylbenzylamine); SPE with 4:6:1 methanol/water/0.2M hydrochloric acid	DPP	8 µg/m^3 for a 50-L sample	98% (1.9% RSD)	Corbini et al. 1991
Air (total diisocyanates)	Air drawn through a glass tube with a glass fiber filter and 0.13–1.1 mg of MAP adsorbent; acetylation with acetic anhydride; extraction with 65:35 (v/v) ACN/ triethylammonium phosphate/formate	RP-HPLC/UV/FD (Method 5525)	17 ng/sample; for a 15-L sample	97–99% (1.0–3.5% RSD)	NIOSH 2003
Air (2,4-TDI)	Air drawn through a tube with 200 mg of Tenax-TA adsorbent; thermal desorption	capGC/FID-ITD	<0.001 µg/sample for a 1-L sample	99.5% (3.4–7% RSD)	Bianchi and Joyner 1997

Table 7-2. Analytical Methods for Determining TDI and MDI in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (2,4-TDI)	Air drawn into traps containing silica gel coated with phosphoric acid; elution with sodium hydroxide and methanol; separation with 60:40 (v/v) phosphate buffer/ methanol	HPLC/UV	0.2 µg/m^3 for a 20-L sample	100% (<3% RSD)	Colli et al. 1993
Air (TDI)	Air collected into a glass tube; simultaneous absorption and derivatization with p-aminophenol	HPLC/ ECHD	94 pg/sample	75–80% (<2% RSD)	Meyer and Tallman 1983
Air (TDI vapor and aerosols)	Air collected onto a denuder coated with dimethylpolysiloxane (adsorbent) and dibutylamine (derivatization reagent) in series with a glass fiber filter; extraction with ACN	LC-ESI/ MS-MS	1.9 ng/m^3 (2,4-TDI); 1.5 ng/m^3 (2,6-TDI)	99.4% (2,4-TDI); 99.7% (2,6-TDI)	Nordqvist et al. 2005
Air (MDI vapor and aerosols)	Air collected onto a denuder coated with N-4-nitrobenzyl-N-1-propylamine in series with a glass fiber filter; extraction with ACN	HPLC/UV	0.7 µg/m^3 (vapor phase); 3.3 µg/m^3 (condensed phase)	No data	Rando and Poovey 1994
Air (diisocyanates)	Air drawn through a glass tube with a glass fiber filter and di-n-butylamine in toluene; extraction with ACN	LC/MS	5–10 ng/mL	92% (2,6-TDI); 96% (2,4-TDI); 86% (MDI)	Bobeldijk et al. 2008
Occupational air (TDI)	Air collected on a 15-mg 1-2MPP-impregnated glass fiber filter; extraction with 1 mL ACN with 0.5% acetic anhydride	RP-HPLC/ UV	0.1 ng per injection	No data	Rosenberg and Savolainen 1986b

Table 7-2. Analytical Methods for Determining TDI and MDI in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air (diisocyanates)	Air collected using midget impinge flasks and derivatized with di-n-butylamine in toluene followed by a glass fiber filter; evaporation; dissolution in ACN	LC/MS	0.002 µg/m^3 (TDI and MDI)	~95% (<4% RSD for TDI); (12% RSD for MDI)	Karlsson et al. 2000
Occupational air (diisocyanates)	Air collected with an impinger and derivatized with 1-2MPP in toluene; acetylation; evaporation; dissolution in ACN/methanol buffer	HPLC/ ECHD/UV (Method 5521)	0.2 µg/sample (2,4- and 2,6-TDI) 0.09 µg/sample (MDI)	No data	NIOSH 1994
Occupational air (diisocyanates)	Air collected using an impinger and derivatized with tryptamine in DMSO; dissolution in ACN/ sodium acetate buffer	HPLC/FD/ ECHD (Method 5522)	0.1 µg/sample (2,4-TDI); 0.2 µg/sample (2,6-TDI); 0.3 µg/sample (MDI)	90.5% (2,4-TDI); 102.8% (2,6-TDI); 96.4% (MDI)	NIOSH 1996
Occupational air (MDI)	Air drawn into traps containing silica gel coated with phosphoric acid; elution with sodium hydroxide and methanol	GC/NPD	0.7 µg/m^3 for a 20-L sample	100% (<5% RSD)	D'Eril et al. 1995
Occupational vapor (2,4-TDI)	Air drawn through a glass tube with a 1-2PP/methylene chloride adsorbent; extraction with methanol/water	RP-HPLC/ UV	1 ppb for a 15-L sample	106.3% (10% RSD)	Chang and Burg 1982
Chemical products (TDI and MDI)	Derivatized with 9-(methyl aminomethyl)-anthracene (1% v/v) in dichloromethane	HPLC/UV	50 ppb (MDI) 5 ppb (TDI)	92–97% (<5% RSD)	Rastogi 1989

1-2MPP = 1-(2-methoxyphenyl)piperazine; 1-2PP = 1-(2-pyridyl)piperazine; ACN = acetonitrile; capGC = capillary gas chromatography; DMSO = dimethyl sulfoxide; DPP = differential-pulse polarography; ECHD = electrochemical detector; ED = electron capture detector; ESI = electrospray interface; FD = fluorescence detector; FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; ITD = ion-trap detector; LC = liquid chromatography; MAP = 1-(9-anthracenylmethyl)piperazine; MDI = methylenediphenyl diisocyanate; MS = mass spectrometry; NPD = nitrogen-phosphorus detector; RP-HPLC = reverse-phase high performance liquid chromatography; RSD = relative standard deviation; SPE = solid-phase extraction; TDI = toluene diisocyanate; UV = ultraviolet absorbance detection

produce ultraviolet (UV)-absorbing urea derivatives that can be easily analyzed by HPLC (OSHA 1980). Another nitro reagent method describes the analysis of diisocyanate monomers by collecting air onto fiberglass filters impregnated with 4-nitro-N-propylbenzylamine, followed by solid-phase extraction and determination of total diisocyanate concentration by differential-pulse polarography (Corbini et al. 1991).

A modified Marcali technique was described that allowed for the ability to isolate specific diisocyanates. Colli et al. (1993) reported this method for the determination of 2,4-TDI concentrations in air. In this procedure, air is collected in traps containing silica gel coated with phosphoric acid to form 2,4-TDA, followed by elution with methanol and sodium hydroxide and analysis using HPLC and UV detection. A similar method was described for the determination of MDI in workplace air, particularly spraying operations, which employs analysis by GC and a nitrogen-phosphorus detector (D'Eril et al. 1995).

Other sampling techniques collect air onto a solid sorbent media by using an impinger and a reagent-impregnated glass-fiber filter. These methods employ the use of a derivatizing agent, such as 1-(2-methoxyphenyl)piperazine, to form stable derivatives of the diisocyanates for HPLC and electrochemical detection (Rosenberg and Savolainen 1986b). Three NIOSH methods (Methods 5521, 5522, and 5525) have been used to analyze diisocyanates and employ the use of HPLC with UV, or UV and fluorescence detection (NIOSH 1994, 1996, 2003). Derivatizing agents in these methods include 1-(2-methoxyphenyl)piperazine, 1-(9-anthracenylmethyl)piperazine, and tryptamine. Several methods use 1-(2-pyridyl)piperazine as a derivatizing agent to form stable urea derivatives for detection by HPLC with UV (Chang and Burg 1982; OSHA 1989a, 1989b). Karlsson et al. (2000) described a method using a di-n-butylamine derivatizing agent followed by LC/MS analysis. This method was validated by Bobeldijk et al. (2008).

A method employing a chemosorptive denuder in series with a glass fiber filter in order to sample personal exposure to TDI vapor and aerosols was described (Nordqvist et al. 2005). This method used a dimethylpolysiloxane denuder coating with dibutylamine as a derivatizing agent. Analysis is performed using LC with an electrospray interface with MS. The advantages of this method include a wide sampling concentration range and accurate vapor-particulate-phase distribution measurements (Nordqvist et al. 2005). Rando and Poovey (1994) described a similar method using a denuder in series with a glass fiber

filter coated with nitro reagent, N-4-nitrobenzyl-N-1-propylamine, for collection and derivatization of MDI vapor and aerosol followed by HPLC analysis and UV detection.

Bianchi and Joyner (1997) describe a method for detecting TDI in air that collects samples directly onto an adsorbent tube packed with Tenax-TA followed by thermal desorption and then uses capillary GC with simultaneous flame ionization and ion-trap detection.

A method for determining TDI and MDI in various types of chemical products, such as adhesives, insulating foam, sealing waxes, surface coatings, etc., has been described (Rastogi 1989). This method involves the reaction of the chemical product with 9-(methyl aminomethyl)-anthracene to form urea derivatives from the diisocyanates present, followed by HPLC and UV detection.

Analytical methods for the detection of diisocyanates in other media were not located. Diisocyanates hydrolyze rapidly in water, so it is therefore unlikely that significant amounts would be found in other environmental media, such as water, soil, sediment, or food.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of TDI and MDI is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of TDI and MDI.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. TDI-protein adducts in biological samples are hydrolyzed to form TDA. (Tinnerberg et al. 1997). Methods of measuring this biomarker of exposure are available (Austin 2007; Carbonelle et al. 1996).

Effect. Respiratory exposure to diisocyanates can lead to occupationally induced asthma. Workers diagnosed with diisocyanate-induced asthma manifest characteristic physiological responses after specific bronchoprovocation, which correlate to changes in their airways (Bernstein 1996).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods for measuring diisocyanates in air are available (Levine et al. 1995; NIOSH 1994, 1996, 2003). Diisocyanates hydrolyze rapidly in water and it is unlikely that significant amounts would be found in environmental media, such as water, soil, and sediment.

7.3.2 Ongoing Studies

L2 Diagnostics, LLC (A. Wisnewski, Principal Investigator) are developing innovative biomonitoring approaches to exposure surveillance for MDI. Specifically, they aim to develop blood tests that measure two different MDI exposure biomarkers. The first biomarker is MDI-specific antibodies (IgG), produced by the immune system in response to exposure. The second biomarker is the chemical (MDI) itself conjugated to albumin, the major "protein adduct" *in vivo* (NIH RePORTER 2014).