# 6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring hexamethylene diisocyanate, its metabolites, and other biomarkers of exposure and effect to hexamethylene diisocyanate. 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.

## 6.1 BIOLOGICAL SAMPLES

Some of the methods used for determining HDI in biological media are reported in Table 6-l. Methods for the biological monitoring of exposures to HDI have been based on determination of the corresponding amine, hexamethylene diamine. Methods are available for determination of HDI in urine and plasma; however, no methods were found for mother's milk, or tissues.

The analysis methods use acid or base hydrolysis of the compound, extraction into solvent (usually toluene), derivatization, followed by chromatographic analysis. Determination is based on sensitive gas chromatography/mass spectrometry (GC/MS) techniques. Alternately, GC with specific detectors or high performance liquid chromatography (HPLC) may be sued. The method based on hydrolysis and basic extraction of the liberated amine into toluene provides sensitive measurements, but the extraction recovery is low (Dalene et al. 1990). A method using hydrolysis followed by a two-phase derivatization procedure showed good recovery (above 90%) but less sensitivity (Dalene et al. 1994a). Very good sensitivity was reported for determination using HPLC-MS (Dalene et al. 1994b); however, this method requires state-of-the-art instrumentation.

Analysis of the degradation products of the oligomeric form of HDI, HDI-BT, may be possible by the above procedures; no experimental data are available.

## Table 6-1. Analytical Methods for Determining Biological Degradation Products of Hexamethylene Diisocyanate

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine[a] | Heat with sulfuric acid; adjust pH to ≈9.2; cleanup on SPE silica cartridge; solvent extraction (toluene); derivatization with heptafluorobutyric anhydride) | GC/MS | 0.2 pmol per injection | No data | Rosenberg and Savolainen 1986 |
| Urine[a] | Heat with aqueous hydrochloric acid; cool; pH adjustment; solvent extraction; derivatization with heptafluorobutyric anhydride | GC/MS (CI mode using ammonia) | >100 µg/L | 34±4% | Brorson et al. 1990; Dalene et al. 1990 |
| Urine[a] | Alkaline hydrolysis; derivatization with trifluoroethyl chloroformate | GC/MS (CI mode using ammonia); GC/ TSD | 0.5 µg/L (GC/MS); 20 µg/L (GC/TSD) | 97±5% | Dalene et al. 1994a |
| | | HPLC-thermospray/ MS | 1 µg/L | No data | Dalene et al. 1994b |
| Plasma, urine[a] | Acid hydrolysis; derivatization with pentafluoroproprionic acid | GC/MS (CI-NI) | ≈0.04 µg/L | No data | Skarping et al. 1996 |

[a] Analyte is hexamethylene diamine

CI = chemical ionization; CI-NI = monitoring negative ions in the chemical ionization mode; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; pmol = picomole; SPE = solid-phase extraction; TSD = thermionic specific detection

6. ANALYTICAL METHODS

## 6.2 ENVIRONMENTAL SAMPLES

Methods are available for the determination of HDI in occupational and ambient air. A summary of representative methods is shown in Table 6-2. Much of the methodology involves drawing air through an impinger or sorbent-containing derivatizing reagent. The sample is then analyzed by HPLC with ultraviolet (UV) and/or electrochemical detection. A good review of these techniques is available. Two NIOSH methods (Methods 5505 and 5521) have been used to analyze for the isocyanates (NIOSH 1984). Both use HPLC with UV, or UV and electrochemical detection. Collection is carried out by passing sampled air through toluene containing l-(2-methoxyphenyl)-piperazine to derivatize the HDI. They were specified for analysis of monomeric and oligomeric forms of isocyanates providing a total isocyanate concentration in air as well as some speciation. Method 5505 has been removed from the current NIOSH manual. Problems have been reported for Method 5521. The ratio criterion may not be suitable for qualitative identification of HDI oligomers (Key-Schwartz 1995).

There are also other techniques which use a similar sampling and analysis scheme. A number of methods used 9-(N-methylaminomethyl)anthracene as a reagent to derivatize the HDI allowing subsequent detection of a stable derivative by UV absorbance or fluorescence detection (Andersson et al. 1983; Gudehn 1984; Kormos et al. 198 1; Sang6 and Zimerson 1980). The “nitro reagent” or N-4-nitrobenzyl-N-n-propylamine has also been used for derivatization prior to analysis by HPLC/UV or differential pulse polarography (Corbini et al. 1991; Dunlap et al. 1976; Graham 1980; Hakes et al. 1986). Various column types and eluent conditions were used in the HPLC separations cited above. The 1-(2-methoxyphenyl)-piperazine used as a derivatization reagent in the NIOSH methods was also used on a sorbent (Schmidtke and Seifert 1990) and inert supports (Huynh et al. 1992; Sesana et al. 1991) avoiding the use of toluene in the collection impinger. Another derivatization agent used was 1-(2-pyridyl)piperazine either in a toluene solution (Ellwood et al. 1981; Goldberg et al. 198 1) or impregnated on glass fiber filters (Rudzinski et al. 1994). Samples were analyzed using TLC, capillary zone electrophoresis, or HPLC techniques. In addition to those listed in Table 6-2, there are methods which may be used for determination of the oligomeric form of HDI or HDI-BT (biuret trimer) (Bagon et al. 1984; Ellwood et al. 1981; Goldberg et al. 1981; Levine et al. 1979).

Additional novel analytical techniques include coating a polystyrene strip with cholinesterase, exposing the strip to an atmosphere (passive sampling), then immersing the strip in a cuvette with reagent for assay

## Table 6-2. Analytical Methods for Determining Hexamethylene Diisocyanate in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Occupational air (total isocyanates) | Air collected/derivatized through 1-(2-methoxyphenyl)piperazine in toluene; acetylation; evaporation | HPLC with UV detection | ≈0.2 µmol/ sample | 96% average | NIOSH 1984 NIOSH Method 5505 |
| Occupational air (total isocyanates) | Air collected/derivatized through 1-(2-methoxyphenyl)piperazine in toluene; acetylation; evaporation | HPLC with electrochemical and UV detection | ≈0.3 µg per sample | 123% avg. | NIOSH 1984 NIOSH Method 5521 |
| Occupational air | Air collected/derivatized through 9-(*N*-methylaminomethyl)anthracene in toluene; evaporation | HPLC with UV or fluorescence detection | 0.1 µg/m$^3$ | No data | Sangö and Zimerson 1980 |
| Occupational air | Air collected/derivatized through 9-(*N*-methylaminomethyl)anthracene impregnated XAD-2; desorption with *N,N*-dimethylformamide | HPLC with UV absorbance detection | 25 ng/m$^3$ for 20 L air | 81–91% | Andersson et al. 1983; Gudéhn 1984 |
| Occupational air | air collected in mini-impinger containing "nitro reagent" in toluene; evaporation | HPLC with UV detection | 1.5 ppb for 20 L air | No data | Dunlap et al. 1976 |
| Occupational and indoor air (HDI and oligomers) | Collection/derivatization on 1-(2-methoxy-phenyl)piperazine coated sorbent; desorption with methanol; concentration | HPLC with heart cutting with UV or electro-chemical detection | 60 ng/m$^3$ (UV); 1 ng/m$^3$ (electro-chemical) | ≤75–99% | Schmidtke and Seifert 1990 |
| Occupational air (HDI and oligomers) | Air collected/derivatized through tubes with sintered glass (or GFFs) impregnated with 1-(2-methoxyphenyl)piperazine; solvent elution; reaction with acetic anhydride; evaporation | HPLC with UV detection | 0.31 µg / sample; 3.4 µg/m$^3$ (lowest tested—GFFs) | 95–107% | Sesana et al. 1991; Huynh et al. 1992 |
| Air (HDI and oligomers) | Air collected/derivatized through 1-(2-pyridyl)-piperazine in toluene; evaporation; | HPLC with UV detection | 0.7 µg/m$^3$ for 10 L air | No data | Goldberg et al. 1981 |
| Air | Air collected/derivatized through sodium hydroxide in ethanol; neutralization; derivatization with heptafluorobutyric anhydride; solvent extraction | GC/thermionic specific detection | 10 pg/µL injected | ≈100% (simulated sample) | Skarping et al. 1988 |

## Table 6-2. Analytical Methods for Determining Hexamethylene Diisocyanate in Environmental Samples (continued)

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Air (total isocyanate) | Air collected/ derivatized through tryptamine in 2,2,4-trimethylpentane; evaporation; derivatization with acetic anhydride | HPLC with fluorescence and electrochemical detection | 1 ppb for 120 L air | 90±5% (simulated sample) | Wu et al. 1990 |
| Occupational air | Air exposed to polystyrene strip with adsorbed cholinesterase; assayed in cuvette with Ellman's reagent and acetylthiocholine | UV absorbance (412 nm) | ≈20 ppb (1-min exposure) | No data | Brown et al. 1984 |
| Air (total isocyanates) | None—direct air monitor | Proprietary paper tape chemistry | ≈5 ppb (lowest reported); 50 ppb max. | No data | Dharmarajan and Rando 1980 |

ECD = electron capture detector; GC = gas chromatography; GFF = glass fiber filter(s); HPLC = high performance liquid chromatography; MS = mass spectrometry; NIOSH = National Institute of Occupational Safety and Health; nitro reagent = *N*-4-nitrobenzyl-*N*-*n*-propylamine; UV = ultraviolet

by UV absorbance measurement (Brown et al. 1984). The disadvantage is that it does not differentiate between isocyanate species, but it is reasonably sensitive. Also available is a dedicated instrument, the Chemcasette® 7005, which uses proprietary paper tape chemistry and was reviewed for total isocyanates (Dharmarajan and Rando 1980). The only direct measurement technique for HDI using chromatography involved collection by impinger containing isooctane, then analysis by GC using the nitrogen sensitive thermionic specific detector (Skarping et al. 1985).

Methods for analysis of HDI in other media were not found. However, since HDI hydrolyzes rapidly in water, it is unlikely that significant amounts of HDI monomer would be found in water, soils, sediment, or food, except near sources of release.

## 6.3 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 hexamethylene diisocyanate is available. Where adequate information is not available, ATSDR, in conjunction with the 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 hexamethylene diisocyanate.

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.

### 6.3.1 Identification of Data Needs

**Methods for Determining Biomarkers of Exposure and Effect.** There is currently only one biomarker hexamethylene diamine, HDA, which is used for the monitoring exposure of individuals to HDI. This biomarker is convenient as it is readily excreted in urine and it can be detected at low concentration levels as indicated in Table 6-1. Additional recovery data are needed for HDA in order to

improve exposure estimates, especially since the half-life of HDA in urine is difficult to determine for persons suspected of being exposed to HDI. No other biomarker has been cited as an alternative.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** Currently there is a fairly broad selection of analytical methods for the analysis of HDI in air. Since it degrades very quickly upon contact in water and soil, and degrades more slowly by reaction with hydroxyl radicals and water vapor in air, it is important for analyses to be focused on air as a medium. It is probably most important at this point to improve the speciation (chromatographic separations) of isocyanate forms including prepolymers which may provide toxic effects to individuals. This will allow more accurate measurement and control of hazards in the workplace and general environment. Better data on recovery would be useful for some air sampling techniques as air sampling is of greatest importance for exposure assessment. It is also important that more information is determined on chemical and physical properties for the biuret trimer form since little is currently available in the literature.

### 6.3.2 Ongoing Studies

No ongoing studies related to methods for detection of HDI were found.