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 4,4′-methylenedianiline, its metabolites, and other biomarkers of exposure and effect to 4,4′-methylenedianiline. 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

Methods are available for the determination of 4,4′-methylenedianiline in blood and urine. Representative methods are summarized in Table 6-l. Most methods involve an extraction step, purification and fractionation procedures, and analysis, usually by gas chromatography (GC) or high performance liquid chromatography (HPLC).

The determination of 4,4′-methylenedianiline in blood, serum, or urine requires special precautions because the compound and some of its metabolites are heat labile (Cocker et al. 1988; Shintani 1992). During sample treatment, care should be taken to assure that no loss of 4,4′-methylenedianiline or its metabolites has occurred during evaporative steps used for the concentration in organic solutions and during hydrolysis of urine (Cocker et al. 1988; Shintani 1992).

Determination of 4,4′-methylenedianiline in blood/serum requires pretreatment steps involving deproteinization with a suitable reagent (e.g., perchloric acid), centrifugation for separation of 4,4′-methylenedianiline from the precipitate, and concentration (Shintani 1992). Determination of 4,4′-methylenedianiline in urine often uses solvent extraction and multiple clean-up steps (Avery 1989). An improved method uses solid phase extraction columns (octadecyl bonded silica) for the

Table 6-1. Analytical Methods for Determining 4,4′-Methylenedianiline in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Alkaline hydrolysis; solvent extraction; clean-up by acid-base extraction; re-extraction of basic sample in n-heptane-isoamyl alcohol; evaporation to dryness.	GC/ECD	10 ng/mL	72	Tortoreto et al. 1983
Serum	Solid-phase extraction (reverse phase C_{18}, phenyl, cyclohexyl columns)	HPLC/EICD	3 ng/mL	100	Shintani 1991, 1992
Serum and urine	Solid-phase extraction (C_{18}-bonded silica column); derivatization with PFPA.	Capillary GC/MS-NICI	0.1 ng/mL (water)	105	Avery 1989
Urine (MDA and N-acetyl and N-glucuronide metabolites)	Hydrolysis (10 M NaOH) at 80°C for 90 minutes; extraction of cooled sample with diethyl ether; concentration and derivatization with PFBA.	Capillary GC/MS	5 ng/mL	>80	Cocker et al. 1988
Urine (total: free, acetylated and conjugated)	Hydrolysis; extraction on C_{18} solid phase columns; concentration.	HPLC/EICD; confirmation by GC/MS	2.5 ng/mL	68	Peterson et al. 1991
Urine (total: free, acetylated and conjugated)	Acid hydrolysis; extraction with toluene; derivatization with PFPA.	Capillary GC/MS-SIM	2 ng/mL	96	Tiljander et al. 1990
Urine (total: MDA, N-acetyl-MDA, N,N′-diacetyl-MDA)	Acid hydrolysis; extraction with toluene; derivatization with PFPA.	HPLC/UV (285 and 258 nm)	8 ng/mL	97	Tiljander and Skarping 1990
Urine (MDA and conjugates)	Alkaline hydrolysis; extraction with toluene; optional cleanup; derivatization with PFPA.	Micro LC/UV (258 nm)	0.4 ng/mL	89	Brunmark et al. 1992
Azo-, azoxy- and nitroso metabolites of MDA in microsomal incubation media	Protein precipitation followed by solid-phase column extraction; evaporation to dryness.	HPLC/PSMS; off-line HPLC tandem FAB/MS	No data	No data	Kajbaf et al. 1992

EICD = electrochemical detector; FAB = fast atom bombardment; GC = gas chromatography; ECD = electron capture detector; Hb = hemoglobin; HFBC = heptafluorobutyryl chloride; HPLC = high performance liquid chromatography; LC = liquid chromatography; MDA = 4,4′-methylenedianiline; MS = mass spectrometry; NaOH = sodium hydroxide; NICI = negative ion chemical ionization; PFPA = pentafluoropropionic anhydride; PSMS = plasma spray mass spectrometry; SIM = selected ion monitoring; UV = ultraviolet detection

separation of 4,4′-methylenedianiline from interfering components in blood and hydrolyzed urine (Peterson et al. 1991; Shintani 1992).

Due to the polar nature of 4,4′-methylenedianiline, derivatization of the amine groups is required to produce a species which can be analyzed by GC without peak tailing. This derivatization may not be necessary if HPLC is used (Peterson et al. 1991). The common derivatizing agents are trifluoroacetic anhydride, pentafluoropropionic anhydride (PFPA), and heptafluorobutyric anhydride (HFBA).

The commonly used HPLC techniques include ultraviolet absorbance detection (UV) (Brumnark et al. 1992; Tiljander et al. 1990; Tiljander and Skarping 1990) and electrochemical detection (Peterson et al. 1991; Shintani 1992). Generally, the detection limit of 4,4′-methylenedianiline with electrochemical detection is in the range of 2-3 ng/mL (Peterson et al. 1991; Shintani 1992). This method is approximately two orders of magnitude better than the HPLC/UV method, which has a detection limit of 150 ng/mL (Shintani 1992). However, with special techniques, such as precolumn separation of derivatized 4,4′-methylenedianiline and microliquid chromatography, it is possible to lower the detection limits with UV in the range 0.4 ng/mL-8.0 ng/mL (Brunmark et al. 1992; Tiljander and Skarping 1990; Tiljander et al. 1990). Interferences from biological materials may co-elute with 4,4′-methylenedianiline, so confirmation is recommended. A second wavelength has been used with UV detection (Tiljander and Skarping 1990) and GC/mass spectrometry (Peterson et al. 1991).

GC may be used with electron capture detection (ECD) (Tortoreto et al. 1983), thermionic specific detection (Skarping et al. 1983), and mass spectrometry (MS) (Avery 1989; Cocker et al. 1988). Detection limits in the range of l-10 ng/mL are attainable by GC/MS (Avery 1989; Cocker et al. 1986a, 1988). For urine and serum, a detection limit of ≤1 ng/mL was obtained with solid phase extraction, derivatization, and GC/negative-ion chemical ionization MS (Avery 1989). The detection limit for GC/ECD is l-10 ng/mL (Skarping et al. 1983; Tortoreto et al. 1983). Although the detection limit with the thermionic specific detectors is about 10 times higher than electron capture detection (Skarping et al. 1983), both thermionic specific detector and MS detectors are more selective for 4,4′-methylenedianiline determination (Cocker et al. 1986a; Skarping et al. 1983).

Besides the parent 4,4′-methylenedianiline, N-acetyl-, N,N’-diacetyl-, and the N-glucuronide of 4,4′-methylenedianiline may also be present in urine as metabolites (Cocker et al. 1986a, 1988;

Peterson et al. 1991). N-acetyl-4,4′-methylenedianiline has been identified in human urine (Cocker 1986a). Three metabolites have been identified in rabbit liver microsomal incubations: azodiphenylmethane (azo), azoxydiphenylmethane (azoxy), and 4-nitroso-4'-aminodiphenylmethane (nitroso) compounds (Kajbaf et al. 1992). The formation of metabolites is shown schematically in Figure 2-3. Methods that can be used for the determination of metabolites are included in Table 6-l.

It is possible to determine the free amine (4,4′-methylenedianiline) and the total 4,4′-methylenedianiline (free, acetylated, and conjugated) in urine. The treatment of urine with strong base or strong acid hydrolyzes acetylated and conjugated 4,4′-methylenedianiline to the free 4,4′-methylenedianiline (Cocker et al. 1988; Tiljander and Skarping 1990). Therefore, the determination of free and total 4,4′-methylenedianiline is possible by determining the amount of 4,4′-methylenedianiline in unhydrolyzed and hydrolyzed urine (Peterson et al. 1991). Methods for the determination of free and total 4,4′-methylenedianiline are included in Table 6-l.

Biological monitoring for 4,4′-methylenedianiline and its metabolites is used to assess exposure to 4,4′-methylenedianiline. Available methods are summarized in Table 6-2. The methods are similar to the methods described in Table 6-1, except that very sensitive and specific MS techniques are used for analysis. In addition, the monitoring strategy must take into account the route of exposure (Cocker et al. 1994). The corresponding hemoglobin adducts have also been measured. Adducts are analyzed by GC/MS after hydrolysis, extraction and derivatization of hemoglobin precipitated from lysated red blood cells (Bailey et al. 1990; Schutz et al. 1995). Very low detection limits have been reported; however, little other performance information is available (Bailey et al. 1990; Schutz et al. 1995).

6.2 ENVIRONMENTAL SAMPLES

Analytical methods available for the determination of 4,4′-methylenedianiline in environmental samples are listed in Table 6-3. As in the case of biological samples, 4,4′-methylenedianiline in environmental samples has been determined by HPLC/UV (Emes and Hanshumaker 1983; Gunderson and Anderson 1988; Mazzu and Smith 1984), HPLC/electrochemical detection (Concialini et al. 1983), and GC/thermionic detection (Audunsson and Mathiasson 1983). Although analysis of 4,4′-methylenedianiline by HPLC does not require derivatization, analysis of environmental samples is frequently performed after derivatization to reduce amine reactivity on HPLC column packing

Table 6.2 Analytical Methods for Determining 4,4'-Methylenedianiline in Biological Materials

Sample matrix	Sample preparation	Analytical method	Sample detection limit	Accuracy % recovery	Reference
Plasma and urine (MDA)	Alkaline hydrolysis; extraction with toluene; PFPA derivatization.	Capillary GC/MS-NICI	0.2 nmol/L	97 (urine) 96 (plasma)	Brumark et al. 1995
Urine (AcMDA)	Hydrolysis at pH 13, 80°C; solvent extraction; HFBA derivitization.	Capillary GC/ MS	No data	No data	Cocker et al. 1986a
Blood plasma and urine (MDA and AcMDA)	Alkaline hydrolysis; PFPA derivitization	Capillary GC/MS-NICI	0.2 nmol/L	97% (urine) 96% (plasma)	Brunmark et al. 1995
Blood and urine (MDA and conjugates)	Acid hydrolysis; solvent extraction; PFPA derivatization	capillary GC/MS-NICI	3 nmol/L	Not reported	Dalene et al. 1995
Serum and urine (MDA)	Acid hydrolysis; solvent extraction; PFPA derivatization	capillary GC/MS-NICI-SIM	0.05 µg/L	Not reported	Skarping et al. 1995
Blood (hemoglobin-MDA and -AcMDA adducts)	Precipitation of hemoglobin from lysated red blood; alkaline hydrolysis; extraction with ethyl acetate; PFPA derivatization.	Capillary GC/MS-SIM	<10 pmol/g Hb	>80	Bailey et al. 1990
Blood (hemoglobin-MDA and -AcMDA adducts)	Centrifugation; precipitation of hemoglobin from lysated red blood cells; SPE or solvent extraction; HFBA derivatization	capillary GC/MS-NICI-SIM	<20 fmol/sample (MDA); 100 fmol/sample (AcMDA)	Not reported	Schutz et al. 1995
Plasma and urine (MDA and isomers 2,4'-MDA and 2,2'-MDA and methylated MDA	Acid hydrolysis; solvent extraction; PFPA derivatization	capillary GC/MS-NICI	<10 ng/L (instrumental)	Not reported	Skarping and Dalene 1995

AcMDA = N-acetyl-MDA; GC = Gas Chromatography; Hb = hemoglobin; HFBC = heptafluorobutyryl chloride; HPLC = high performance liquid chromatography; MDA = 4,4'-methylenedianiline; MS = mass spectrometry; NICI = negative ion chemical ionization; PFPA = pentafluoropropionic anhydride; SIM = selected ion monitoring; UV = ultraviolet (detection)

Table 6-3. Analytical Methods for Determining 4,4′-Methylenedianiline in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air	Collection on acid-coated glass-fiber filter; extraction with 0.26 N sodium hydroxide/5% acetonitrile; derivatization with acetic anhydride.	HPLC/UV	<1.0 µg/m^3	>90	Gunderson and Anderson 1988
Occupational air	Collection in impingers containing 0.05 M sulfuric acid; addition of sodium hydroxide pellets to make basic; extraction with toluene.	GC/TSD	2 µg/m^3	>80	Audunsson and Mathiasson 1983
Occupational air (NIOSH Method 5029)	Collection on acid-coated glass-fiber filter; ultrasonic extraction with 0.1 N methanolic potassium hydroxide.	HPLC/UV and EICD	0.2 µg/m^3 for 100 L sample	>80	NIOSH 1994
Solution of neat compound	Acidification; derivatization to diazo-compound with N-(1-naphthyl)-ethylenediamine.	Spectrophotometric at 555 nm	No data	95–105	Norowitz and Keliher 1986
Polyurethane extract (autoclaved and soaked in water)	Extraction with diethyl ether; derivatization with triethylamine and benzoyl chloride; concentration.	HPLC/UV	<70 ng/g in polyurethane and <3.5 µg/L for extract	>60	Mazzu and Smith 1984
Aqueous extract of polyurethane film	Extraction with diethyl ether; derivatization with triethylamine and benzoyl chloride; concentration.	HPLC/UV (280 and 254 nm)	0.05 µg/L for extract	>89	Ernes and Hanshumaker 1983
Water	Extraction of sample spiked with tetradeuterated analogue from C_{18} cartridge; derivatization with PFPA.	Capillary GC/NICI-MS and EI/MS	2 ng/L	No data	Benfenati et al. 1992

EI/MS = electron impact mass spectrometry; GC = gas chromatography; EICD = electrochemical detector; HPLC = high performance liquid chromatography; MS = mass spectrometry; NICI = negative ion chemical ionization; PFPA = pentafluoropropionic anhydride; TSD = thermionic specific detection; UV = ultraviolet detection

materials (Emes and Hanshumaker 1983; Gunderson and Anderson 1988; Mazzu and Smith 1984). Because of lower matrix interference environmental samples (air or water), the detection limits can be an order of magnitude lower than those achieved for biological samples (Avery 1989). The available analytical methods are capable of detecting 4,4′-methylenedianiline in air at concentrations lower than the ACGIH threshold limit value of 0.08 mg/m^3 (10 ppb) (for time-weighted average for 8 hours) and a short-term exposure limit of 0.8 mg/m^3 (100 ppb) (Audunsson and Mathiasson 1983; Boeniger 1984a; Gunderson and Anderson 1988).

Special precautions should be taken during sampling of workplace air for 4,4′-methylenedianiline. The compound can be present in workplace air, both in the vapor and particle phase. Therefore, air sampling methods must be able to collect 4,4′-methylenedianiline present in both phases. Glass fiber filter/silica gel sampling tubes (Boeniger 1984a) or acid-impregnated glass fiber filters (Gunderson and Anderson 1988) are capable of collecting 4,4′-methylenedianiline in both phases. In certain workplace environments, such as polyurethane industries that produce methylenediphenyl diisocyanate (MDI) or polymeric MDI, the air may contain both 4,4′-methylenedianiline and MDI. Sampling methods that use impingers containing acids (Audunsson and Mathiasson 1983) or acid-coated glass fiber filters (Gunderson and Anderson 1988) would hydrolyze MD1 to 4,4′-methylenedianiline (Audunsson and Mathiasson 1983; Gunderson and Anderson 1988). Therefore, if a distinction is to be made between 4,4′-methylenedianiline and MDI, either MD1 or 4,4′-methylenedianiline should be determined in the sample alone and could be subtracted from the total (following acid hydrolysis) for the determination of individual MD1 and 4,4′-methylenedianiline concentrations. Methods for such determinations are available (Audunsson and Math&son 1983; Boeniger 1984a).

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 4,4′-methylenedianiline 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 4,4′-methylenedianiline.

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. The total amount of 4,4′-methylenedianiline (free and conjugated) in urine is used as an indicator of 4,4′-methylenedianiline exposure in workers (Bnmmark et al. 1995; Cocker et al. 1994). Total MDA in plasma is also an appropriate method for biological monitoring of 4,4′-methylenedianiline. Methods for the determination of total 4,4′-methylenedianiline in human urine and blood by alkaline hydrolysis of urine are available and shown in Table 6-1. However, no quantitative correlation has been established between level of workplace exposure and urinary level of free and/or conjugated 4,4′-methylenedianiline. Adducts of 4,4′-methylenedianiline with hemoglobin can also be used as an indicator of exposure (Bailey et al. 1990; Schutz et al. 1995). These methods are suitable for the monitoring of occupational exposure. It would be helpful to assess whether the existing analytical methods are capable of detecting the compound in biological samples at levels at which biological effects might occur.

There is no specific biomarker of effect that can be attributed to 4,4′-methylenedianiline exposure. Exposure to the compound is usually associated with occurrence of jaundice, bile duct inflammation, suppression of bile excretion, and clinical hepatitis (NIOSH 1986). However, none of these effects is specific to 4,4′-methylenedianiline exposure. It would be useful to establish that a certain effect could be specifically attributed to 4,4′-methylenedianiline exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Few methods are available for analysis of 4,4′-methylenedianiline in environmental media. Methods are available for determination in occupational air (Audunsson and Mathiasson 1983; Boeniger 1984a; Gunderson and Anderson 1988). Analytical methods of sufficient sensitivity, precision, and accuracy are available for the determination of 4,4′-methylenedianiline in occupational air at levels at least an order of magnitude lower than the ACGIH level of 0.8 mg/m^3

(Hoeniger 1984a; Gunderson and Anderson 1988). However, the compound has not been measured in ambient air. Methods are also available for the determination of extractable 4,4′-methylenedianiline in polyurethanes (Emes and Hanshumaker 1983; Mazzu and Smith 1984). Standardized analytical methods for the determination of 4,4′-methylenedianiline in water, soil, and other environmental media are not available. Therefore, it would be most useful to develop standardized analytical methods of sufficient sensitivity, precision, and accuracy for its determination, at least, in contaminated environmental media. Most important, analytical methods should be developed to determine the concentrations of 4,4′-methylenedianiline in soil and groundwater at Superfund waste disposal sites.

Experimental data on the environmental fate of 4,4′-methylenedianiline are very scarce. Consequently, the environmental degradation products of the compound have not been identified. Therefore, before the evaluation of this data need, it would be more important to conduct more studies to assess the environmental fate of 4,4′-methylenedianiline.

6.3.2 Ongoing Studies

No ongoing studies involving methods for determination of 4,4′-methylenedianiline were located.