# 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 MBOCA, its metabolites, and other biomarkers of exposure and effect to MBOCA. 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 enviromental 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 MATERIALS

Very few biological materials have been analyzed for the presence of MBOCA or its metabolites. MBOCA and its metabolites have been measured in urine of exposed humans and experimental animals. Hemoglobin adducts have also been measured in the blood of exposed animals. The most frequently used techniques are gas chromatography (GC) with electron capture detection (ECD) and high-performance liquid chromatography (HPLC) with electrochemical detection (ED). Detailed methodologies from selected studies are presented in Table 6-1.

Only a small amount of absorbed MBOCA is excreted in urine as MBOCA in animals, and probably in humans as well (see Section 2.3). The methods used to detect MBOCA in urine are *somewhat* limited since they commonly measure unmetabolized MBOCA--not its metabolic by-products. Some methods have been developed to directly measure major MBOCA metabolites in urine. Other analytical methods pretreat urine samples with acid, base and/or heat to release MBOCA from its various conjugates. Another approach is to analyze the longer-lived complexes, such as MBOCAhemoglobin adducts. The specific methods used are noted in the following text and in Table 6-1.

Hemoglobin adducts of MBOCA and its metabolites have been detected in animals dosed with the chemical (Chen et al. 1991; Sabbioni and neumann 1990). The methods used were HPLC/ED, gas chromatography/mass spectrometry (GC/MS), and GC/ECD. Sample preparation for all three methods required hemoglobin to be isolated from the blood of the test animals and hydrolyzed to release the bound MBOCA. Insufficient data were provided to compare the different methods, but MBOCA was detected and quantified in the blood of dosed rats by all three methods. GC/MS in the negative chemical ionization mode, with a detection limit of 2 pg, appeared to be the most sensitive of the methods tested (Sabbioni and neumann 1990).

Both GC and HPLC have been used to separate MBOCA and its metabolites from urine. Most recently, HPLC has become the method of choice to selectively detect MBOCA and its metabolites in urine. The most sensitive and specific detection methods for HPLC are ED (Ichikawa et al. 1990; NIOSH 1986b; Okayama et al. 1988; Trippel-Schulte et al. 1986; Vantulder et al. 1981) and photoconductivity detection (PCD) (Ducos et al. 1985). Of these, ED has been the most frequently used detection method. Ultraviolet detection (UV) has also been paired with HPLC (McKerrell et al. 1987; Angerer and Schaller 1985; Trippel-Schulte et al. 1986) but is less sensitive and less selective than either ED or PCD (Trippel-Schulte et al. 1986).

## TABLE 6-1. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Biological Materials

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Blood (hemoglobin adducts) | Centrifuge sample to extract red blood cells; lyse with EDTA; centrifuge to remove debris and precipitate hemoglobin with ethanol; centrifuge and retain precipitate; hydrolyze with NaOH and SDS; extract with ether; evaporate; reconstitute in methanol | HPLC/ED | 25 pg (MBOCA) | 84 (MBOCA) | Sabbioni and Neumann 1990 |
| | | GC/MS (EI, SIM) | 50 pg (Ac-MBOCA) | 80 (Ac-MBOCA) | |
| | | GC/MS (NCI) | 30 pg | NR | |
| | | | 2 pg | NR | |
| Blood (hemoglobin adducts) | Centrifuge sample to extract red blood cells; lyse with EDTA; precipitate hemoglobin with acetone/HCl; centrifuge and retain precipitate; wash with acetone/HCl, acetone, and ether; reconstitute in SDS and add HCl to hydrolyze; extract with hexane; evaporate; derivatize with heptafluorobutyric anhydride in isooctane; terminate reaction with $NH_4OH$ | GC/ECD | NR | NR | Chen et al. 1991 |

## TABLE 6-1. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Biological Materials *(continued)*

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine | Acidify sample with citric acid; adjust to pH 9.5 with NaOH; extract with diethyl ether; wash with sodium bicarbonate; dry with anhydrous sodium sulfate; evaporate and redissolve in acetone; evaporate; fractionate using TLC; remove region of silica gel containing MBOCA and extract with acetone; evaporate; derivatize with TFA; evaporate and reconstitute in triphenylamine in carbon disulfide | GC/FID | 1 μg/L | 70–78 | Van Roosmalen et al. 1979, 1981 (IARC Method 8) |
| Urine | Add internal standard to sample; add 1 mL of 1 M NaOH; extract with diethyl ether; evaporate; derivatize with HFBC | GC/ECD | 2.7 μg/L | 85–90 | Gristwood et al. 1984 |
| Urine | Extract with diethyl ether; derivatize with HFBC | GC/ECD | 2.4 g/g creatinine | NR | Thomas and Wilson 1984 |

## TABLE 6-1. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Biological Materials *(continued)*

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine | Hydrolyze sample with NaOH; extract with methanol and diethyl ether/hexane; concentrate; add TEA, HFBA, and MDA; extract derivative with hexane in $KH_2PO_4$ buffer; centrifuge; cleanup organic phase on Florisil® if needed | HRGC/ECD | 1 μg/L | 79–89 | NIOSH 1984 |
| Urine (*N*-acetyl-MBOCA) | Add internal standard to sample; hydrolyze with NaOH; extract with diethyl ether; evaporate; derivatize with PFPA; evaporate and redissolve in toluene | GC/MS (EI) | 6.7 μg/L | NR | Cocker et al. 1988 |

**TABLE 6-1. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Biological Materials *(continued)***

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine | Hydrolyze sample with acid and heat; adjust pH to >9 with NaOH; extract sequentially with diethyl ether and HCl; adjust pH with NaOH; extract with diethyl ether; dry with $Na_2SO_4$; evaporate and redissolve in acetonitrile/water; elute from reverse-phase column with acetonitrile/water | HPLC/UV | 3 μg/L | 101 | Angerer and Schaller 1985 |
| Urine | Hydrolyze sample with NaOH and heat; extract with hexane; centrifuge; evaporate; reconstitute with ammonium acetate/acetonitrile; elute from reverse-phase column with ammonium acetate/acetonitrile | HPLC/UV | 10 μg/L | 94–98 | McKerrell et al. 1987 |

**TABLE 6-1. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Biological Materials *(continued)***

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine | Precipitate proteins from sample with TCA; centrifuge; extract with ether; concentrate using HPLC precolumn and elute from analytical column with ammonium acetate/acetonitrile mixture | HPLC/UV<br>HPLC/ED | 51.5 ng<br>12.1 ng | 79–80<br>42–44 | Trippel-Schulte et al. 1986 |
| Urine | Stabilize sample with citric acid; elute from HPLC column with ammonium phosphate-buffered acetonitrile | HPLC/ED | 5 μg/L | 97–111 | NIOSH 1986b |
| Urine (human) | Stabilize sample with citric acid; add ethanol and sodium bicarbonate; extract with diethyl ether; concentrate organic layer; dilute with acetonitrile/sodium acetate; elute from reverse-phase column with acetonitrile/sodium acetate | HPLC/ED | 1–10 μg/L | NR | Vantulder et al. 1981 |

**TABLE 6-1. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Biological Materials *(continued)***

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Urine | Mix sample with methanol/heptane sulfonate/acetic acid; centrifuge; cleanup supernatant on ODS® column; elute from HPLC column with methanol/water | HPLC/ED | 1 μg/L | 97–100 | Okayama et al. 1988 |
| Urine | Saturate citric acid-preserved sample with sodium bicarbonate; cleanup on Extrelut cartridge; extract with methylene chloride; evaporate; redissolve in methylene chloride; elute from HPLC column with isooctane (or hexane)-isopropanol-methanol | HPLC/PCD | <1 μg/L | 85–86 | Ducos et al. 1985 |

Ac-MBOCA = *N*-acetyl-4,4'-methylenebis-(2-chloroaniline); ECD = electron capture detection; ED = electrochemical detection; EDTA = ethylene diamine tetraacetic acid; EI = electron impact; FID = flame ionization detection; GC = gas chromatography; HCl = hydrochloric acid; HFBA = hepta-fluorobutyric anhydride; HFBC = heptafluorobutyryl chloride; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; $KH_2PO_4$ = monopotassium phosphate; M = molar; MDA = 4,4'-methylenediamine; MS = mass spectrometry; NaOH = sodium hydroxide; $Na_2SO_4$ = sodium sulfate; NCI = negative chemical ionization; $NH_4OH$ = ammonium hydroxide; NR = not reported; PCD = photoconductivity detection; PFPA = pentafluoropropionic anhydride; SDS = sodium dodecyl sulfate; SIM = selected ion monitoring; TCA = trichloroacetic acid; TEA = triethylamine; TFA = trifluoroacetic anhydride; TLC = thin layer chromatography; UV = ultraviolet detection

Although not the preferred method, GC may still be used to measure MBOCA and its metabolites in urine. Analysis of a heptafluorobutyryl derivative of MBOCA using GC/ECD has been frequently used (Gristwood et al. 1984; NIOSH 1984; Thomas and Wilson 1984), but GC/flame ionization detection (FID) has also been used to analyze a trifluoroacetyl derivative (Van Roosmalen et al. 1979, 1981). The sensitivity of GC with either ECD or FID is in the low-ppb (μg/L) range, but the electron capture detector is more selective and gave higher recovery of analyte. GC and MS are frequently used to confirm the identity of isolated MBOCA compounds (Chen et al. 1991) and a method for quantifying the metabolite n-acetyl-MBOCA in urine using GCMS has been developed (Cocker et al. 1988). The sensitivity of this method is also in the low-ppb range.

## 6.2 ENVIRONMENTAL SAMPLES

As with biological materials, the two primary methods used to separate MBOCA from other compounds in Environmental samples are HPLC and GC. Most of the analytical methods found describe detection and measurement of MBOCA in air, the medium of primary interest for monitoring potential worker exposures in facilities manufacturing or using the chemical. Table 6-2 presents details on selected analytical methods for determining MBOCA in Environmental samples. Air samples have been collected either in impingers containing a sorbent liquid (Ebell et al. 1980; nieminen et al. 1983; Skarping et al. 1985) or on solid sorbent cartridges (Ichikawa et al. 1990; James et al. 1985; Sawicki et al. 1975; Yasuda 1975). Air samples have also been collected on glass fiber filters designed to trap and stabilize MBOCA in both particulate and vapor form (NIOSH 1985, 1986b; Purnell and Warwick 1981; Rappaport and Morales 1979). MBOCA is desorbed from the collection medium with an organic solvent. For GC analysis, the extract is usually reacted with a perfluoro fatty acid anhydride to make the corresponding MBOCA derivative that is then analyzed. The most commonly used detectors are FID, ECD, and thermionic nitrogen-specific detection (TSD, but TSD and ECD are preferred because of their increased sensitivity (sub- to low-ppb [pg/m-] range) and selectivity compared to FID (low-ppb range) (Ichikawa et al. 1990; Sawicki et al. 1975; Skarping et al. 1985; Yasuda 1975). Sample preparation for HPLC generally requires only desorption from the collection medium prior to separation on the HPLC column. Two detection methods, UV and ED, have been paired with HPLC for the analysis of air samples containing MBOCA (James et al. 1985; nieminen et al. 1983; NIOSH 1986b; Purnell and Warwick 1980, 1981; Rappaport and Morales 1979). Of these, ED is clearly the most sensitive (ppt [rig/m”] compared to sub to low ppb for UV) and selective detection method, and HPLC/ED is the method recommended by NIOSH for the detection of low levels of MBOCA in air (NIOSH 1986b; Purnell and Warwick 1980, 1981). Both the HPLC and GC methods gave high recoveries and precision making them reliable methods for determination of MBOCA in air samples. HPLC/UV and GC/FID can be used to determine MBOCA in incinerator effluents indicating that even highly contaminated air samples can be analyzed with these methods (James et al. 1985). While no specific information was located on the analysis of similar materials using the other methods, it is likely that they too can be used for highly complex samples.

HPLC/ED can be used to measure MBOCA in surface water, groundwater, soil, and sludge and is the IARC-recommended method for these media (Rice and Kissinger 1981, 1982). Liquid samples require only filtering prior to concentration on a reverse-phase HPLC column and separation on an analytical column. Solid samples must be mixed with water and filtered prior to the concentration step. Sensitivity is in the low-ppt range and precision is excellent. no recovery data were reported. An alternative EPA-recommended method of determining MBOCA in waste water requires more extensive sample handling prior to analysis by GC/TSD (EPA 1981a). This method has a sensitivity

## TABLE 6-2. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Environmental Samples

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Air | Collect sample on Gas-chrom® S cartridge; desorb with acetone | GC/FID | 2 $\mu g/m^3$ | 92 | Sawicki et al. 1975; Yasuda 1975 |
| Air | Collect sample on silica gel; desorb with methanol; evaporate; derivatize with HFBA | GC/ECD | 0.1 $\mu g/m^3$ | NR | Ichikawa et a. 1990 |
| Air | Collect sample in impinger containing HCl/acetic acid/water solution; add NaOH; extract with chloroform; derivatize with TFAA; evaporate; redissolve in toluene | GC/ECD; GC/TSD | 40 $\mu g/m^3$ | NR | Ebell et al. 1980 |
| Air | Collect sample in impinger containing alkaline ethanol; add phosphoric acid; evaporate; add phosphate buffer and toluene to extract; derivatize with PFPA; analyze toluene layer | HRGC/TSD | 1 $ng/m^3$ | 100 | Skarping et al. 1985 |

## TABLE 6-2. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Environmental Samples *(continued)*

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Air | Collect sample on glass fiber filter/silica gel cartridge; extract with methanol; centrifuge; elute from reverse-phase column with acetonitrile/water | HPLC/UV | ≈3 $\mu g/m^3$ | 86–97 | Rappaport and Morales 1979 |
| Air | Collect sample on impinger containing KOH/ethanol; add HCl; filter out KCl precipitate; evaporate; redissolve in ethanol/water; elute from HPLC column with acetate-buffered tetrahydrofuran/acetonitrile/water | HPLC/UV | 1–5 $\mu g/m^3$ | NR | Nieminen et al. 1983 |
| Air | Collect sample on a glass fiber filter; desorb with methanol; elute from reverse-phase column with phosphate-buffered methanol | HPLC/UV<br>HPLC/ED | 7.5 $\mu g/m^3$<br>0.1 $\mu g/m^3$ | 86–98<br>86–98 | Purnell and Warwick 1980, 1981 |

## TABLE 6-2. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Environmental Samples *(continued)*

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Air | Collect sample on glass fiber filter; desorb with KOH/methanol; elute from HPLC column with acetonitrile/water | HPLC/UV | 0.015 μg/filter | NR | NIOSH 1986b |
| | | HPLC/ED | ≤0.05 μg/filter | NR | |
| Waste water | Extract sample with methylene chloride; dry over anyhdrous $NaSO_4$; concentrate; reconstitute with hexane | GC/TSD | 1 μg/L | 44–93 | EPA 1981a |
| Surface water, groundwater, soil | Mix solid samples with water; filter water or water from soil sample; concentrate on reverse-phase HPLC sampling column; elute to analytical column with methanol/ammonium acetate | HPLC/ED | 25 ng/L | NR | Rice and Kissinger 1981, 1982 (IARC Method 4) |

## TABLE 6-2. Analytical Methods for Determining 4,4'-Methylenebis(2-chloroaniline) (MBOCA) in Environmental Samples *(continued)*

| Sample matrix | Preparation method | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Laboratory wastes | Add ascorbic acid to waste solution; adjust pH to 8 with NaOH; centrifuge; add methanol to supernatant and centrifuge; elute from HPLC column with ammonium acetate/methanol | HPLC/UV/ED | 5 ng (UV); 4.6 ng (ED) | NR | Barek et al. 1985 |
| Surface wipe | Collect samples using Whatman filter tabs; desorb in 0.1N KOH in methanol; elute from HPLC column with acetonitrile/water | HPLC/ED | 0.008 μg/wipe | NR | NIOSH 1986b |

ECD = electron capture detection; ED = electrochemical detection; FID = flame ionization detection; GC = gas chromatography; HCl = hydrochloric acid; HFBA = heptafluorobutyric anhydride; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; KCl = potassium chloride; KOH = potassium hydroxide; N = normal; NaOH = sodium hydroxide; $NaSO_4$ = sodium sulfate; NR = not reported; PFPA = pentafluoropropionic anhydride; TFAA = trifluoroacetic anhydride; TSD = thermionic nitrogen-specific detection; UV = ultraviolet detection

in the low-ppb range, but analyte recovery varies widely. Both methods are selective for MBOCA. MBOCA has been detected in laboratory wastes using HPLC/UV/ED (Barek et al. 1985). While ED was found to be more sensitive than UV for many chemicals in the wastes, it was comparable for detection of MBOCA. Either detector could be used to monitor the destruction of aromatic amines in laboratory wastes. no details on recovery or precision were provided.

Surface wipe and hand monitoring samples taken from areas in a factory in which MBOCA was used were analyzed for the chemical using HPLCKJV and HPLC/ED (NIOSH 1986b). Because of its increased sensitivity, HPLC/ED was used for all samples containing low levels (≤2 μg/mL) of MBOCA and HPLC/UV was used to analyze those with higher levels. Recovery and precision were both high using these methods.

## 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 MBOCA 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 MBOCA.

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 measurement of MBOCA in urine has been used to monitor populations exposed occupationally to the chemical. The most frequently used methods are HPLC/ED and GC/ECD (Gristwood et al. 1984; Ichikawa et al. 1990; NIOSH 1984, 1986b; Okayama et al. 1988; Thomas and Wilson 1984; Trippel-Schulte et al. 1986; Vantulder et al. 1981). These methods are sensitive and have produced reliable results in several monitoring studies to estimate exposure of workers who have contact with MBOCA during manufacturing operations.

There are no known adverse health effects associated with exposure to MBOCA other than its weak association with bladder cancer. While hemoglobin adduct formation has been demonstrated in exposed animals (Chen et al. 1991; Sabbioni and neumann 1990), the relevance of these adducts with bladder cancer or with other adverse health effects is not known. The methods that have been tested for measuring hemoglobin adducts include HPLC/ED, GC/ECD, and GCMS (Chen et al. 1991; Sabbioni and neumann 1990). The limited information on these methods suggests that they are sensitive and reliable. However, since there is no clear relationship between the presence of these adducts in blood and adverse health consequences, no additional methods development is recommended at this time.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** numerous methods have been developed for the measurement of MBOCA in air (Ebell et

al. 1980; Ichikawa et al. 1990; nieminen et al. 1983; NIOSH 1986b; Purnell and Warwick 1980, 1981; Rappaport and Morales 1979; Sawicki et al. 1975). The most sensitive and reliable of these are GC with TSD or ECD and HPLC with ED (Ebell et al. 1980; Ichikawa et al. 1990; NIOSH 1986b; Purnell and Warwick 1980, 1981). These methods are useful for the measurement of low concentrations (ppt) of MBOCA in air, and no additional methods development is needed. HPLC/ED and GC/TSD can be used to determine the presence of MBOCA in water and soil (EPA 1981a; Rice and Kissinger 1981, 1982), surface wipe and hand monitoring samples (NIOSH 1986b), and laboratory waste (Barek et al. 1985). While these methods are sensitive for measuring MBOCA in ENVIRONMENT media, they are of unknown reliability. Some additional data on the accuracy and precision of measurements in liquid and solid media would be useful.

### 6.3.2 On-going Studies

No on-going studies focused on analytical method development for MBOCA were located. However, several of the on-going studies on MBOCA (FEDRIP 1991) involve the development of methods to better identify MBOCA adducts, which should make the process of monitoring exposed individuals easier. NIOSH is the sponsoring institution of three studies involving MBOCA that are currently in progress. Two of the three studies involve the development of more sensitive methods to monitor and evaluate the effects following MBOCA exposure. F.B. Daniel from NIOSH is the principal investigator of the study on "Biomonitoring for Populations Occupations Exposed to Aromatic Amines." The investigator will analyze hemoglobin and DNA adduct formation by MBOCA in order to propose a methodology for monitoring workplace exposure. For the second project, L.L. Lowry from NIOSH is the principal investigator of the study entitled "Biological Monitoring Methods Research and Evaluation." This study involves the development and evaluation of biological monitoring methods in urine to determine their effectiveness in predicting worker exposure to MBOCA.

The NTP has a study in progress on "Aryl Amine Adducts in Blood as Indicators of Exposure" (NTP 1991a). In this study, blood samples from 100 workers will be analyzed for hemoglobin *o*-toluidine adducts. MBOCA will be used to develop an HPLC method for separation and isolation of mitochondrial or total aryl amine-DNA adducts. In addition, the *in vitro* activation of potential carcinogens will be studied, and a mathematical model for MBOCA distribution, metabolism, and adduct formation will be prepared. The overall objective of the project is to develop a more sensitive adduct isolation procedure to be used for biological monitoring. The contact person for this project is K. Cheever (NTP 1991a).