# 7. ANALYTICAL METHODS

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

Methods are available for measuring levels of DCBs in blood, urine, tissue, and breath. Representative methods are summarized in Table 7-1. Methods include sample collection, preparation, cleanup, and determination. Sample preparation techniques are usually required to separate the compound of interest from the complex biological sample medium. Gas purge and solvent extraction are used most frequently to separate DCBs from blood, urine, and tissues. The breath matrix is relatively simple and does not require preparation steps; however, special techniques such as use of a spirometer are required to provide pure air for inhalation and a mechanism for collection of exhaled air. Gas chromatography (GC) is used most frequently to detect DCBs in biological materials. Detectors used to identify DCBs in biological materials include the electron capture detector (ECD) (Bristol et al. 1982; Jan 1983), the photoionization detector (PID) (Langhorst and Nestrick 1979), and mass spectrometry (MS) (Ashley et al. 1992; Michael et al. 1980). ECD and PID provide some selectivity, but confirmation using a different GC column or detector is often recommended. MS provides identification as well as quantitation of analytes.

Separation of DCBs from biological samples may be accomplished by extraction with hexane (Bristol et al. 1982; Jan 1983), or carbon tetrachloride (Langhorst and Nestrick 1979), or by purging with an inert gas and trapping on a sorbent material. Solvent extraction permits concentration, thereby increasing sensitivity, but the extraction solvents can interfere with the analysis, and evaporative losses can result in low recovery. Gas purge techniques may be static (headspace) or dynamic (purge-and-trap). The static

## Table 7-1. Analytical Methods for Determining Dichlorobenzenes in Biological Materials

| Sample matrix (analyte) | Sample preparation | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Blood (1,3-DCB) | Headspace purge; thermal desorption | cap. GC/MS | 3 ng/mL | 86.3 | IARC Method 25; Pellizzari et al. 1985 |
| Blood (model compounds) | Headspace purge; thermal desorption | cap. GC/MS | Low-ppb | 86–120 (model compounds) | Michael et al. 1980 |
| Blood (1,2-DCB) | Solvent extraction; silica gel column clean-up | GC/PID | 3.6 ppb | 85 | Langhorst and Nestrick 1979 |
| Blood (1,3-DCB) | Solvent extraction; silica gel column clean-up | GC/PID | 2.8 ppb | 82 | Langhorst and Nestrick 1979 |
| Blood (1,4-DCB) | Solvent extraction; silica gel column clean-up | GC/PID | 3.0 ppb | 89 | Langhorst and Nestrick 1979 |
| Blood (1,2-DCB) | Solvent extraction | GC/ECD | 1.4 ppb | 76.6 | Bristol et al. 1982 |
| Blood (1,3-DCB) | Solvent extraction | GC/ECD | 1.3 ppb | 74.5 | Bristol et al. 1982 |
| Blood (1,4-DCB) | Solvent extraction | GC/ECD | 2 ppb | 81.6 | Bristol et al. 1982 |
| Blood (1,2-DCB) | Purge and trap | cap. GC/MS | 0.05 ppb | 77–122 | Ashley et al. 1992 |
| Blood (1,3-DCB) | Purge and trap | cap. GC/MS | 0.04 ppb | 130–162 | Ashley et al. 1992 |
| Blood (1,4-DCB) | Purge and trap | cap. GC/MS | 0.04 ppb | 93–98 | Ashley et al. 1992 |
| Blood, urine (unspecified DCBs) | Purge-and-trap, thermal desorption cap | GC/MS | No data | No data | Barkley et al. 1980 |
| Urine (1,2-DCB) | Solvent extraction; silica gen column clean-up | GC/PID | 0.90 ppb | 83 | Langhorst and Nestrick 1979 |
| Urine (1,3-DCB) | Solvent extraction; silica gen column clean-up | GC/PID | 0.70 ppb | 78 | Langhorst and Nestrick 1979 |
| Urine (1,4-DCB) | Solvent extraction; silica gen column clean-up | GC/PID | 0.75 ppb | 81 | Langhorst and Nestrick 1979 |
| Urine (model compounds) | Headspace purge; thermal desorption | cap. GC/MS | Low-ppb | 48–110 (model compounds) | Michael et al. 1980 |

## Table 7-1. Analytical Methods for Determining Dichlorobenzenes in Biological Materials

| Sample matrix (analyte) | Sample preparation | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Adipose tissue (model compounds) | Maceration; headspace purge; thermal desorption | cap. GC/MS | Low-ppb | 13–80 (model compounds) | Michael et al. 1980 |
| Human milk (chloro-benzene) | Headspace purge; thermal desorption | GC/MS | 0.6 | 62.9 | Erickson et al. 1980 |
| Human milk (unspecified DCBs) | Solvent extraction; cleanup with sulfuric acid, Florisil | GC/ECD | No data | >80 | Jan 1983 |
| Adipose tissue (unspecified DCBs) | Solvent extraction; cleanup with sulfuric acid, Florisil | GC/ECD | No data | >80 | Jan 1983 |
| Tissue (1,3-DCB) | Maceration; headspace purge; thermal desorption | cap. GC/MS | 6 ng/g | 56.5 | IARC Method 25; Pellizzari et al. 1985 |
| Breath (unspecified DCBs) | Collection using a spirometer; adsorption on Tenax traps; thermal desorption cap | GC/MS | No data | No data | Barkley et al. 1980 |
| Breath (1,4-DCB) | Collection into canisters using spirometer; cryofocussing; thermal desorption | cap. GC/MS-SIM | low-μg/$m^3$ | 49–80 | Thomas et al. 1991 |

cap. = capillary; ECD = electron capture device; GC = gas chromatography; MS = mass spectrometry; PID = photo-ionization detector; SIM = selected ion monitoring

headspace technique is relatively simple, but may be less sensitive than the purge-and-trap method. The purge-and-trap method, while providing increased sensitivity, requires more complex instrumentation and may result in artifact formation (Seto 1994).

Although a variety of methods are available for determination of DCBs in blood, few are well characterized and validated. A method has been developed which utilizes headspace purge followed by thermal desorption of the trapped, purged analytes. DCBs are then determined by capillary GC/MS (Michael et al. 1980; Pellizzari et al. 1985). Recovery is very good (>85%) and detection limits are in the low-ppb range for model compounds (Michael et al. 1980; Pellizzari et al. 1985). A sensitive and reliable method for identification and quantitation of DCBs in samples of whole blood has been developed by Ashley and coworkers at the Centers for Disease Control and Prevention (CDC) (Ashley et al. 1992). The method involves purge-and-trap of a 10 mL blood sample with analysis by capillary GC/high resolution MS. Anti-foam procedures are utilized as well as special efforts to remove background levels of volatile organic compounds (VOCs) from reagents and equipment. The method is sensitive enough (ppt levels) to determine background levels of VOCs in the population. Percent recoveries were 77–122% for 1,2-DCB, 130–162% for 1,3-DCB, and 93–98% for 1,4-DCB.

Methods are available for monitoring DCBs in urine and tissues, particularly adipose tissue and mother's milk. Solvent extraction, silica gel column clean-up, and GC/ECD or GC/PID analysis has been used for urine (Langhorst and Nestrick 1979), mother's milk (Jan 1983), and adipose tissue (Jan 1983). Recovery is good (>80% recovery) and detection limits are in the low-ppb range (Jan 1983; Langhorst and Nestrick 1979). Headspace purge followed by capillary GC/MS analysis has been utilized for urine (Michael et al. 1980), mother's milk (Erickson et al. 1980), and tissue (Pellizzari et al. 1985). Recovery, where reported, is adequate (>60%) (Erickson et al. 1980), and detection limits are in the low-ppb range (Erickson et al. 1980).

Breath samples are usually collected through a spirometer onto a sorbent cartridge (Barkley et al. 1980) or into passivated canisters (Thomas et al. 1991). Analytes are concentrated cryogenically from a portion of the canister contents or after thermal desorption from the sorbent, then analyzed by GC/MS. Recovery of 1,4-DCB using Tenax cartridges was 86–101% and the detection limit was about 1 μg/$m^3$. The method is sufficiently sensitive and reliable for monitoring exposure to DCBs. Recovery for collection of 1,4-DCB in canisters was 49–80% and the detection limits were in the low-μg/$m^3$ range (Thomas et al. 1991). The spirometer system utilizing canisters is compact, and may be useful as a field screening method (Thomas et al. 1991).

## 7.2 ENVIRONMENTAL SAMPLES

Methods are available for determining DCBs in a variety of environmental matrices. A summary of representative methods is shown in Table 7-2. Validated methods, approved by agencies and organizations such as EPA, ASTM, APHA, and NIOSH, are available for air, water, and solid waste matrices. These methods for analysis of drinking water, waste water, and soil/sediment samples are included in Table 7-2. Many of the methods published by APHA and ASTM for water are equivalent to the EPA methods.

GC is the most widely used analytical technique for quantifying concentrations of DCBs in environmental matrices. Various detection devices used for GC include the flame ionization detector (FID), ECD, Hall electroconductivity detector (HECD), and PID. Confirmation using a second column is usually recommended. MS provides identification as well quantitation for GC analysis. Because of the complexity of the sample matrix and the usually low concentrations of VOCs in environmental media, sample concentration is generally required prior to GC analysis. Methods suitable for determining trace amounts of DCBs in aqueous and other environmental media include three basic approaches to the pretreatment of the sample: gas purge-and-trap technique, headspace-gas extraction, and extraction with solvent. Care must be taken during sample collection and processing to avoid evaporative losses. Contamination is another potential analytical problem and monitoring is required. 1,4-DCB is a relatively common chemical compound and can contaminate reagents and glassware.

Charcoal adsorbent is used for collection of DCBs in occupational air. The compounds are desorbed with carbon disulfide and analyzed by GC/FID. The method is sufficiently sensitive and reliable for determining occupational exposure to DCBs (NIOSH 1994).

Ambient air samples are collected on adsorbents such as Tenax (Wallace 1987), or multisorbent (Heavner et al. 1992; Oliver et al. 1996), or in passivated canisters (EPA 1988a). Tenax traps are thermally desorbed, concentrated cryogenically, and analyzed by capillary GC/MS (Wallace et al. 1987). Recovery is good (81–110%), precision for side-by-side samples is acceptable (9–45% RSD), and the detection limit is ≈1 $\mu g/m^3$ (Wallace 1987). Multisorbent traps may be solvent desorbed and analyzed by capillary GC/MS. Recovery and precision are good and detection limits as low as 0.019 ppb have been reported (Oliver et al. 1996). Collection of air samples in passivated stainless steel canisters is also widely utilized

## Table 7-2. Analytical Methods for Determining Dichlorobenzenes in Environmental Samples

| Sample matrix (analyte) | Sample preparation | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Occupational air (1,2-DCB) | Collection on charcoal tubes; desorption with $CS_2$ | GC/FID | 0.01 mg/ sample[a] | ±13.7 | Method 1003 NIOSH 1994 |
| Occupational air (1,4-DCB) | Collection on charcoal tubes; desorption with $CS_2$ | GC/FID | 0.01 mg/ sample[a] | ±12.5 | Method 1003 NIOSH 1994 |
| Ambient air (VOCs including DCBs) | Collection in canisters; cryofocussing; thermal desorption | cap. GC with FID, ECD or MS | No data | No data | Method TO-14 EPA 1988a |
| Air-emission sources (selected compounds) | MM5 sampling train (condensate, filter, adsorbent); condensate, impinger and rinses, solvent extraction, evaporation; XAD-2 adsorbent and filters, Soxhlet extraction, concentration | cap. GC/MS | No data | -13 to -16 | Method 0010 EPA 1994f |
| Air-emission sources (volatile organics) | VOST sampling train (sorbent traps); thermal desorption | GC/MS | No data | No data | Method 0030 EPA 1994h |
| Drinking water (1,2- and 1,3-DCB) | Purge and trap | GC/HECD; conf. on second col. or GC/MS | <0.01 µg/L for most VOCs | 95 | Method 502.1 EPA 1991a |
| Drinking water (1,4-DCB) | Purge and trap | GC/HECD; conf. on second col. or GC/MS | <0.01 µg/L for most VOCs | 90 | Method 502.1 EPA 1991a |
| Drinking water (1,2-DCB) | Purge and trap | GC/PID-HECD; conf. by GC/MS | 0.03–0.05 µg/L (PID); 0.02–0.04 µg/L (HECD) | 97–102 (PID); 98–100 (HECD) | Method 502.2 EPA 1991b |
| Drinking water (1,3-DCB) | Purge and trap | GC/PID-HECD; conf. by GC/MS | 0.02 µg/L (PID); 0.02–0.07 µg/L (HECD) | 97–104 (PID); 97–106 (HECD) | Method 502.2 EPA 1991b |
| Drinking water (1,4-DCB) | Purge and trap | GC/PID-HECD; conf. by GC/MS | 0.01–0.03 µg/L (PID); 0.01–0.04 µg/L (HECD) | 97–103 (PID); 97–98 (HECD) | Method 502.2 EPA 1991b |
| Drinking water (1,2-DCB) | Purge and trap | GC/PID; conf. on second col. or GC/MS | 0.02 µg/L | 75–85 | Method 503.1 EPA 1991c |

## Table 7-2. Analytical Methods for Determining Dichlorobenzenes in Environmental Samples

| Sample matrix (analyte) | Sample preparation | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Drinking water (1,3-DCB) | Purge and trap | GC/PID; conf. on second col. or GC/MS | 0.006 µg/L | 91 | Method 503.1 EPA 1991c |
| Drinking water (1,4-DCB) | Purge and trap | GC/PID; conf. on second col. or GC/MS | 0.006 µg/L | 91–107 | Method 503.1 EPA 1991c |
| Drinking water | Purge and trap | cap. GC/MS | 0.03–0.05 µg/L | 93–97 | Method 524.2 EPA 1992a |
| Drinking water | Purge and trap | cap. GC/MS | 0.05–0.12 µg/L | 87–100 | Method 524.2 EPA 1992a |
| Drinking water | Purge and trap | cap. GC/MS | 0.03–0.04 µg/L | 93–103 | Method 524.2 EPA 1992a |
| Waste water | Purge and trap | GC/HECD; conf. on second col. or GC/MS | 0.15 µg/L | ND–208 | Method 601 EPA 2002c |
| Waste water | Purge and trap | GC/HECD; conf. on second col. or GC/MS | 0.32 µg/L | 7–187 | Method 601 EPA 2002c |
| Waste water | Purge and trap | GC/HECD; conf. on second col. or GC/MS | 0.24 µg/L | 42–143 | Method 601 EPA 1984c; EPA 2002c |
| Waste water | Purge and trap | GC/PID; conf. on second col. or GC/MS | 0.4 µg/L | 37–154 | Method 602 EPA 2002d |
| Waste water | Purge and trap | GC/PID; conf. on second col. or GC/MS | 0.3 µg/L | 50–141 | Method 602 EPA 2002d |
| Waste water | Purge and trap | GC/PID; conf. on second col. or GC/MS | 0.3 µg/L | 42–143 | Method 602 EPA 1984f; EPA 2002d |
| Waste water | Solvent extraction; optional Florisil column clean-up | GC/ECD | 1.14 µg/L | 9–160 | Method 612 EPA 2002b |
| Waste water | Solvent extraction; optional Florisil column clean-up | GC/ECD | 1.19 µg/L | DL–150 | Method 612 EPA 2002b |
| Waste water | Solvent extraction; optional Florisil column clean-up | GC/ECD | 1.34 µg/L | 13–137 | Method 612 EPA 1984c; EPA 2002b |
| Waste water (1,2- and 1,4-DCB) | Purge and trap | GC/MS | No data | 18–190 | Method 624 EPA 1984d; EPA 2002a |
| Waste water (1,3-DCB) | Purge and trap | GC/MS | No data | 59–156 | Method 624 EPA 1984d; EPA 2002a |
| Waste water | Purge and trap | cap. GC/MS | 0.031 µg/L | 106 | Method 6200B APHA 1998 |

## Table 7-2. Analytical Methods for Determining Dichlorobenzenes in Environmental Samples

| Sample matrix (analyte) | Sample preparation | Analytical method | Sample detection limit | Percent recovery | Reference |
|---|---|---|---|---|---|
| Waste water | Purge and trap | cap. GC/MS | 0.045 µg/L | 108 | Method 6200B APHA 1998 |
| Waste water | Purge and trap | cap. GC/MS | 0.033 µg/L | 106 | Method 6200B APHA 1998 |
| Waste water/ Drinking water (1,2-DCB) | Purge and trap | cap GC/HECD, PID | 0.023 µg/L (HECD); 0.031 µg/L (PID) | 93 (HECD); 67 (PID) | Method 6200 APHA 1998 |
| Waste water/ Drinking water (1,3-DCB) | Purge and trap | cap GC/HECD, PID | 0.017 µg/L (HECD); 0.028 µg/L (PID) | 95 (HECD); 70 (PID) | Method 6200 APHA 1998 |
| Waste water/ Drinking water (1,4-DCB) | Purge and trap | cap GC/HECD, PID | 0.059 µg/L (HECD); 0.061 µg/L (PID) | 91 (HECD); 70 (PID) | Method 6200 APHA 1998 |
| Drinking water (VOCs) | Purge and trap | GC | low µg/L | 99 | Method D 3871 ASTM 1994 |
| Solid waste (VOCs) | Closed system purge and trap and extraction | GC/ECD, FID, MS | Not reported | Not reported | Method 5035 EPA 1996c |
| Solid waste (1,2-DCB) | Purge and trap, direct injection, headspace, or vacuum distillation | GC/HECD, PID | 0.02 µg/L (HECD); 0.05 (PID) | 100 (HECD); 102 (PID) | Method 8021B EPA 1996d |
| Solid waste (1,3-DCB) | Purge and trap, direct injection, headspace, or vacuum distillation | GC/HECD, PID | 0.02 µg/L (HECD); 0.02 (PID) | 106 (HECD); 104 (PID) | Method 8021B EPA 1996d |
| Solid waste (1,4-DCB) | Purge and trap, direct injection, headspace, or vacuum distillation | GC/HECD, PID | 0.01 µg/L (HECD); 0.07 (PID) | 98 (HECD); 103 (PID) | Method 8021B EPA 1996d |
| Solid waste (1,2-DCB) | Solvent extraction | Single or dual cap. GC/ECD | 270 ng/L | 102 | Method 8121 EPA 1994l |
| Solid waste (1,3-DCB) | Solvent extraction | Single or dual cap. GC/ECD | 250 ng/L | 103 | Method 8121 EPA 1994l |
| Solid waste (1,4-DCB) | Solvent extraction | Single or dual cap. GC/ECD | 890 ng/L | 104 | Method 8121 EPA 1994l |

[a]Estimated limit of detection

cap. = capillary; conf. = confirmation; col. = column; DL = detection limit; ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HECD = Hall electrolytic conductivity detector; MS = mass spectrometry; ND = not detected; PID = photoionization detector; VOC = volatile organic compound

(EPA 1988a), but performance data are unavailable. Passive sampling devices are also widely used, due in part to their ease of use and small size (Lewis et al. 1985).

For water, soil, or sediment samples, DCBs are purged from the sample with an inert gas such as helium or nitrogen, and then passed through the sorbent (EPA 1984a, 1984b, 1991a, 1991b, 1991c, 1992a, 1994a, 1994f). The analytes are thermally desorbed and analyzed by GC/HECD, GC/PID, GC/ECD, or GC/MS techniques. Detection limits for waste waters and solid wastes are in the low-ppb range, which is probably well below levels of health concern. Detection limits for drinking water samples are generally in the ppt range (0.006–0.05 μg/L) (EPA 1991a, 1991b, 1991c, 1992a).

Several physical parameters may interfere with analytical accuracy. High sampling flow rates and high temperature and humidity may cause decreased adsorption of DCB vapor on the solid sorbent (APHA 1995a). Interference by other VOCs with similar retention times may be resolved by using different GC column materials and temperatures or be using MS techniques.

The use of capillary columns rather than packed column GC has improved resolution and sensitivity and shortened the analysis time (Washall and Wampler 1988). However, more stringent sample clean-up procedures are required for capillary column GC (Oliver and Nicol 1982b). The development of methods using whole column cryotrapping (Pankow and Rosen 1988; Pankow et al. 1988) and cryogenic refocusing (Washall and Wampler 1988) provide even greater sensitivity and resolution for GC analysis.

## 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 dichlorobenzenes 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 dichlorobenzenes.

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.*** Exposure to DCBs may be evaluated by measuring the levels of these compounds in blood, breath, milk, and adipose tissue, and by measuring the level of 2,5-dichlorophenol, a metabolite of 1,4-DCB, or the levels of 2,3-dichlorophenol, 3,4-dichlorophenol, 3,4-dichlorocatechol, and 4,5-dichlorocatechol, metabolites of 1,2-DCB, in urine (Bristol et al. 1982; Erickson et al. 1980; Jan 1983; Kumagai and Matsunaga 1995, 1997; Langhorst and Nestrick 1979; Mage et al. 2004; Pellizzari et al. 1985). Sensitive analytical methods are available for measurements in blood. Development of methods with improved specificity and sensitivity for other tissues and breath would be valuable in identifying individuals with low-level exposure. Development of standardized procedures would permit comparison of data and facilitate the study of correlations between exposure and measured levels biological samples. Interlaboratory studies are also needed to provide better performance data for methods currently in use.

***Effect.*** There are no known health effects such as elevated liver enzymes that are uniquely associated with exposure to DCBs. Therefore, the identification of specific health effects and the development of analytical methods to determine biomarkers of effect for DCBs would be useful.

**Methods for Determining Parent Compounds and Degradation Products in Environmental Media.** Air is the environmental medium of most concern for human exposure to DCBs. Exposure from drinking water may also be of concern in some areas, such as near hazardous waste sites. Existing analytical methods can measure DCBs in these and other environmental media at background levels (EPA 1988a, 1984a, 1984b, 1991a, 1991b, 1991c, 1992a, 1994a, 1994f; NIOSH 1994). The accuracy and precision of the methods for water and wastes are well documented and MS provides adequate specificity. Performance data for measurements in ambient and indoor air would be helpful. Development of techniques to improve the accuracy and ease of sample preparation and transfer for these methods would also be helpful.

### 7.3.2 Ongoing Studies

No ongoing studies involving analytical techniques for DCBs were found in a search of the Federal Research in Progress database (FEDRIP 2005).