7. ANALYTICAL METHODS

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

Since the majority of the analytical data on dichloropropenes are for the 1,3- isomer, the focus of this chapter is on methods that measure for 1,3-dichloropropene. Environmental analytical methods for 1,1- and 1,2-dichloropropene have been located; however, most of these are adequately described in the context of measuring for 1,3-dichloropropene. Analytical methods for measuring 2,3- and 3,3-dichloropropene in biological or environmental media were not located in the available literature.

7.1 BIOLOGICAL MATERIALS

The primary method for determining human exposure to 1,3-dichloropropene is measurement of the mercapturic acid metabolites N-acetyl-S-(cis-3-chloropropenyl-2)-L-cysteine (or cis-DCP-MA) and N-acetyl-S-(trans-3-chloropropenyl-2)-L-cysteine (or trans-DCP-MA) in the urine (Osterloh et al. 1984, 1989a, 1989b). Van Welie et al. (1989) describes a procedure whereby these metabolites are extracted from urine samples and analyzed using gas chromatography (GC) followed by sulfur-selective detection with a flame-photometric detector (FPD). During this study, the urine samples were collected from applicators before, during, and up to 24 hours after finishing soil fumigation with 1,3-dichloropropene. These samples were stored in the dark at 4 °C until they were transported (within 2 days); thereafter, they were stored at -18 °C. Methods for the analysis of these metabolites in human blood have not been located in the available literature.

Kastl and Hermann (1983) developed an analytical procedure for determining the level of cis- and trans-1,3-dichloropropene in whole rat blood. Blood is extracted, 200 µL n-hexane is added, and the sample is vortexed and centrifuged at 800 g for 1 minute. Samples are either directly injected onto a GC column for GC/mass spectrometry (MS) analysis or diluted with hexane for GC/electron capture detection (ECD) analysis. Percent recoveries of the GC analysis range from 80.8 to 98.5 for the cis isomer and from 81.3 to 98.2 for trans-1,3-dichloropropene. For GC/MS analysis, percent recoveries are between 83.1 and 94.9 for cis- and 88.7 and 98.8 for trans-1,3-dichloropropene. The GC/ECD method is sensitive to cis and trans isomeric concentrations in rat blood of 5.88–1.17×10^4 and 5.35–1.07×10^4 ng/mL, respectively. The GC/MS method is sensitive to cis- and trans-1,3-dichloropropene concentrations in rat blood of 5.18×10^1–1.29×10^4 and 4.71×10^1–1.18×10^4 ng/mL, respectively.

Fisher and Kilgore (1989) extracted the glutathione conjugate of 1,3-dichloropropene from the blood of rats. After collection, the blood was frozen and stored at -20 °C until analysis. Solutions of 1 mL whole blood and 2 mL 10 mM HCl in an acetone dry-ice slurry were repeatedly frozen and thawed and then finally centrifuged. The supernatant (1 mL) was deproteinated using 0.33 mL of 70% perchloric acid and then centrifuged again. The resulting clear supernatant was either injected into the high performance liquid chromatography (HPLC) or stored at -20 °C. Schneider et al. (1998a) described a method for analyzing 1,3-dichloropropene epoxides in mouse liver. Livers were homogenized in 2 mL 100 mM sodium phosphate buffer. Ethyl acetate containing 2 µg of internal standard was added followed by homogenization and centrifugation. After removal of the organic layer, the pellet was extracted using ethyl acetate without the internal standard and analyzed using GC/MS. Recoveries for cis/trans-1,3-dichloropropene and cis/trans-1,3-dichloropropene epoxides were 81–95%. Bond et al. (1985) described a method for analyzing 2,3-dichloropropene in the urine, feces, and tissues (including blood) of rats. Tissue samples from rats exposed to C-14 labeled 2,3-dichloropropene were homogenized in ice-cold distilled water and added to acetonitrile. Following centrifugation and extraction, the supernantant was diluted in water to give a final concentration of 50% water and 50% acetonitrile. Analysis was performed using a liquid scintillation spectrometer. Recovery in spiked samples was >95%.

Table 7-1 summarizes the methods used to detect 1,3-dichloropropene in biological materials, including a procedure for detecting 1,3-dichloropropene in foods (Daft 1989).

Analytical methods for measuring 1,1-, 1,2-, 2,3-, or 3,3-dichloropropene in biological media were not located in the literature.

Table 7-1. Analytical Methods for Determining cis- and trans-1,3-Dichloropropene and Metabolites in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Addition of internal standard, extraction using ethyl acetate, dissolve in methanol, methylation using ethereal diazomethane, redissolve in ethyl acetate	GC/FPD	107 ng/mL (trans-DCP-MA); 115 ng/mL (cis-DCP-MA)	69 (trans-DCP-MA); 70 (cis-DCP-MA)	van Welie et al. 1989
Rat blood	Extract with hexane vortex and centrifuge	GC/MS	5.18 ng/mL (cis); 4.71 ng/day (trans)	83.1–94.9 (cis); 88.7–98.8 (trans)	Kastl and Hermann 1983
Rat blood	Extract with hexane vortex and centrifuge	GC/ECD	5.88 ng/day (cis); 5.35 ng/mL (trans)	80.8–98.5 (cis); 81.3–98.2 (trans)	Kastl and Hermann 1983
Food	Extract composited, table-ready foods with isooctane or acetone-aqueous phosphoric acid-isooctane mixture	GC-ECD/HECD	No data	45–112	Daft 1989, 1990

ECD = electron capture detection; FPD = Flame-photometric detection; GC = gas chromatography; HECD = Hall electron capture detection; MS = mass spectrometry

7.2 ENVIRONMENTAL SAMPLES

Procedures for detecting cis- and trans-1,3-dichloropropene in water and soil samples at hazardous waste sites are outlined in the method for semivolatiles in the Contract Laboratory Program (CLP) Statement of Work for Organics Analysis (EPA 1999). The required quantification limits for both cis- and trans-1,3-dichloropropene are 10 ppb for water samples and 10 ppb for soil samples in this monitoring program.

For the most part, soil and sediment samples are analyzed in a similar manner to water samples, with the exception that a small amount of water is added to soil and sediment samples. At this point, all three matrices are subjected to a purge-and-trap cycle. An inert gas is bubbled through the sample, volatilizing 1,3-dichloropropene. The gas stream is then passed though an adsorbent tube, which recollects the 1,3-dichloropropene. The sorbent tube is attached to a GC, heated, and backflushed with an inert gas to desorb the halocarbons onto a GC column. Quantification can be accomplished using either a flame ionization detector or an MS, depending on the total concentration of organics in the sample.

EPA's Test Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater (EPA 1982) and Test Methods for Solid Waste (EPA 1986) are very similar to those already outlined. However, the purge-and-trap cycle may be bypassed for aqueous process wastes with expected concentrations in excess of 10,000 μg/L. In these instances, the sample may be directly injected into the GC system with a 10 μL syringe (EPA 1986). EPA-Office of Solid Waste Methods 8021B and 8260B can be applied to solid waste (EPA 1996a, 1996b). Method 8021B uses GC followed by a photoionization detector (PID) and a Hall electron capture detector (HECD) connected in series (EPA 1996a).

It is important to note the discrepancies in detection limits between the standardized methods. CLP cites a detection limit of 10 ppb, yet gives no information regarding the percent recoveries (EPA 1999). The EPA procedures for solid wastes (EPA Method 8010) and municipal and industrial waste waters (EPA Method 601), however, maintain a detection limit of 0.34 ppb. The percent recovery, according to the Solid Waste Manual, ranges from 22 to 178 (EPA 1986). Therefore, results from EPA Method 8010 must be interpreted with caution. For municipal and industrial waste waters, the average percent recoveries for the cis- and trans-isomers are reportedly 86.7 and 73.5 with standard deviations of 6.0 and 17.2%, respectively (EPA 1982). Again, the precision at which the trans-isomer can be measured is questionable.

Other standardized methods used for detection of 1,3-dichloropropene in water samples by purge and trap followed by GC/MS include EPA Methods 524.2, 624, and 1624, Standard Methods 6200B and 6200C, ASTM Method D5790, and USGS-NWQL Method O-4127-96 (EPA 1995a, 2001b, 2005b; NEMI 1997b, 2001; USGS 1998). Detection limits and percent recoveries for determination of both isomers in water range were 0.02–10 ppb and 78–110%, respectively, using these methods.

A few methods have appeared in the available literature. Leiber and Berk (1984) outlined a method for determining 1,3-dichloropropene in ambient air. Tenax-GC sampling tubes are used for sample collection. Solvent desorption is accomplished with isooctane containing 4.0 μg/L of 1,3,5-tribromo-benzene, followed by heat treatment at 90 °C for 15 minutes; the mixture is then left to stand for 12 hours. After centrifugation, an aliquot of the resulting solution is injected onto the GC column. Sample analysis by capillary GC/ECD was validated for the range of 0.4–4.0 ppm, with a mean percent recovery of 100. Table 7-2 summarizes the methods for detecting cis- and trans-1,3-dichloropropene in environmental media.

Several of the environmental methods mentioned above for measuring 1,3-dichloropropene (EPA-OSW method 8021B and 8260B, ASTM method D5790, Standard Methods 6200B and 6200C, and USGS-NWQL Method O-4127-96) also include 1,1-dichloropropene as an analyte (EPA 1996a, 1996b; NEMI 1997a, 1997b, 2001; USGS 1998). Table 7-3 provides information specific to the measurement of 1,1-di-chloropropene in environmental media using these methods. In addition, EPA-NERL method 502.2 can be used to measure 1,1-dichloropropene in water using GC followed by either photoionization detection or electrolytic conductivity detection (EPA 1995a).

EPA method 524.2 was the only method identified for measuring 1,2-dichloropropene in environmental media. This method uses purge and trap followed by GC/MS to analyze for the substance in water. The sample detection limit and percent recovery are 0.02 ppb and 98%, respectively (NEMI 1992). Analytical methods for measuring 2,3- and 3,3-dichloropropene in environmental media were not located in the available literature.

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 dichloropropenes is available. Where adequate information

Table 7-2. Analytical Methods for Determining 1,3-Dichloropropene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Adsorb (Tenax-GC); desorb (isooctane); inject aliquot	GC/ECD	0.4–4.0 ppm	100	Leiber and Berk 1984
Water	Purge and trap	GC/MS (EPA CLP Method)	10 ppb	No data	EPA 1999
Water	Purge and trap	GC/MS (EPA Method 8010)	0.34 ppb	22–178	EPA 1986
Wastewater	Purge and trap	GC/MS (EPA Method 601)	0.20 ppb 0.34 ppb	100 (cis) 100 (trans)	EPA 2001a
Soil	Add water, heat, purge and trap, thermal desorption	GC/MS (EPA CLP Method)	10 ppb	No data	EPA 1999
Solid waste	Purge and trap, direct injection, vacuum distillation	GC/PID and/or HECD (EPA-OSW Method 8021B)	No data	No data	EPA 1996a
Air, water, solid waste	Purge and trap (aqueous, solid, and waste oil), direct injection (waste oil), automatic static headspace (solid), closed system vacuum distillation (aqueous, solid, oil, and tissue), or desorption from trapping media (air)	GC/MS (EPA-OSW Method 8260B)	No data	No data	EPA 1996b
Water	Purge and trap	GC/MS (EPA Method 524.2)	0.02 ppb (cis) 0.048 ppb (trans)	100 (cis) 110 (trans)	EPA 1995a
Water	Purge and trap	GC/MS (EPA Method 624)	5 ppb (cis) Not available (trans)	100 (cis) 100 (trans)	EPA 2005b
Water	Purge and trap	GC/MS (EPA Method 1624)	Not available (cis) 10 ppb (trans)	Not available (cis) Not available (trans)	EPA 2001b
Water	Purge and trap	GC/MS (ASTM Method D5790)	0.21 ppb (cis) 0.2 ppb (trans)	93% (cis) 85% (trans)	NEMI 2001
Water	Purge and trap	GC/MS (Standard Methods 6200B)	0.04 ppb (cis) 0.05 ppb (trans)	99% (cis) 101% (trans)	NEMI 1997a

Table 7-2. Analytical Methods for Determining 1,3-Dichloropropene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Purge and trap	GC (Standard Method 6200C)	0.06 ppb (cis) 0.02 ppb (trans)	78% (cis) 78% (trans)	NEMI 1997b
Water	Purge and trap	GC/MS (USGS-NWQL Method O-4127-96)	0.048 ppb (cis) 0.072 ppb (trans)	93% (cis) 85% (trans)	USGS 1998

ECD = electron capture detection; FID = flame ionization detector; GC = gas chromatography; HECD = Hall electron capture detection; MS = mass spectrometry; PID = photoionization detector

Table 7-3. Analytical Methods for Determining 1,1-Dichloropropene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Purge and trap	GC/PID or ELCD (EPA-NERL Method 502.2)	0.02 ppb	103%	EPA 1995b
Solid waste	Purge and trap, direct injection, vacuum distillation	GC/PID and/or ELCD (EPA-OSW Method 8021B)	0.02 ppb	103%	EPA 1996a
Air, water, solid waste	Purge and trap (aqueous, solid, and waste oil), direct injection (waste oil), automatic static headspace (solid), closed system vacuum distillation (aqueous, solid, oil, and tissue), or desorption from trapping media (air)	GC/MS (EPA-OSW Method 8260B)	Not available	102%	EPA 1996b
Water	Purge and trap	GC/MS (ASTM Method D5790)	0.18 ppb	107%	NEMI 2001
Water	Purge and trap	GC/MS (Standard Method 6200B)	0.04 ppb	110%	NEMI 1997a
Water	Purge and trap	GC (Standard Method 6200C)	0.01 ppb	74%	NEMI 1997b
Water	Purge and trap	GC/MS (USGS-NWQL Method O-4127-96)	0.028 ppb	Not available	USGS 1998

ELCD = electrolytic conductivity detection; FID = flame ionization detector; GC = gas chromatography; MS = mass spectrometry; PID = photoionization detector

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 dichloropropene isomers.

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

Although the following discussion covers 1,1-, 1,2-, 1,3-, and 2,3-dichloropropene, testing to fill data gaps for 1,3-dichloropropene should take priority, since it is the only isomer currently in production at a significant volume.

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Van Welie et al. (1989) has described a method for determining the mercapturic acid metabolites N-acetyl-S-(cis-3-chloropropenyl-2)-L-cysteine (or cis-DCP-MA) and N-acetyl-S-(trans-3-chloropropenyl-2)-L-cysteine (or trans-DCP-MA) in the urine. Additional study and the development of standardized methods regarding the detection of dichloropropene metabolites in human biological materials (urine, blood, and tissue) are needed.

Effect. There are no known biomarkers of effect that are unique to dichloropropenes. Therefore, standardized analytical methods for their determination are not warranted.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining of 1,3-dichloropropene in environmental matrices have appeared in the literature. Of these, standardized methods exist only for the analysis of surface water, soil, or sediment samples (EPA 1982, 1986, 1999). For sediments and soils, the levels of accuracy have not been reported. Both the accuracy and precision at which the trans-isomer can be measured in water is questionable. Therefore, refinement of the current procedures and establishing standardized methods for analyzing other media such as air will aid in determining levels of human exposure to 1,3-dichloro-

propene. A limited number of methods are available for determining 1,1- and 1,2-dichloropropene in environmental media, while no methods were located for 2,3- or 3,3-dichloropropene. Development of standardized methods for determining levels of 1,1-, 1,2-, 2,3-, and 3,3-dichloropropene in environmental materials would be helpful.

A limited number of methods is available to determine 1,3-dichloropropene in biological materials (Daft 1989; Kastl and Hermann 1983), and none of the methods have been standardized. The establishment of standardized methods for determining of 1,3-dichloropropene in biological materials, together with methods that are unique to 1,3-dichloropropene exposure, would be helpful in determining the levels of and exposure to the general population. No methods for determining 1,1-, 1,2-, 2,3-, or 3,3-dichloropropene in biological materials have been located. Development of standardized methods for determining levels of these isomers in biological materials would be helpful.

7.3.2 Ongoing Studies

Ongoing studies related to analytical methods for dichloropropenes were not located in the Federal Research in Progress database (FEDRIP 2006).

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of dichloropropenes and other volatile organic compounds in blood. These methods use purge and trap methodology, high-resolution gas chromatography, and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.