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 HCCPD, its metabolites, and other biomarkers of exposure and effect to HCCPD. 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

Few methods are available for measuring HCCPD in biological materials. Representative examples of analytical methods for HCCPD in biological samples are summarized in Table 6- 1.

Determination of HCCPD involves two steps, sample preparation and sample analysis. Prior to analysis, HCCPD must be separated from the biological sample matrix and prepared for introduction into the analytical instrument. Separation is usually effected by extraction with an organic solvent or a mixture of solvents such as hexane, acetonitrile, toluene/acetonitrile, petroleum ether, or hexane/methylene chloride (DeLeon et al. 1980a; EPA 1984b, 1991a; Gill et al. 1996). Clean-up procedures such as fractionation using solid-phase extraction (SPE) materials may be required for some biological matrices (Gill et al. 1996).

Gas chromatography (GC) is the most common method for detecting and measuring HCCPD in biological materials (DeLeon et al. 1980a; EPA 1984b, 199 la). The chromatograph separates complex mixtures of organics and allows individual compounds to be identified and quantified by a detector. Because of its sensitivity, the electron capture detector (ECD) has often been used to identify HCCPD. Mass spectrometry (MS) can provide compound identification and has been used to provide confirmation of GC/ECD results. Modern GUMS techniques provide sensitivity comparable to GC/ECD (DeLeon et al.

Table 6-1. Analytical Methods for Determining HCCPD in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Extraction with toluene/acetonitrile	Capillary column GC/ECD; GC/MS	50 ng/mL	28.8–54.5	DeLeon et al. 1980a
Blood	Extraction with hexane/dichloromethane: SPE fractionation	Capillary column GC/MSD or ECD	low ppb	47–50	Gill et al. 1996
Urine	Acidify with hydrochloric acid; extraction with petroleum ether	Capillary column GC/ECD; GC/MS	10 ng/mL	35–51.8	DeLeon et al. 1980a

ECD = electron capture detector; GC = gas chromatography; MS = mass spectrometry; MSD = mass selective detector; SPE = solid-phase extraction

1980a; Gill et al. 1996). Current methods provide adequate recovery (~50%) and sensitivity (low ppb range) for the measurement of HCCPD in blood (DelLeon et al. 1980a; Gill et al. 1996).

Accurate analysis of HCCPD in biological samples is complicated by the tendency of the chemical to degrade during storage. It was reported that up to 3 1% of HCCPD in a urine sample containing 10 ppb of the chemical could be degraded when the sample was stored overnight in a cooler. Degradation may also occur when sample extracts are concentrated (EPA 1984b).

6.2 ENVIRONMENTAL SAMPLES

Methods are available for the determination of HCCPD in most important environmental matrices. Representative methods for quantifying HCCPD in each of these media are summarized in Table 6-2. Validated methods, approved by agencies and organizations such as EPA, the American Society for Testing and Materials (ASTM), APHA, and NIOSH, are available for air, water, and solid waste matrices. These methods for analysis of air, drinking water, waste water, and soil/sediment samples are included in Table 6-2. Many of the methods published by APHA (1995) for water are equivalent to the EPA methods.

Most environmental analyses have been performed using multiresidue methods involving solvent extraction of the analytes from the sample matrix, clean-up to remove interfering compounds, and determination by GC with ECD or MS.

HCCPD in workplace air or ambient air is sampled by pulling a volume of air through a sorbent trap. The adsorbent is extracted with solvent, then analyzed by GCYECD (Boyd et al. 1981; EPA 1988a; NIOSH 1994). Recovery, where reported is good (80-99.7%). Detection limits depend upon the volume of air sampled; detection limits in the low ppb range can be achieved (Boyd et al. 1981; EPA 1988a; NIOSH 1994).

HCCPD is usually extracted from water with organic solvents for analysis (Eichelberger et al. 1983; EPA 1982a, 1984a, 1989b, 1995c). Newer methods utilize adsorption onto cartridges or disks with subsequent solvent desorption (EPA 1995c). Analysis is by capillary column GC/ECD (EPA 1989b) or GUMS (Eichelberger et al. 1983; EPA 1995c). Where GC/ECD is used, confirmation using a second dissimilar column or GUMS is recommended (EPA 1995c). Recovery for these methods is good (≥70%) and precision, where reported, is also good (>15% RSD). Detection limits are in the low ppb to ppt range.

Table 6-2. Analytical Methods for Determining HCCPD in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Workplace air	Collection on sorbent tubes; extraction with hexane	GC/ECD	5 ppb[a]	99.7	NIOSH 1994
Workplace air	Collection on solid sorbent; extraction with hexane	GC/ECD	25 ng	>80	Boyd et al. 1981
Air	Collection on PUF; extraction with ether/hexane	GC/ECD	No data	No data	EPA 1988a
Drinking water	Extraction with hexane	Capillary column GC/ECD	0.13 µg/L	69–191	EPA 1989b (Method 505)
Drinking water	Extraction through LSE disk or cartridge; elution with solvent	Capillary column GC/MS	0.072–0.16 µg/L	84–86	EPA 1995c (Method 525.2)
Drinking water	Extraction with methylene chloride or hexane	GC/MS	50 ng/L	79–88	Benoit and Williams 1981
Drinking water	Solvent extraction	capillary column GC/ECD; confirmation using dissimilar column or GC/MS	0.016–0.018 µg/L	98–103	EPA 1995c (Method 551.1)
Water	Extraction with methylene chloride; exchange to hexane	GC/ECD	No data	No data	EPA 1982a (Method 612)
Water	Extraction with methylene chloride	GC/MS	No data	No data	APHA 1992 (Method 6410B)
Water	Extraction with methylene chloride	Capillary column GC/MS	1–10 µg/L	38	Eichelberger et al. 1983 (EPA Method 625, 625.1)
Water	Isotope dilution; acid/base extraction with methylene chloride	GC/MS	10 µg/L	No data	EPA 1984a (Method 1625)

Table 6-2. Analytical Methods for Determining HCCPD in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water; soil; wastes	Extraction with methylene chloride; exchange to hexane	Dual capillary column GC/ECD; GC/MS	240 ng/L[b]	30–32	EPA 1996d (Method 8121)
Water; soil; wastes	Automated Soxhlet extraction	Capillary column GC/MS	10 μg/L (ground water); 660 μg/L (soil; sediment)	19	EPA 1996d (Method 8270C)
Soils	Microwave-assisted extraction	Capillary column GC/MSD	No data	27	Lopez-Avila and Benedicto 1996
Food (fish, milk, butter, corn oil)	Extraction with acetonitrile; clean-up using Florisil	GC/ECD	No data	≥80	Yurawecz and Puma 1986

[a] Based on 5-L air sample. Estimated detection limit is 5 ng/sample.
[b] Method detection limit (MDL) in reagent water. Estimated quantitation limits for other matrices are 10 MDL in groundwater; 670–10,000 MDL in soil, and 100,000 MDL in nonaqueous wastes.

ECD = electron capture detection; FID = flame ionization detector; GC = gas chromatography; LSE = liquid-solid extraction; MS = mass spectrometry; MSD = mass selective detector; PUF = polyurethane foam

Soxhlet extraction is used most commonly to extract HCCPD from solid matrices such as soils, sediments and solid wastes (EPA 1996d, 1996e). The extracts are analyzed by capillary column GUECD (EPA 1996d) or GUMS (EPA 1996d, 1996e; Lopez-Avila and Benedict0 1996). Recovery is poor (19-30%); detection limits are in the ppb range (EPA 1996d, 1996e; Lopez-Avila and Benedict0 1996).

Accurate determination of HCCPD in environmental samples is also complicated by the susceptibility of the chemical to photochemical and thermal decomposition (EPA 1991b). HCCPD decomposes rapidly upon exposure to light (see Section 5.3.2) and, therefore, samples to be analyzed for this chemical must be stored protected from light (Benoit and Williams 198 1). Thermal decomposition may occur in the inlet of the gas chromatograph (APHA 1992). HCCPD also reacts chemically in acetone solution (APHA 1992). Degradation may occur during storage processing (Eichelberger et al. 1983). Thus, recovery of HCCPD is sometimes low (see Table 6-2) and some methods used for semivolatile compounds are considered inappropriate for quantification of this chemical (APHA 1992; Otson and Williams 1981).

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA 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 HCCPD 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 HCCPD.

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 or eliminate 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. Methods for extracting and identifying HCCPD from urine and blood using GC or GUMS have been developed (DeLeon et al. 1980a; Gill et al. 1996). However, in spiked samples, compound recovery was low (approximately 50%). HCCPD metabolites have been identified in rat urine and feces after exposure to HCCPD, but the metabolites were not characterized (Dorough and Ranieri 1984; Mehendale 1977; Yu and Atallah 1981). Metabolites seem to be predominantly polar. Research efforts to identify HCCPD metabolites and/or reaction products in blood, urine, fecal matter, and tissues could help to identify stable derivatives that could be used as biomarkers of exposure.

Inhalation exposure to HCCPD causes the appearance of electron-lucent granules in the Clara cells in the epithelial lining of the lungs at exposure concentrations as low as 0.01 ppm (Rand et al. 1982b). Although these cellular changes might be used as a biomarker of effect, obtaining tissues for analysis would involve invasive procedures. Thus, Clara cell changes are not recommended as a suitable biomarker of effect. Additional research concerning the mechanism of toxicity is needed in order to identify suitable biomarkers of effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods are available to detect and quantify HCCPD in air, water, soil, wastes, and food (APHA 1992; Benoit and Williams 1981; Boyd et al. 1981; Eichelberger et al. 1983; EPA 1982a, 1984a, 1988a, 1995, 1996d, 1996e; Lopez-Avila and Benedicto 1996; NIOSH 1994; Yurawecz and Puma 1986). Air is the medium of most concern for human exposure to this chemical. Exposure may also occur from water, especially in the vicinity of hazardous waste sites or industrial sources. The existing analytical methods can provide determinations for HCCPD at levels sufficiently low to meet regulatory requirements (Boyd et al. 1981; EPA 1989b, 1991b; NIOSH 1985). However, its tendency to photochemical and thermal degradation and chemical reactivity in some solvents limits the accuracy of analyses of this chemical in all media. Improved methods of extraction and analysis that minimize these reactions would enhance recovery of HCCPD from environmental samples and provide a better estimate of environmental levels, especially in drinking water and soil at hazardous waste sites. In addition, methods to measure degradation products of HCCPD in environmental samples would be useful to determine the environmental impact of this chemical.

6. ANALYTICAL METHODS

6.3.2 Ongoing Studies

No reports of ongoing studies were located.