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 HMX, its metabolites, and other biomarkers of exposure and effect to HMX. 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 MATERIALS

Reverse-phase high performance liquid chromatography (HPLC) is the preferred method for detecting and quantifying HMX in rodent plasma (Army 1985g), and has also been used to analyze rodent urine and fecal samples (Army 1986). This method should be applicable to analysis of human biological samples, as well. The liquid chromatograph separates mixtures of organics and allows individual compounds to be identified and quantified by a detector. An ultraviolet (UV) detector is used for quantitation of HMX. Thin layer chromatography (TLC) has also been used for analysis of ^{14}C-HMX in urine and fecal samples, but this method is not applicable to unlabeled HMX analysis (Army 1986).

Prior to analysis, HMX must be separated from the biological sample matrix and prepared for introduction into the analytical instrument. Separation from plasma is accomplished by extraction with methylene chloride, followed by drying to remove the solvent, and dissolution in a mixture of perchloric acid and acetonitrile prior to injection onto the HPLC column (Army 1985g). Extraction with acetonitrile separates HMX from urine and fecal samples (Army 1986). Details of methods for HMX analysis in biological samples are summarized in Table 6- 1.

TABLE 6-1. Analytical Methods for Determining HMX in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma (rodent)	Extract with methylene chloride; dry under nitrogen stream; dissolve residue in perchloric acid/acetonitrile	HPLC/UV	20 ng/mL	105–108	Army 1985g
Urine/feces (rodent)	Extract with acetonitrile; dry; dissolve in methanol/water; elute with acetonitrile/water	HPLC/UV	No data	No data	Army 1986

HPLC = high performance liquid chromatography; UV = ultraviolet detector

6.2 ENVIRONMENTAL SAMPLES

Several methods have been developed to detect HMX in water and soil. Representative methods for quantifying HMX in these media are summarized in Table 6-2. Only one analytical method for HMX in air was located (OSHA 1987). This method has been only partially evaluated and is presented for information and trial use. The method is summarized in Table 6.2.

The primary method for determining HMX in water and soil samples is by HPLC analysis (Army 1984c, 1985i, 1991c; Bauer et al. 1986, 1990; Jenkins et al. 1986, 1989; Maskarinec et al. 1984; Phillips et al. 1994). The U.S. EPA has issued a reverse-phase high-performance liquid chromatographic (RP-HPLC) method (Method 8330) for the determination of nitroaromatic and nitramine explosives in environmental samples (EPA 1992). The American Society for Testing and Materials (see ASTM 1991) and the Association of Official Analytical Chemists (AOAC 1990) have also adopted an HPLC method as the standard method for determining explosive residues in soil and water (AOAC 1990; Army 1984c; Bauer et al. 1986, 1990; Jenkins et al. 1986, 1989). Gas chromatography (GC) and thin-layer chromatography (TLC) may also be employed (Glover and Hoffsommer 1973; Hable et al. 1991).

Separation of HMX from environmental samples is usually accomplished by extraction with an organic solvent such as methanol, isoamyl acetate, or acetonitrile (Army 1984c, 1985i; Bauer et al. 1986, 1990; Bongiovanni et al. 1984; Jenkins et al. 1989; Phillips et al. 1994). Method 8330 recommends a salting-out procedure with sodium chloride and acetonitrile for low concentrations of explosive residues in surface or groundwater, direct injection for water samples of higher concentrations, and extraction by acetonitrile in an ultrasonic bath for soil and sediment samples (EPA 1992). Research to improve these extraction methods is still in progress. A recent study was conducted to evaluate the use of supercritical fluids for the extraction of explosive residues from soils in an attempt to improve the efficiency, specificity, and time and solvent requirements of extraction (Thorne 1994). This study, however, concluded that supercritical fluid extraction was not a practical method for the routine extraction of explosive residues from soils.

Identification and quantification is most frequently done by UV (Army 1984c, 1985i; Bauer et al. 1986, 1990; Bongiovanni et al. 1984; Glover and Hoffsommer 1973; Jenkins et al. 1989), but an

TABLE 6-2. Analytical Methods for Determining HMX in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Draw air through fiber filter; extract with acetone	HPLC/UV	10.5 ng/injection	93–96	OSHA 1987
Water	Adsorb on Porapak resin; desorb with acetone; exchange with ethanol	HPLC/ED	≈1 µg/L[a]	No data	Maskarinec et al. 1984
Water	Adsorb on Porapak resin; desorb with acetonitrile dilute with reagent grade water	Reverse-phase HPLC	0.21 µg/L	107	Jenkins 1992
Water	Filter through Empore styrene-divinyl benzene disk; extract with aceto-nitrile; dilute with reagent grade water	Reverse-phase HPLC	0.33 µg/L	81	Jenkins 1992
Water	Extract with benzene; dry; dissolve in acetone; elute with benzene/acetone	TLC/UV	20 µg/L	5–15	Glover and Hoffsommer 1973
Water	Extract with sodium chloride and acetonitrile; evaporate and exchange to water; elute with water/methanol/tetra-hydrofuran	Reverse-phase HPLC/UV	0.271 µg/L	118	Army 1991c
Water	Adjust pH to 6; mix with	HPLC/UV	No data	29	Major 1992

TABLE 6-2. Analytical Methods for Determining HMX in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	sodium chloride; trap with C18 disposable cartridge; elute with methanol				
Water	Extract with acetonitrile and sodium chloride; mix with salt water; separate acetonitrile phase; dilute with reagent grade water	Reverse-phase HPLC	0.19 µg/L	106	EPA 1992; Jenkins 1992
Waste water	Mix with methanol/acetonitrile; elute with water/methanol/ acetonitrile	Reverse-phase HPLC/UV	26 µg/L	95	Bauer et al. 1986; AOAC 1990; Army 1984c; Jenkins et al. 1986; EPA 1992
Drinking water	Extract with isoamyl acetate; inject using direct flash injection technique	Capillary column GC/ECD	6 µg/L	81–92 (avg. = 86)	Hable et al. 1991
Soil	Grind soil sample with mortar and pestle; extract with acetonitrile in ultrasonic bath; dilute with aqueous calcium chloride	Reverse-phase HPLC/UV	1.27 µg/g	80–95	Bauer et al. 1986, 1990; Jenkins et al. 1989; EPA 1992

TABLE 6-2. Analytical Methods for Determining HMX in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Grind soil sample; extract with acetonitrile containing 1,3-dinitrobenzene in sonic bath; dilute with aqueous calcium chloride	HPLC/UV	0.15 mg/L	99–112	Major 1992
Soil	Extract with acetonitrile, with sonication; elute with methanol/water	HPLC/UV	0.45 µg/g (ppm)	109.7	Bongiovanni et al. 1984
Soil/Sediment	Freeze or air dry, as required; extract with acetonitrile/methanol/water	Reverse-phase HPLC/UV	≈1 µg/g	92–104	Army 1985i
Food (rodent diet)	Extract with acetonitrile; elute with acetonitrile/water	HPLC/UV	1,250 µg/g (ppm)	94.2–102	Army 1985f

[a] For explosives; not specific for HMX

ECD = electron capture detection; ED = electrochemical detection; GC = gas chromatrography; HPLC = high performance liquid chromatography; TLC = thin layer chromatography; UV = ultraviolet detection

electron capture detector (ECD), thermal energy analyzer (TEA), or electrochemical detector (ED) may also be used (Fine et al. 1984; Hable et al. 1991; Maskarinec et al. 1984). When unequivocal confirmation is required, mass spectrometry (MS) may be employed (Hable et al. 1991).

Accurate determination of HMX in environmental samples is complicated by its low vapor pressure and susceptibility to thermal degradation (EPA 1988), thus compromising the usefulness of GC methods for separation of this compound from complex mixtures. This problem has recently been overcome by limiting contact of the compound with metal parts in the injection port, deactivating the injection port liner by acid treatment, and employing direct flash rather than splitless injection (Hable et al. 1991).

Due to lack of experimental data for HMX, EPA specified a maximum holding time (MHT) for preextraction samples of seven days (the same as for semivolatile organics) (EPA 1992). Subsequently, the stability of HMX in laboratory samples was evaluated (Army 1991b; Grant et al. 1993a, 1993b). The MHT for HMX was experimentally evaluated in reagent-grade water, groundwater, and surface water (Grant et al. 1993a). Samples were held for a period of up to 70 days at both room temperature (22°C) and under refrigeration (2°C). HMX was stable over the entire period for all waters under both storage temperatures (Grant et al. 1993a). Studies to evaluate the MHT in soils demonstrated that HMX was stable over the 56-day study period under all conditions (Grant et al. 1993b). Further studies on MHT for both water samples and soil samples were conducted by Makarinec, Bayne, and Johnson (Army 1991). Based on these studies, the recommended MHT for surface water, groundwater, and soil samples prior to analysis for HMX were 30 days, 50 days, and 56 days respectively (Army 1991b; Grant et al. 1993a, 1993b). All samples should be refrigerated during storage.

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 HMX 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 HMX.

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

Exposure. Methods exist for determining the levels of HMX in plasma, tissues, urine, and feces in animals (Army 1985g, 1986), which are presumably applicable to human exposures, as well. These methods are reasonably accurate, reliable, and specific for determining exposures to HMX. Since HMX is poorly absorbed in the gastrointestinal tract, the levels in plasma, tissues, and urine are likely to be lower than those detected in the feces following oral exposure. The data are too limited to determine if these methods are sufficiently sensitive for measuring the levels at which biological effects are expected to occur. Most people are not exposed to HMX; therefore the background levels of HMX in the general population are not expected to be detectable. Since HMX is metabolized to polar intermediates in the body, studies which identify these intermediates, in conjunction with studies which develop sensitive and reliable methods for detecting and quantifying these intermediates, would be useful for medical surveillance in the future.

Effect. Methods exist for measuring serum enzyme activities and brain wave alterations which could be used as biomarkers of the hepatic and neurological effects of HMX. These methods are sufficiently sensitive for measuring background levels in unexposed populations and could be used to determine the levels at which biological effects occur. However, these types of effects are common to exposures to a large number of exogenous compounds and endogenous diseases and illnesses and, therefore, are not specific biomarkers of effect for HMX exposure. Studies which identify specific biomarkers of effect for HMX, in conjunction with studies that develop sensitive and reliable methods for detecting these biomarkers, would be useful in determining if significant exposure to HMX has occurred.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods are available to detect and quantify HMX in water and soil (Army 1984c, 1985i, 1991c; Bauer et al. 1986, 1990; Bongiovanni et al. 1984; EPA 1992; Glover and Hoffsommer 1973; Hable et al. 1991; Jenkins et al. 1986, 1989, 1992; Major 1992; Maskarinec et al. 1984). Water is the medium of most concern for human exposure to this chemical. Exposure may also occur from soil or sediments in the vicinity of hazardous waste sites or from manufacturing or processing sources. The existing analytical methods can provide determinations for HMX at levels sufficiently low to meet water quality guidelines and below which health effects may occur (Army 1991c; Hable et al. 1991). However, improved methods of extraction and analysis that minimize interferences and decomposition would enhance recovery of lower levels of HMX that may be present in soil and in drinking water at hazardous waste sites and at military manufacturing and processing facilities. Research to improve the available methods is in progress (see Section 6.3.2). No information was located on specific analytical methodology for HMX in air samples. Development of a simple method for sampling and analyzing workplace air would be helpful in assessing the potential for human exposure from this source.

Methods are also available to measure degradation products of HMX in environmental samples (Army 1983), but these products (mainly nitrate, nitrite, and formaldehyde) are released to the environment from many other sources and are therefore not useful determinants of the environmental impact of this chemical.

6.3.2 On-going Studies

On-going research to improve analytical methods for HMX and related compounds includes studies to facilitate efficient elution of HMX using mobile phase modifiers and/or more inert capillary columns, thus enabling detection and quantitation of HMX by these methods. Research continues on developing improved techniques for extraction, concentration, and elution of HMX (Army 1991c; Bauer et al. 1990; Berberich et al. 1988; Hable et al. 1991). These improvements are designed to oVercome problems with sample preparation and increase sensitivity and reliability of the analyses.