7. ANALYTICAL METHODS

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

The most common procedures for the analytical separation of RDX in biological and environmental materials are high-performance liquid chromatography (HPLC) and gas chromatography (GC). These methods have been paired with several types of detectors, including thermal energy analyzer (TEA), electrochemical detector (ED), electron capture detector (ECD), and ultraviolet (UV). The TEA is very selective for nitroso compounds and when paired with either HPLC or GC, gives excellent selectivity, recovery, and precision and high sensitivity (Fine et al. 1984; Lafleur and Morriseau 1980). The limited reports of analysis of materials using HPLC and ED indicate detection limits in the low ppb range and good reliability (Krull et al. 1984; Lloyd 1983). GC coupled with ECD appears to have good sensitivity (low ppb), accuracy, and precision (Bishop et al. 1981, 1988). UV detection has also been used with HPLC separation, but few data are available for comparison with other methods (Burrows and Brueggemann 1985; Strobel and Tontarski 1983). The data suggest that this method has very good accuracy and precision; however, the selectivity may not be as good as that obtained with other detectors. Methods based on mass spectrometry (MS) with sensitivity in the sub-ppb range have been described, but specific information on their reliability is limited (St. John et al. 1975; Tanner et al. 1983). MS is generally accepted to be highly selective. Sample preparation for RDX analytical methods is relatively simple, consisting of collection of the sample from air, water, soil, tissue, fluid, residue, or waste followed by homogenization if necessary, one or two extraction/clean-up steps, and concentration of the sample.

7.1 BIOLOGICAL MATERIALS

Analytical methods specifically used for the determination of RDX in biological fluids and tissues are limited. Methods were located that discussed the analysis of RDX in blood, tissues, urine, and hand swabs. The separation methods employed included high-performance liquid chromatography (HPLC) or gas chromatography (GC). These were combined with detection by thermal energy analyzer (TEA), ultraviolet (UV), electrochemical detector (ED), or electron capture detector (ECD). Both HPLC and high-resolution gas chromatography (HRGC) can rapidly separate RDX from other explosives, but HPLC has the advantage of being run at ambient temperature, which helps prevent breakdown of the analyte. Pertinent data on these methods are presented in Table 7-1.

Detection of RDX in human and animal plasma as well as human urine and cerebrospinal fluid has been accomplished by HPLC/TEA and HPLC/UV (U.S. Army 1981a; Fine et al. 1984; Turley and Brewster 1987). While both methods provide relatively rapid sample turn-around times, HPLC/TEA is the most sensitive and selective of the two, and requires little sample preparation (Fine et al. 1984). The older HPLC/UV method (U.S. Army 1981a) had the problem of coelution of a plasma component with the RDX peak. This was eradicated by clean-up on a C18 bonded-phase extraction column (Turley and Brewster 1987; Woody et al. 1986), but the sensitivity of HPLC/UV was still several orders of magnitude less (limit of detection in low ppb) than that of HPLC/TEA (limit of detection in low ppt). Reported recoveries ranged from 87.7 to 101% (Turley and Brewster 1987; U.S. Army 1981a; Woody et al. 1986). Precision was comparable and ranged from 0.65 to 10% coefficient of variation (CV).

A method of analyzing feces for RDX was located (Woody et al. 1986). This method used HPLC/UV and required extraction of the sample with acetonitrile and sonication. The limit of detection was not reported, although based on the data presented, it was assumed to be in the low ppb range.

One method was located for analysis of tissue samples. The method used HPLC/UV to analyze bovine kidney, muscle/fat, and liver samples for RDX, but it could be used to analyze human tissues (U.S. Army 1981a). Optimal sample preparation methods varied slightly for the different tissues, as did detection limits and precision. In general, the detection limit was in the low ppb and recovery was high (in the range of 87.7–102.9). Precision ranged from 7 to 16% CV. The primary issue with analysis of tissue using this method is the variation in selectivity. Minor differences in sample extraction and contamination from unknown sources can create interferences that drastically affect interpretation of results and may also adversely affect the sensitivity.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma	Extract with methylene chloride and pentane; filter; concentrate	HPLC/TEA	100 ng/L	No data	Fine et al. 1984
Plasma	Add NaCl/acetic acid solution to sample; extract with toluene; add water; evaporate organic phase; combine aqueous phase with acetonitrile-containing internal standard; filter	HPLC/UV	146 µg/L	87.7 (spike levels 0–2,000 ng/g; SD±19–188 ng; CV 7–19)	U.S. Army 1981a
Serum and urine	Mix sample with internal standard; clean up on C_{18}-bonded-phase extraction column, eluting with methanol; concentrate	HPLC/UV	100 µg/L	90±2.0–101±1.1 (1–10 mg/L in serum); 98±1.6–101±1.3 (1–10 mg/L in urine)	Turley and Brewster 1987
Kidney	Add NaCl/acetic acid solution to sample; extract with toluene; add water; evaporate organic phase; combine aqueous phase with acetonitrile-containing internal standard; filter	HPLC/UV	95 ng/g	99.5 (spike levels 0–2,000 ng/g; SD±12–58; CV 2–11)	U.S. Army 1981a
Muscle/fat	Homogenize sample; extract with acetonitrile; concentrate; add internal standard and purified water; filter	HPLC/UV	62 ng/g	102.9 (spike levels 0–2,000 ng/g; SD±2.2–86; CV 3.9–14)	U.S. Army 1981a
Liver	Homogenize sample; add NaCl/acetic acid solution; evaporate; redissolve in acetonitrile-containing internal standard; filter	HPLC/UV	150 ng/g	87.7 (spike levels 0–1,000 ng/g; SD±18–69; CV 7–22)	U.S. Army 1981a
Hand swabs	Wipe hand with swab soaked in acetone; squeeze out acetone and concentrate	HPLC/TEA; HRGC/TEA	10 pg/inj	No data	Fine et al. 1984

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Hand swabs	Wipe hand with swab soaked in ether; extract with ether; centrifuge to remove debris; decant supernatant and evaporate; redissolve in pentane; clean up on Amberlite XAD-7 beads, eluting with ethyl acetate; evaporate; redissolve in pentane and repeat Amberlite XAD-7 clean-up	GC/ECD	50 ng/ swab (1.7 ng/inj)	47 (at 200 ng/swab)	Douse 1982
		TLC	20 ng/ swab	No data	
Hand swabs, standards	Wipe hand with dry swab; extract with methanol/ potassium phosphate; directly inject standards	HPLC/PMDE	8 pg/inj (standards)	No data	Lloyd 1983

CV = coefficient of variation; ECD = electron capture detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; inj = injection; PMDE = pendant mercury drop electrode; TEA = thermal energy analyzer; TLC = thin layer chromatography; UV = ultraviolet

The only other methods for biological matrices located were for analysis of hand swabs. These are of primary importance in forensics, but they could also be used to determine if dermal exposure of workers has occurred. Methods that have been used for the determination of trace amounts of RDX on hands include HPLC with TEA or electrochemical detection and HRGC with TEA or ECD (Douse 1982; Fine et al. 1984; Lloyd 1983). Thin-layer chromatography has also been tested, but because of the large amounts of sample that are required for the analysis, it is useful only as a screening test for high concentration samples (Douse 1982). Separation of the sample by HPLC and HRGC are comparable, but reported recovery for HRGC is low (Douse 1982). This is likely because of decomposition of the sample, but the data are not available to adequately compare the recovery of the two methods. The nature of the detector seems to be the most important factor in determining which of the reported methods is most useful for the analysis of RDX in hand-swab extracts. ECD appears to be less sensitive (ng amounts) than either electrochemical detection using the pendant mercury drop electrode (PMDE) or TEA (pg amounts). In addition, in the method reported, clean-up was required to prevent matrix interference (Douse 1982). For both the PMDE and TEA methods, clean-up of the sample was not required, and both methods were rapid, selective, and of high precision (Fine et al. 1984; Lloyd 1983).

7.2 ENVIRONMENTAL SAMPLES

A large variety of methods have been described for the detection of RDX in environmental samples. These primarily include HRGC combined with ECD, TEA, mass spectrometry (MS), or flame ionization detection (FID); HPLC combined with UV, TEA, MS, photoconductivity (PD), or electrochemical detection; automated multiple development high performance thin-layer chromatography (HPTLC-AMD); liquid chromatography (LC) with thermospray (TSP) and MS; and several stand-alone MS techniques. Other methods have also been proposed, including fluorescent quenching; supercritical fluid chromatography (SFC) with UV. Table 7-2 is a summary of several representative methods for determining RDX in various environmental media.

Several methods for determining RDX in air have been investigated. Based on the limited data available, the two most common methods are GC/ECD and MS. The data reported are not sufficient to make comparisons of sensitivity and reliability between the methods. GC/ECD, however, appears to have good sensitivity (low ppb), accuracy, and precision (Bishop et al. 1981, 1988). An alternate method based on spectrophotometry also provided similar results for accuracy and precision (±12.4% CV) and had a detection limit of the same order of magnitude as that reported using GC/ECD (Eminger and Vejrostova

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect sample on Tenax-plus-filter tubes; desorb with acetonitrile	HRGC/ECD	17 µg/m^3	No data	Bishop et al. 1988
Air	Collect sample on Tenax-GC; desorb with acetonitrile	HRGC/ECD	No data	93–102; 98±4.4 average (6–120 µg test level)	Bishop et al. 1981
Air	Collect sample on glass-fiber filter; extract with ethyl acetate	GC/FID	0.5 mg/m^3	No data (precision ±15%)	U.S. Army 1975
Air	Collect sample in sampling tube of glass-microfibers and silica gel; transfer to H_2SO_4 solution and react with dihydroxynapthalene-disulfonic acid and water; dilute with water	Spectro-photometry	40 µg/m^3	95.7–97.3	Eminger and Vefrostova 1984
Air	Incorporate sample into bulb containing isotopically-labeled RDX; extract with benzene; transfer to capillary tube and evaporate	IDMS	Sub-ppb	No data	St. John et al. 1975
Air	Inject sample directly into instrument	APCI/MS/MS	Sub-ppb	No data	Tanner et al. 1983
Waste water effluents	Add internal standard to sample; elute from reverse-phase column with methanol/water	HPLC/UV	0.2 mg/L	100–102 (measured at 0.67 mg/L; RSD 0.36–9.48% measured at 0.27–2.66 ppm)	U.S. Army 1983c
Groundwater, waste water effluents	Dilute sample with methanol/acetonitrile; filter; elute from reverse-phase column with water/acetonitrile/ methanol	HPLC/UV	22 µg/L	101	Jenkins et al. 1986; U.S. Army 1985c

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater	Collect sample on Hayesep R solid sorbent cartridge; elute with acetone; concentrate; add internal standards; dilute with methanol/ water	HPLC/UV/ UV/PD	5–7.5 µg/L	104–121	U.S. Army 1989a
Surface water, well water	Collect sample on Porapak resin; rinse sorbent with distilled water and elute with acetone; concentrate; add ethanol; concentrate; add methanol/water	HPLC/ED	≈1 µg/L	57–63	Maskarinec et al. 1984
Water	Collect sample on XAD-4 resin; elute with ethyl acetate; concentrate	HRGC/ECD	<0.1 µg/L	97±5 (spike level 4 µg/L)	Richard and Junk 1986
Water	Liquid/liquid extraction using dichloromethane	HPTLC-AMD	10 ng	No data (RSD 1.6–5.9% for 20–130 ng in solution)	Steuckart et al. 1994
Groundwater, drinking water	Extract sample with isoamyl acetate	HRGC/ECD	0.3 µg/L	56–84 (spike level 0.15–3.0 µg/L; RSD 9.3–19)	Hable et al. 1991
Sea water	Add internal standard to sample; extract with benzene; evaporate; redissolve in benzene	GC/ECD	5 ng/L	70±10 (at 103–1,400 ng/L)	Hoffsommer and Rosen 1972
Water	Evaporate sample; redissolve in acetone; filter; concentrate	HRGC/ECD	60 ng/L	85	Haas et al. 1990
Water	Inject sample directly into instrument	MS (CI)	40 mg/L	No data	Yinon and Laschever 1982
Groundwater	Add sample to cyclo-hexanone/ pyrenebutyric acid/ cellulose triacetate/ isodecyl diphenyl-phosphate membrane in cuvette	Fluorescense quenching	≈10 mg/L	No data	Jian and Seitz 1990

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Air-dry, grind, and sieve sample; extract with acetonitrile in ultrasonic bath; add $CaCl_2$; filter; elute from reverse-phase column with water/methanol	HPLC/UV	0.74 µg/g	84–112 (multilaboratory determination)	Bauer et al. 1990; Jenkins and Grant 1987; Jenkins et al. 1989; U.S. Army 1987b (interim AOAC method)
Soil	Adjust sample moisture to 20–30%; homogenize and sieve; extract with acetonitrile and sonication; centrifuge and filter; elute from reverse-phase column with methanol/water	HPLC/UV	0.6 µg/g	103.7 (spike level 0.5–200 µg/g; CV 0.098)	Bongiovanni et al. 1984
Soil	Air-dry sample; extract with acetonitrile; filter; evaporate; redissolve in acetonitrile; elute from reverse-phase column with acetonitrile/water	HPLC/UV	0.005 µg/g	No data	Lyter 1983
Soil	Homogenize sample; extract with acetone; filter	HRGC/ECD	75 ng/g	95	Haas et al. 1990
Soil	Homogenize sample; extract with acetone; evaporate; react with diphenylamine/H_2SO_4	Spectro-photometry	5 mg/L	No data	Haas et al. 1990
Soil	Grind sample; extract with acetone in ultra-sonic bath, centrifuge, add toluene, and dry; remove humic substances with calcium chloride or elution with ethyl acetate/petroleum ether over biobeads	HPTLC-AMD	10 ng	No data (RSD 1.6–5.9% for 20–130 ng in solution)	Steuckart et al. 1994
Soil	Extract of soil sample and enzyme conjugate reagent added to immobilized RDX antibody; D TECH™ RDX test kit required	Immunoassay	5 µg/g	53–114 (spike level 0.53–6.82 mg/g; SD 0.12–1.21; CV 5–46%)	EPA 1996

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Soil samples extracted with acetone; extract passed through ion exchange resin; extract acidified and mixed with zinc dust, color developed using a NitriVer 3 powder pillow	Colorimetric screening using spectro-photometry	1 µg/g	60–140	EPA 2007
Agricultural crops (tomato)	Acid hydrolysis; extract with diethyl ether	HPLC	5 ng/g (laboratory-grown); 17 ng/g (field-grown)	90±4 (laboratory-grown; spike level 5 µg/g dry mass)	Harvey et al. 1997
Agricultural crops (soybean)	Acid hydrolysis; extract with diethyl ether	HPLC	50 ng/g (laboratory-grown)	70±3 (spike level 5 µg/g dry mass)	Harvey et al. 1997
Agricultural crops (corn)	Acid hydrolysis; extract with diethyl ether	HPLC	51 ng/g (laboratory-grown, stover); 13 ng/g (field-grown, kernel)	75±18 (laboratory-grown; spike level 5 µg/g dry mass)	Harvey et al. 1997
Agricultural crops (bush bean)	Acid hydrolysis; extract with diethyl ether	HPLC	8 ng/g (laboratory-grown)	68±11 (spike level 5 µg/g dry mass)	Harvey et al. 1997
Agricultural crops (radish)	Acid hydrolysis; extract with diethyl ether	HPLC	3.2 ng/g (laboratory-grown)	103±38 (spike level 5 µg/g dry mass)	Harvey et al. 1997
Agricultural crops (alfalfa)	Acid hydrolysis; extract with diethyl ether	HPLC	15 ng/g (laboratory-grown)	76±3 (spike level 5 µg/g dry mass)	Harvey et al. 1997
Agricultural crops (lettuce)	Acid hydrolysis; extract with diethyl ether	HPLC	7 ng/g (laboratory-grown)	71±9 (spike level 5 µg/g dry mass)	Harvey et al. 1997
Agricultural crops (hot pepper)	Acid hydrolysis; extract with diethyl ether	HPLC	28 ng/g (field-grown)	No data	Harvey et al. 1997
Agricultural crops (carrot)	Acid hydrolysis; extract with diethyl ether	HPLC	39 ng/g (field-grown)	No data	Harvey et al. 1997
Agricultural crops (green pepper)	Acid hydrolysis; extract with diethyl ether	HPLC	20 ng/g (field-grown)	No data	Harvey et al. 1997

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Agricultural crops (grapes)	Acid hydrolysis; extract with diethyl ether	HPLC	18 ng/g (field-grown)	No data	Harvey et al. 1997
Explosive preparations	Elute from HPLC column with isooctane/ethanol	HPLC/TEA	No data	98–102	Lafleur and Morriseau 1980
Explosives, explosion debris	Dissolve sample in acetone; dilute in methanol	HPLC/TEA; HRGC/TEA	Low pg	No data	Fine et al. 1984
Explosives	Extract sample with acetone; elute from HPLC column with methanol/potassium phosphate	HPLC/EC (PMDE)	8 pg/g	No data (CV 0.8% of 1 ng replicates)	Lloyd 1983
Explosion debris	Extract sample in acetone; clean up on cyclohexyl column; eluting with methylene chloride/hexane; clean up on cyanopropyl column; elute with acetonitrile/water	HPLC/UV	No data	99	Strobel and Tontarski 1983
Munitions products	Dissolve sample in acetonitrile; add water; elute from reverse-phase column with methanol/water	HPLC/UV	No data	No data	Burrows and Brueggemann 1985
Explosives	Extract with acetone; evaporate; redissolve in dichloroethane; elute from HPLC column with dichloroethane/hexane	HPLC/MS (CI)	≈1 ng	No data	Vouros et al. 1977
Explosives, explosive residues	Dissolve in acetone or methanol; elute from HPLC column with methanol/ammonium acetate	HPLC/TSP/MS	Low pg	No data	Berberich et al. 1988

AMD = automated multiple development; APCI = atmospheric pressure chemical ionization; AOAC = Association of Official Analytical Chemists; $CaCl_2$ = calcium chloride; CI = chemical ionization; CV = coefficient of variation; EC = electrochemical detection; ECD = electron capture detection; ED = electrochemical detection; FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HPTLC = high performance thin-layer chromatography; HRGC = high-resolution gas chromatography; H_2SO_4 = sulfuric acid; IDMS = isotope dilution mass spectrometry; MS = mass spectrometry; PD = photoconductivity detection; PMDE = pendant mercury drop electrode; RSD = relative standard deviation; SD = standard deviation; TEA = thermal energy analyzer; TSP = thermospray; UV = ultraviolet detection

1984). MS methods with sensitivity in the sub-ppb range have been described, but specific information on their reliability is limited. MS is generally accepted to be highly selective. Of the two MS methods described, isotope dilution MS (IDMS) (St. John et al. 1975) and MS/MS with atmospheric pressure chemical ionization (APCI) (Tanner et al. 1983), the latter (APCI/MS/MS) is the most rapid and simple to perform because the sample of air containing RDX vapors is directly injected into the instrument. The high sensitivity and selectivity of MS/MS allow the air sample to be injected without prior treatment or concentration. However, the method as presented appears to be primarily useful as a screening technique to determine if more rigorous quantitative analysis is required. IDMS requires some sample preparation in order to incorporate the known amount of labeled analyte in the sample containing the unknown amount of RDX. IDMS has been used to measure the vapor pressure of RDX, which is in the sub-ppb range.

The primary analytical methods for determining RDX in water are HPLC/UV and GC/ECD. These methods have been used to determine the chemical in waste-water effluents, groundwater, well water, drinking water, and seawater. The critical step in the analysis of RDX by HPLC/UV is separation of the sample on a reverse-phase column, which provides good selectivity without risk of thermal breakdown of the analyte (Jenkins et al. 1986; U.S. Army 1983c, 1985c). The method is simple, quick, and reproducible. Sensitivity is in the low- to mid-ppb range, with good recovery and excellent precision (2–7.6% CV). The use of HPLC in combination with photodiode-array detection improves the reliability of peak identification (Emmrich et al. 1993). The HPLC-photodiode-array detection method can provide a detection limit of 0.09 ppb for RDX in aqueous samples concentrated 1,000-fold by liquid-liquid extraction or by solid phase extraction (C-18) (Levsen et al. 1993). The extraction efficiency of RDX from water to acetonitrile can be improved by using salting out agents (U.S. Army 1991). The sensitivity and selectivity of RDX detection was improved by combining a solid sorbent cartridge to concentrate RDX from water and HPLC-tandem ultraviolet and photoconductivity detection (HPLC/UV/PD) (U.S. Army 1989a). The system consisted of a UV absorbance detector set to 254 nm and a photoconductivity detector equipped with a zinc photoionization source. The serial use of the two detectors effectively differentiated RDX from other explosives and from contaminants in the solid sorbent cartridge. In addition, the sensitivity was improved by a factor of about 3. To prevent negative baseline drift and random spikes in the PD, only highly purified water must be used, and the effluent must be exhaustively degassed (U.S. Army 1989a). Automated multiple development high performance thin-layer chromatography (HPTLC-AMD) has also been used to analyze water samples. Liquid-liquid extraction using dichloromethane was used to prepare the samples. A detection limit of 10 ng was obtained (Steuckart et al. 1994).

For analysis by GC/ECD, water samples may be solvent-extracted (Belkin et al. 1985; Haas et al. 1990; Hable et al. 1991; Hoffsommer and Rosen 1972) or collected on a solid sorbent (Richard and Junk 1986). Solvent extraction is most commonly used, but solid sorbent collection has the advantages of being faster and cheaper than solvent extraction (Richard and Junk 1986). Sensitivity for the GC/ECD methods ranges from low to mid ppt, and the recovery and precision are acceptable. Use of the solid sorbent improved recovery and precision compared to solvent-extraction methods (Richard and Junk 1986). Substitution of ED, using a gold-mercury electrode, improved selectivity compared to ECD detection. Sensitivity was not as good, but it remained within an order of magnitude of that found with GC/ECD (Maskarinec et al. 1984). Recovery and precision were comparable. A more recent study indicated that GC/ECD is not useful in the determination of RDX in water samples, as RDX may undergo thermal degradation (Steuckart et al. 1994).

Other methods that have been used to determine RDX in water are MS, fluorescence quenching, COD, and total organic carbon (TOC) (Jian and Seitz 1990; Roth and Murphy 1978; Yinon and Laschever 1982). COD and TOC (Roth and Murphy 1978) are well-established standard methods for determining organic pollution in water, but they are not selective for RDX. MS with chemical ionization (CI) permits direct injection of the water sample into the analytical instrument, but the sensitivity is substantially less than with the HPLC/UV and GC/ECD methods (Yinon and Laschever 1982). Fluorescence quenching also lacks sensitivity, and the method is still under development. However, it does permit *in situ* measurement of samples, and further improvements in the technology may make it a desirable field method (Jian and Seitz 1990). Continuous flow immunosensor (CFI) has been found to produce results comparable to HPLC in detecting RDX in groundwater samples (Bart et al. 1997). CFI utilizes a small column of plastic beads containing immobilized antibodies with the explosive and a fluorescent dye-labeled explosive analog. When the explosive present in the sample displaces the dye-labeled analog in the column, the explosive is detected with a detection limit of approximately 20 ppb (Bart et al. 1997).

The methods that were located for detection of RDX in soil are based primarily on HPLC/UV analysis (Bauer et al. 1990; Bongiovanni et al. 1984; Jenkins and Grant 1987; Jenkins et al. 1989; Lyter 1983; U.S. Army 1987b). All of the methods involve extraction of the sample with acetonitrile, separation using a reverse-phase column, and in most cases, elution with acetonitrile/water. Sensitivity for these methods is in the sub- to low-ppm range with good recovery (84–112%) and precision (2.3–24% CV). A variation of the method involves the soil sample being extracted with acetonitrile in an ultrasonic bath (Jenkins et al. 1989; Steuckart et al. 1994). Soil samples can be ground into mortar and extracted with acetone in an

ultrasonic bath maintained at ambient temperature, centrifuged, added to toluene, and dried over anhydrous sodium sulfate. Steuckart et al. (1994) removed humic substances with either a calcium chloride solution or elution with ethyl acetate/petroleum ether over biobeads. The samples were analyzed by HPTLC-AMD with a detection limit of 10 ng (Steuckart et al. 1994).

Other analytical methods are based on GC/ECD and spectrophotometry (Haas et al. 1990). In both of these methods, the samples were extracted with acetone. The detection limit for spectrophotometric determination of RDX in soil was in the low-ppm range, while the detection limit for GC/ECD was in the mid-ppb range. No information on accuracy and precision were given for the spectrophotometric method; however, the accuracy of GC/ECD was comparable to HPLC/UV.

Methods are available for identification of RDX in agricultural crops. Harvey et al. (1997) utilized HPLC to determine RDX concentrations. The samples underwent acid hydrolysis with hydrochloric acid and extraction with diethyl ether prior to analysis by HPLC. The detection limits and percent recoveries for a variety of crops are listed in Table 7-2 (Harvey et al. 1997). Larson et al. (1999b) used an 18-hour cooled sonication extraction technique using acetonitrile to extract RDX from plant tissues that had been exposed to contaminated irritation water. The samples were then analyzed with HPLC/UV.

Several methods have been used to detect and measure RDX in explosive materials and debris from explosions. The most common separation procedure is HPLC, but HRGC has also been used. These methods have been paired with several types of detectors, including TEA, MS, electrochemical detection, and UV. The TEA is very selective for nitroso compounds and when paired with either HPLC or HRGC, gives excellent selectivity, recovery, and precision and high sensitivity (Fine et al. 1984; Lafleur and Morriseau 1980). GC/MS has been used for confirmation of RDX in samples of explosive materials (Burrows and Brueggemann 1985), and HPLC/MS and MS/MS have been investigated as screening methods for explosives (McLucky et al. 1985; Vouros et al. 1977). A sophisticated method linking HPLC, thermospray (TSP), and MS or MS/MS (with both positive and negative chemical ionization) has also been proposed as an extremely sensitive (low pg range) and selective method for detecting RDX in explosive residues (Berberich et al. 1988; Verweij et al. 1993). However, there is no evidence that any MS-based method is currently used to quantitatively measure RDX in explosives or explosion debris. A relatively new method being investigated uses supercritical fluid extraction chromatography (SFC) to separate RDX from other analytes and contaminants followed by detection by UV/FID (Griest et al. 1989). The method is slower but more selective than HPLC/UV. The precision for standard solutions

was excellent. However, more work is needed to improve the mobile phase and column packing material before samples in complex matrices can be analyzed.

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 RDX is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to ensure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of RDX.

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. Few methods exist for monitoring exposure to RDX. Methods have been reported for detection of the analyte in plasma (Fine et al. 1984; Turley and Brewster 1987; U.S. Army 1981a; Woody et al. 1986), urine (Turley and Brewster 1987; Woody et al. 1986), cerebrospinal fluid (Woody et al. 1986), feces (Woody et al. 1986), and tissues (U.S. Army 1981a), as well as on hands (Douse 1982; Fine et al. 1984; Lloyd 1983). The available methods can detect levels in urine and plasma from exposure to concentrations below those that would be encountered in most manufacturing situations. In general, these methods are reliable and accurate; however, the development of the LC-MS methodology could be useful as a definitive method to validate the specificity of the HPLC methods. The data are insufficient to permit correlation of RDX levels in the urine or blood with exposure levels.

Effect. There are no known sensitive biomarkers of effect for RDX. Therefore, no methods recommendations can be made for this chemical.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist to detect and quantify RDX in air (Bishop et al. 1988; Eminger and Vejrostova 1984; St. John et al. 1975; Tanner et al. 1983; U.S. Army 1974), water (Haas et al. 1990; Hable et al. 1991; Jian and Seitz 1990; Maskarinec et al. 1984; Richard and Junk 1986; Steuckart et al. 1994; U.S. Army 1983c, 1985c, 1989a; Yinon and Laschever 1982), soil (Bongiovanni et al. 1984; Haas et al. 1990; Steuckart et al. 1994; U.S. Army 1987b), agricultural crops (Harvey et al. 1997; Larson et al. 1999b), explosive materials (Burrows and Brueggemann 1985; Fine et al. 1984; Lafleur and Morriseau 1980; Lloyd 1983), and debris from explosions (Fine et al. 1984; Strobel and Tontarski 1983). These methods are relatively sensitive and reliable and can be used to detect levels of the compound in the environment that cause known adverse health effects. There are some problems involving reduced sensitivity and selectivity with all of the commonly used methods. Several proposed improvements in current methods, such as combining various analytical methods to increase selectivity, sensitivity, reliability, and/or accuracy (Berberich et al. 1988; Krull et al. 1984; U.S. Army 1989a), and investigations of new methods (Griest et al. 1989; Jian and Seitz 1990) will be useful in forensics and in monitoring environmental contamination from manufacture and disposal of RDX.

7.3.2 Ongoing Studies

The information in Table 7-3 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2009).

Table 7-3. Ongoing Studies on RDX

Investigator	Affiliation	Research description	Sponsor
Ram, M	Triton Systems, Inc. Auburn University	*In situ* near real-time detection of RDX in soil	U.S. Army
Li, J	University of Florida	Enhanced quadrupole resonance technology for explosive detection	NSF
Indacochea, JE	University of Illinois at Chicago	Development of a nanostructured-based sensor system for reliable detection of improvised explosive devices	NSF
Scherer, JJ	NovaWave Technologies	Ultrasensitive, real-time explosives sensor	NSF

NSF = National Science Foundation

Sources: DOD 2009; EPA 2008b; FEDRIP 2009