

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring tetryl in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify tetryl. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect tetryl in environmental samples are the methods approved by federal 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 refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

No analytical methods specifically used for the determination of tetryl in biological fluids and tissues were located. One attempt to develop a method for detecting tetryl in animal tissues using highperformance liquid chromatography (HPLC) with ultraviolet (UV) detection was unsuccessful because of suspected metabolism and binding of the parent compound and/or metabolites to macromolecules (Army 1981a). However, methods were located for the detection of the tetryl metabolites, picric acid and picramic acid, in urine and for the analysis of tetryl in hand swabs. Table 6-1 is a summary of methods used to determine tetryl metabolites in urine and tetryl in hand swabs.

Methods to detect picric acid and picramic acid in rabbit urine using calorimetric assays have been reported (Zambrano and Mandovano 1956). The detection limits were in the sub- to low ppm range, but no information on recovery or precision was given.

Methods for the analysis of tetryl in hand swabs are used in forensics, but they could also be used to determine if dermal exposure of workers has occurred. The methods that have been used for the determination of trace amounts of tetryl on hands include high resolution gas chromatography (HRGC) with electron capture detection (ECD) or thermal energy analyzer (TEA), or HPLC with TEA or electrochemical detection (ED) (Douse 1982, 1985; Fine et al. 1983, 1984; Lloyd 1983a, 1983b). Thin-layer chromatography (TLC) has also been tested, but because of the large amounts of sample

TABLE 6-1. Analytical Methods for Determining Tetryl in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (picric acid)	Add lead acetate to sample; filter and add sulfuric acid; filter and extract with chloroform; filter organic phase; add ammonia to an aliquot of the extract; add Grinbert reagent (ferrous sulfate/tartaric acid/water) to bottom of test tube	Colorimetric	1 mg/L	NR	Zambrano and Mandovano 1956
Urine (picramic acid)	Add sulfuric acid and sodium nitrate to sample; cool and add ammonia saturated with β -naphthol	Colorimetric	0.05 mg/L	NR	Zambrano and Mandovano 1956
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, elute with ethyl acetate; evaporate; redissolve in pentane and repeat Amberlite XAD-7 [®] clean-up	HRGC/ECD	50 ng/swab (1.7 ng/inj)	25	Douse 1982
		TLC	60 ng/swab	NR	
Hand swabs	Wipe hand with dry swab; extract with methanol/potassium phosphate; directly inject standards	HPLC/ED (PMDE)	24 pg/inj	44-91	Lloyd 1983a, 1983b
Hand swabs	Wipe hand with swab soaked in acetone; squeeze out acetone and concentrate	HPLC/TEA; HRGC/TEA	Low pg	NR	Fine et al. 1984

ECD = electron capture detection; ED = electrochemical detection; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; inj = injection; NR = not reported; PMDE = pendant mercury drop electrode; TEA = thermal energy analyzer; TLC = thin-layer chromatography

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required for the analysis, it is useful only as a confirmatory test (Douse 1982). Both HPLC and HRGC can rapidly separate tetryl from other explosive compounds, but HPLC has the advantage of being run at ambient temperature. This decreases temperature-related breakdown of the analyte and yields higher recoveries. The type of detector seems to be the most important factor in determining which of the reported methods is most useful in the analysis of tetryl in hand swabs. ECD appears to be less sensitive (ng amounts) than either TEA or electrochemical detection using pendant mercury drop electrode (PMDE) (pg amounts) (Douse 1982; Fine et al. 1983; Lloyd 1983a, 1983b). In addition, a clean-up step using ECD is usually required to prevent matrix interference (Douse 1982, 1985). Both TEA and PMDE appear to have greater selectivity than ECD, and because of the selectivity of the detector, a clean-up step was not required (Fine et al. 1983; Lloyd 1983b). Both TEA and PMDE methods are rapid, selective, and have high precision (Fine et al. 1983; Lloyd 1983b).

6.2 ENVIRONMENTAL SAMPLES

A variety of methods have been described for the detection of tetryl in environmental samples, including air, fresh water and sea water, soil, sediment, ocean floor fauna, and explosives. Table 6-2 is a summary of several representative methods for determining tetryl in environmental media. A single method of analyzing tetryl in air was located (NIOSH 1977). The method utilized spectrophotometric analysis. Air samples were collected on a cellulose membrane filter and then extracted with N,N-diethylethanolamine.

The primary analytical methods for determining tetryl in water are gas chromatography (GC), GC/ECD, HRGC/ECD, HPLC/ED, HPLC/JV, and mass spectrometry (MS) (Army 1981c, 1984c, 1986c, 1988, 1989a; Belkin et al. 1985; Feltes and Levsen 1989; Hoffsommer and Rosen 1972; Yinon and Laschever 1982). These methods have been used to determine tetryl in sea water, well water, surface water, groundwater, and drinking water. Both GC and HPLC methods have been applied to the analysis of tetryl. One drawback with GC analysis is that thermally labile chemicals, such as tetryl, are subject to a significant amount of heating. This introduces the likelihood of partial degradation (Army 1986c). Liquid chromatographic methods, by contrast, do not volatilize the sample; therefore, thermal degradation was not a complication (Army 1986c). Following concentration of samples by liquid-liquid extraction or by solid phase extraction with C-18 adsorbents, HPLCAJV method can attain a detection limit of 0.05 ppb for tetryl in water samples (Levsen et al. 1993).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect sample on a cellulose membrane filter; extract with N,N-diethylethanolamine	Spectrophotometry	0.15 mg/m ³	NR	NIOSH 1977 (Method S225)
Water	Filter and extract sample with toluene	GC/ED	23.9 mg/L	NR	Army 1981c
Water	Inject sample directly into instrument	MS (CI)	40 mg/L	NR	Yinon and Laschever 1982
Water	Dilute aqueous sample 1:1 with acetonitrile; filter; inject into instrument	HPLC/UV	26 µg/L	96.7	Army 1988
Sea water	Add internal standard to sample; extract with benzene; evaporate; redissolve in benzene	GC/ECD	≈20 ng/L	70	Hoffsommer and Rosen 1972
Well water, surface water	Collect sample on Amberlite XAD-4 [®] resin; rinse sorbent with distilled water and elute with acetone; concentrate; add methanol/water	HPLC/ED	2 µg/L	30–120	Army 1986c
Surface water (brooks, ponds)	Collect sample on Amberlite XAD-2/4/8 [®] resin; dry; desorb with dichloromethane; dry over anhydrous sodium sulfate; solvent exchange to methanol and concentrate; elute from reversed phase column with methanol/water	HPLC/UV	50 ng/L	85–105	Feltes and Levsen 1989
Drinking water	Extract with toluene; inject into instrument	HRGC/ECD	1 ppb	100–107	Belkin et al. 1985

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater	Collect sample on Hayesep-R® solid sorbent cartridge; elute with acetone and concentrate; add internal standard; solvent exchange to methanol/water	HPLC/UV/PC	5–14 µg/L	62–82	Army 1989a
Water	Analyze tetryl using a monoclonal antibody with the required reactivity and specificity	Immunoassay	2 ppm	NR	Krogsrud and Lang 1990
Soil	Air dry, grind, and homogenize sample; extract with acetonitrile in ultrasonic bath; flocculate with aqueous CaCl ₂ and filter clarified solution; elute from reverse phase column with methanol/water	HPLC/UV	0.12 µg/g	83	Jenkins et al. 1989 (Proposed AOAC method)
			0.12 µg/g	96.8	Army 1990a
			NR	70–100	Bauer et al. 1990
Soil	Stabilize soil samples at 20–30% moisture; homogenize; extract in acetonitrile with sonication; filter; elute from reverse-phase column with methanol/water	HPLC/UV	4.59 µg/g	103.5	Bongiovanni et al. 1984
Soil	Air dry, grind, and homogenize; extract with acetonitrile in ultrasonic bath; filter; elute from reversed-phase column with methanol/water	HPLC/UV	5.5 µg/g	97.1	Army 1987b
Soil	Air dry and sieve sample; extract with toluene; clean up on Florisil	GC/ECD	1.5 µg/g	NR	Army 1981a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediment	Extract with benzene; centrifuge; evaporate; redissolve in benzene; clean-up with TLC; extract with benzene; evaporate; redissolve in benzene	GC/ECD	NR	NR	Navy 1972
Ocean floor fauna (rat tail fish, sea cucumber)	Homogenize sample in benzene; centrifuge; filter supernatant; clean up with TLC; extract with benzene; evaporate; redissolve in benzene	GC/ECD	≈740 pg/g	NR	Navy 1972
Explosives	Dissolve sample in acetone; dilute in methanol	HRGC/TEA HPLC/TEA	25 pg low pg	NR NR	Fine et al. 1984
Explosive debris	Extract sample with acetone; clean up on cyclohexyl column; wash with acetonitrile/water; elute with methylene chloride/hexane onto a cyanopropyl column; wash with hexane; elute with acetonitrile/water	HPLC/UV	NR	96	Strobel and Tontarski 1983
Explosives, explosive residues	Dissolve in acetone or methanol; elute from HPLC column with methanol/ammonium acetate	HPLC/TSP/MS	Low pg	NR	Berberich et al. 1988
Explosives	NR	HRGC/ECD	40 pg	NR	Douse 1981
Tetryl, picric acid, and 2,4,6-trinitroanisol	Irradiate single crystals of explosives with high-power laser pulses	MS	NR	NR	Bhasu et al. 1991

CaCl₂ = calcium chloride; CI = chemical ionization; ECD = electron capture detector; ED = electrochemical detection; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; NR = not reported; PC = photo-conductivity detector; TEA = thermal energy analyzer; TLC = thin-layer chromatography; TSP = thermospray; UV = ultraviolet detection

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GC/ECD has been used to analyze tetryl in sea water with sensitivity in the ppt range (Hoffsommer and Rosen 1972). Recovery of tetryl was adequate (70%) (Hoffsommer and Rosen 1972). The sensitivity of the HRGC/ECD method for analysis of tetryl in drinking water was in the low ppb range (Belkin et al. 1985). The sample preparation was simple and yielded recoveries of 100% (Belkin et al. 1985). Precision was very good, as shown by the relative standard deviation (RSD) range of 5.4- 7.5% for tetryl (Belkin et al. 1985).

Tetryl has also been measured in well water and surface water by HPLC/ED, using a gold-mercury electrode (Army 1986c). The technique provides a higher degree of selectivity than either UV or ECD. Sensitivity is in the low ppb range, but precision was not reported. Tetryl was best recovered using the Amberlite XAD-4® resin. A limitation with this method was that tetryl was not stable in acetone; therefore, the acetone eluates from the resin must be analyzed as quickly as possible (Army 1986c). Reversed-phase HPLC/UV is a highly selective technique that yields excellent recovery (Army 1988; Feltes and Levsen 1989). It can be used as an alternative method to gas chromatography (Feltes and Levsen 1989). Sensitivity was in the ppb-to-ppt range, and the precision was acceptable (<5-21% RSD) (Army 1988; Feltes and Levsen 1989). Tetryl was found to be unstable in a methanol-water matrix if left for an extended period of time, but was stable in a water-acetonitrile matrix. Therefore, dilution with acetonitrile prior to filtration was recommended (Army 1988). Tetryl has also been analyzed in groundwater by HPLC using UV and photo-conductivity (PC) in tandem (HPLC/UV/PC) (Army 1989a). This method allowed a solid sorbent cartridge to be used to collect the sample, which improved selectivity (Army 1989a). The serial use of the detectors effectively differentiated tetryl from other explosives and from contaminants in the solid sorbent cartridge. Sensitivity was in the low ppb range. Of all the explosives studied, tetryl was the only compound that showed evidence of chemical decomposition. Harsh conditions during the Hayesep-R® treatment were reflected in reduced recoveries (62-82%) and precision (7-47% RSD) (Army 1989a). To prevent negative baseline drift and random spikes in the PC, only highly purified water must be used and the eluent must be exhaustively degassed.

Other methods that have been used to determine tetryl in water are MS and immunoassays (Krogsrud and Lang 1990; Yinon and Laschever 1982). MS with chemical ionization (CIMS) permits direct injection of the water sample into the analytical instrument, but sensitivity was substantially less (ppm range) than with GC/ECD (ppt range) (Yinon and Laschever 1982). However, its selectivity makes it a good method for screening samples for further analysis (Yinon and Laschever 1982). A method has

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been developed for the analysis of tetryl in water using monoclonal antibodies (Krogsrud and Lang 1990). Because the detection limit achieved by the tetryl immunoassay was significantly higher (2 ppm) than that obtainable by more conventional methods such as HPLC, the assay will more likely be used as a field screen rather than a quantitative laboratory method (Krogsrud and Lang 1990).

Tetryl can be analyzed in soil using HPLC/UV (Army 1987b, 1990a; Bauer et al. 1990; Bongiovanni et al. 1984; Jenkins et al. 1989). It has also been qualitatively analyzed using HPLCAJV, GCYECD, and GC/MS (Army 1980, 1981c). The preparation steps for HPLC analysis generally involved drying, grinding, homogenizing, and extracting with acetonitrile in an ultrasonic bath (Army 1987b; Bauer et al. 1990; Bongiovanni et al. 1984; Jenkins et al. 1989). The method was rapid and selective and generally gave high recoveries from complex samples (Army 1987b; Bongiovanni et al. 1984; Jenkins et al. 1989). Supercritical carbon dioxide extraction also provides high recovery (Engelhardt et al. 1993). The lower recovery ($\approx 70\%$) and precision ($\approx 18\%$ RSD) that were found in some laboratories were attributed to thermal degradation of tetryl (Bauer et al. 1990). The problem was corrected when the sonic bath temperatures were maintained near ambient levels (Bauer et al. 1990). Similarly, tetryl is not stable during methanolic Soxhlet extraction (Jenkins and Walsh 1994). Sensitivity in the low ppm to low ppb range has been reported and precision was excellent (0.1-6% RSD) (Army 1987b; Bauer et al. 1990; Bongiovanni et al. 1984; Jenkins et al. 1989).

GC/ECD has been used to measure tetryl in ocean floor sediment and fauna (Navy 1972). Preparation steps generally involved homogenization and extraction with benzene, filtering, clean-up with TLC, and concentration. The sensitivity for detecting tetryl in ocean floor fauna using this method was estimated to be in the ppt range. Precision and accuracy were not reported (Navy 1972).

An attempt to develop an HPLC/UV method for detecting tetryl in plant stems was unsuccessful because of binding of the compound to macromolecules in the tissue (Army 1981a). A methodology based on solvent extraction of plant tissues followed by silica gel fractionation and HPLCKJV detection was used for the determination of tetryl in plant tissues (Harvey et al. 1993). This method allowed 82.7% recovery of tetryl from fortified bush bean leaves.

Several methods have been used to detect and measure tetryl in explosive materials or mixtures and in debris from explosions. The most common separation procedures used are HPLC and HRGC. These methods have been paired with TEA, UV, ECD, and MS (Army 1988; Douse 1981; Fine et al. 1984;

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Strobel and Tontarski 1983; Tamiri and Zitrin 1986). Several of the methods used were similar to those used to analyze for tetryl in hand swabs. The identification of tetryl in explosive debris usually involves extracting the debris with a suitable polar solvent such as acetone or methanol. TEA was very selective for nitroso compounds and nitramines (e.g., tetryl) and when paired with either HPLC or HRGC gave excellent selectivity, precision (2.6% RSD when paired with HRGC), and high sensitivity (low pg) (Fine et al. 1984). Because of the selectivity of this technique, there was no need for sample clean-up before analysis (Fine et al. 1984). The use of a dual column clean-up technique using both polar and nonpolar sorbents gave excellent recovery (96%) with HPLC/UV (Strobel and Tontarski 1983). Sensitivity and precision were not reported (Strobel and Tontarski 1983). With HPLC, tetryl elutes very near TNT, but a separation of TNT and tetryl may be achieved using a methanol/water (50/50) mobile phase (Army 1977). The level of sensitivity for the HPLC/UV method permits a lower limit of detection in the ppb range (Army 1977). The sensitivity of ECD was comparable to TEA (pg amounts) and precision was very good (5% RSD) (Douse 1981). A sophisticated method linking HPLC, thermospray (TSP), and MS (with both positive and negative chemical ionization) has also been proposed as a sensitive (low pg range) and selective method for detecting tetryl in explosives and explosive debris (Berberich et al. 1988). Other methods include photochemical decomposition, followed by ion-pair chromatography with electrochemical detection (Engelhardt et al. 1993) and micellar electrokinetic capillary chromatography (Kleiboehmer et al. 1993).

HRGC/MS has been used to identify tetryl in explosive debris (Tamiri and Zitrin 1986). Tetryl was reported to decompose by hydrolysis during the analysis, but its decomposition product, N-methylpicramide, was well defined and served as evidence for the presence of tetryl (Tamiri and Zitrin 1986). The authors of this report concluded that the hydrolysis of tetryl during the GC analysis could take place at the injector, which was held at relatively high temperatures (Tamiri and Zitrin 1986). MS can be used by itself for the identification of tetryl and its decomposition products, picric acid and 2,4,6-trinitroanisol, generated by laser irradiation (Bhasu et al. 1991). Chemical ionization mass spectrometry (CIMS) has also been successfully used to identify tetryl (Zitrin and Yinon 1976).

Other methods that have been used to determine tetryl in explosive material and debris from explosions are thin layer chromatography (TLC), supercritical fluid chromatography (SFC)AJV, and nuclear magnetic resonance (NMR) spectrometry (Griest et al. 1989; Margalit et al. 1986; Parker et al. 1975). TLC has been used to confirm the presence of tetryl in explosive residues using various solvent systems (Parker et al. 1975). The TLC method is suited to the analysis of bomb scene

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residues (Parker et al. 1975). SFC is complementary to GC and HPLC because of its ability to mobilize compounds not readily chromatographed by GC and because it can be interfaced with GC-like ionization detectors and mass spectroscopy more easily than can HPLC (Greist et al. 1989). The precision for the standard solutions was very good (2.8% RSD) with sensitivity in the low-ppm range (Griest et al. 1989). However, more work is needed to improve the mobile phase and column packing material before samples in complex matrices can be analyzed by this method. NMR spectrometry has also been applied to the analysis of explosive samples (Margalit et al. 1986). For unexploded samples, NMR was found to be a simple, fast, and reliable method, allowing for identification of mixtures without preseparation. Some of the postexplosion samples, which required sample preparation, were also successfully analyzed by NMR. Although sensitivity problems still exist, NMR shows promise for the difficult field of postexplosion analysis (Margalit et al. 1986).

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 tetryl 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 tetryl.

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 may be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods have been developed for detecting tetryl metabolites (picric and picramic acid) in urine (Zambrano and Mandovano 1956) and analyzing hand swabs for explosives (Douse 1982; Fine et al. 1984; Lloyd

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1983b), including tetryl. The calorimetric assays for tetryl metabolites in urine have been used only in animal studies and lack sensitivity. In addition, they are only semiquantitative, and no data on recovery or precision were provided. Analysis methods for tetryl in hand swabs have been used primarily in forensics and not on workers or the general population. No methods for measuring tetryl or its metabolites in the tissues, fluids, or excreta of humans or animals were located. One attempt to develop a method failed because of suspected binding of tetryl to macromolecules in the animal tissue (Army 1981a). There are no known sensitive biomarkers of exposure or effect for tetryl. More sensitive methods for analyzing tetryl metabolites in urine are needed in order to assess the utility of urinary metabolites in monitoring human exposure. Development of a method for extracting tetryl and metabolites from biological samples are needed so that important information on tetryl metabolism and bioconcentration can be obtained and so that biomarkers of tetryl exposure can be better defined.

Methods for Determining Parent Compounds and Degradation Products in Environmental

Media. One spectrophotometric method for detecting tetryl in air was located (NIOSH 1977). Methods exist to detect and quantify tetryl and/or its degradation products in water (Army 1986c, 1988, 1989a; Belkin et al. 1985; Feltes and Levsen 1989; Hoffsommer and Rosen 1972; Yinon and Laschever 1982), soil and sediments (Army 1987b, 1990a; Bauer et al. 1990; Bongiovanni et al. 1984; Jenkins et al. 1989; Navy 1972), ocean floor fauna (Navy 1972), and explosive materials and debris from explosions (Army 1988; Douse 1981; Fine et al. 1984; Griest et al. 1989; Strobel and Tontarski 1983; Tamiri and Zitrin 1986). These methods are relatively sensitive, selective, and reliable, and can be used to detect low levels of the compound in the environment (specifically water and soil). The most sensitive and selective methods for detecting tetryl in water and soil are HPLC-based with detection by UV and ECD (Army 1986c, 1987b, 1988, 1989a; Bongiovanni et al. 1984; Feltes and Levsen 1989; Lyter 1983). However, precision data are needed to assess the reproducibility of these methods. Methods are needed to detect and measure tetryl in fish, plants, and other aquatic and terrestrial organisms. One attempt to develop an HPLC/UV method for determining tetryl in plant tissue was unsuccessful because of binding of the compound to macromolecules in the plant tissue (Army 1981a). A method reportedly used to determine tetryl in ocean floor fauna did not give recovery data (Navy 1972). A reliable, well-characterized method for extracting and measuring tetryl in biological tissue is needed in order to investigate bioaccumulation in aquatic and terrestrial species. The most sensitive and selective methods for detecting tetryl in explosive materials and debris from explosions are HPLC/TEA and HPLC/ECD (Army 1977; Douse 1981; Fine et al. 1984). Recovery and precision data are needed in order to adequately assess the reproducibility of these methods.

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Improvements that have been made in current methods and the improvements proposed for newer methods (Griest et al. 1989; Krogsrud and Lang 1990) will be useful in monitoring environmental contamination from manufacture and disposal of tetryl and in forensics.

6.3.2 Ongoing Studies

No ongoing analytical methods studies were located.