6. ANALYTICAL METHODS

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

Methods for the analysis of ethion and its metabolites in human biological samples are given in Table 6-1. Ethion can be recovered from saliva, urine, and plasma by extraction and analysis by gas chromatography with flame photometric detection (GC/FPD), or GC in conjunction with mass spectrometry (GC/MS) (Nigg et al. 1993; Singh et al. 1986). The metabolites of ethion for which methods have been developed include diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). These metabolites are recovered from urine and saliva via extraction, solid phase extraction (SPE), and derivatization with tetrabutylammonium hydroxide before analysis using GC/FPD (Nigg et al. 1993). Ethion is also metabolized to form the oxygen analogs (monoxon, dioxon) but no methods for these compounds were found for biological samples of human origin. A method for the oxygen analogs in animal tissues has been described (Ivey and Mann 1975) and would serve as a good starting point for methods to be validated for human samples. A recent publication (Mahajna et al. 1996) has shown that O,O-dialkyl phophosphorodithioate insecticides (such as ethion) can undergo cleavage at the S-R (S-CH_3 of ethion) and be methylated. However, no methods have been described for human samples.

Table 6-1. Analytical Methods for Determining Ethion and Metabolites in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Saliva (Ethion, DEP, DETP, DEDTP)	Ethion: Extraction with hexane and addition of methanol followed by centrifugation (repeat 3 times); evaporation of solvent to dryness with re-dissolution in hexane just prior to analysis. Metabolites: Addition of NaOH to aqueous residue from ethion extraction; addition of methanol and removal of water; acidification and addition of ammonium sulfate; isolation using SPE; addition of tetrabutylammonium hydroxide just prior to GC analysis.	GC/FPD	Ethion: <1 ppm Metabolites: <260 ppb	80±10% at 1 ppm DEP: 68±3% DETP: 77±2% DEDTP: 69±1% at 260 ppb	Nigg et al. 1993
Urine (DEP, DETP, DEDTP)	Acidification and addition of ammonium sulfate; isolation using SPE; addition of 20% methanol in acetone to eluate; addition of tetrabutylammonium hydroxide just prior to GC analysis.	GC/FPD	<260 ppb	DEP: 81±3% DETP: 92±2% DEDTP 51±5%	Nigg et al. 1993
Plasma	Extraction with ethyl acetate followed by centrifugation; water removal and evaporation of solvent; re-dissolution in small volume of ethyl acetate	GC/MS (selected ion monitoring)	no data	57% at 200 ng/mL	Singh et al. 1986
Urine	Adjustment of pH to 7.4; centrifugation; extraction with ethyl acetate; centrifugation; evaporation of ethyl acetate, re-dissolution in small volume	GC/MS (selected ion monitoring)	<10 ng/mL	7,580% at 10 ng/mL	Singh et al. 1986

DEDTP = diethyl dithiophosphate; DEP = diethyl phosphate; DETP = diethyl thiophosphate; FPD = Flame photometric detector; GC = gas chromatography; MS = mass spectrometry; NaOH = sodium hydroxide; SPE = solid phase extraction

6.2 ENVIRONMENTAL SAMPLES

A variety of methods for the analysis of ethion, and to a lesser extent, ethion oxidation products (oxygen analogs), in environmental samples have been described. Ethion has limited volatility, so it is not typically measured in air. Methods have been reported for water (EPA 1992; EPA 1997c; Lopez-Avila et al. 1997; Valor et al. 1997) and are based on solvent extraction or solid phase microextraction (SPME). SPME is a technique in which a polymer-coated fiber is placed into the water and organic compounds adsorb to the film. The organic compounds are subsequently released via thermal desorption in the GC. The choice of fiber coating (polar vs. non-polar) can have a large impact on the recovery of the target analytes (Lopez-Avila et al. 1997) and is thus an important consideration in methods utilizing SPME.

Since ethion is used on plants, it is not surprising that analytical methods have been developed for many types of vegetative material, including many used as foods (see Table 6-2). The majority of methods are based on solvent extraction during or following homogenization of the sample (e.g., FDA 1997; Johnson et al. 1997; Sicbaldi et al. 1997; Torres et al. 1996) followed by GC with some form of selective detection. Supercritical fluid extraction (SFE) has also been reported as a means to isolate ethion from the plant matrix (Lehotay and Valverde-Garcia 1997; Pearce et al. 1997). The applicability of SFE has been reviewed by Lehotay (1997).

Foods of animal origin have also been investigated for ethion and some of its metabolites/decomposition products. Methods have been developed, for milk (Erney et al. 1997; Di Muccio et al. 1996); beef, turkey, and chicken (Ivey and Mann 1975; Lino and da Silveira 1994); and fatty foods, including dairy and meats (FDA 1997).

As with any analytical method, it is important to verify that the method blanks are free of interfering compounds; it is also important to be certain that the matrix does not have an impact on the recovery of the target analytes relative to solvent standards. Erney et al. (1997) have described how co-extracted matrix components can have a protective effect on the analytes. That is, these matrix components can reduce any thermal decomposition in the injector port and give rise to recoveries well in excess of 100% relative to solvent standards. The use of spiked matrix samples during method development and validation can bring such interferences to light.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Milk	Precipitation of protein using acetone and acetonitrile followed by extraction of the aqueous phase with dichloromethane.	GC/FPD	No data	No data	Erney et al.1997
Milk	Extraction with acetonitrile and acetone, cleanup using SPE; extract taken to dryness followed by redissolution in 1 mL acetone.	GC/FPD	0.005 mg/kg	90	DiMuccio et al. 1996
Water	Adjustment of pH of sample to 9, liquid-liquid extraction with hexane and water removal using sodium sulfate.	GC/MS	<10 ppt	113 at 10 ppt	Termonia and Termonia 1997
Water	SPME from aqueous phase	GC/TSD	No data	82 (18%RSD)	Lopez-Avila et al. 1997
Water, wastewater	SPME from aqueous phase	GC/NPD	30 ng/L	Tap water: 108±2%; Sea water: 98±2%; Wastewater: 103±8%	Valor et al. 1997
Leaves	Addition of water and wetting agent to pre-weighed leaf sections; extraction with hexane; water removal.	GC/FPD	0.5 ng	No data	Thompson and Brooks 1976
Oranges	Addition of water and wetting agent to pre-weighed leaf sections; extraction with hexane; water removal.	GC/ECD	0.5 ng	No data	Thompson and Brooks 1976

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Oranges	Homogenization of oranges; addition of C_{18} to 0.5 g of homogentae, mixing (Matrix Solid Phase Dispersion). Introduce to column and elute with ethyl acetate; volume reduction	GC/ECD	95 µg/kg	93±7% at 539 µg/kg	Torres et al. 1996
Oranges, sweet potatoes, and green beans	Homogenization of sample with drying agent. Supercritical fluid extraction.	GC/ion trap MS	4 ng/g	74–77	Lehotay and Valverde-García 1997
Fruits and vegetables	Maceration of sample in presence of acetone; filtration and addition of sodium sulfate followed by extraction with hexane; extraction with ethyl acetate and cleanup using Florisil.	GC/Thermionic NPD	<0.1 mg/kg	peaches 88 at 0.2 mg/kg	Ferreira and Fernandes 1980
Grass	Homogenization with acetone; filtration; extraction with dichloromethane; evaporation to less than 1 mL, dilution to 5.0 m; filtration clean up using GPC.	GC/MS	2 µg/kg	87±2%	Johnson et al. 1997
Vegetation	Homogenization followed by mixing with diatomaceous earth; extraction with ethyl acetate; filtration; vaporization to dryness then redissolution in acetone.	GC/NPD	0.001 µg/g	Apples 90–95 at 1 µg/g	Sicbaldi et al. 1997
Rice grains	Using supercritical fluid extraction, ethion is extracted using CO_2 + 5% methanol, 45EC, 315 bar, and dynamic flow.	SFE/GC-AED	0.01 mg/kg	75-120%	Skopec ZV et al. 1993

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Strawberries	Homogenization of sample and mixing with Extralute; supercritical fluid extraction and expansion into acetone	GC/FPD; GC/NPD	0.1 mg/kg	82–117 (3–12% RSD)	Pearce et al. 1997
Beef and turkey fat, skin, muscle, liver, kidney, heart, gizzard; (ethion, ethion mono-oxon, ethion di-oxon)	Fat: Blending of fat with sodium sulfate and 5% acetone in hexane; mixing with celite, heating; filtration; partitioning with acetonitrile; solvent evaporation; redissolution and column cleanup. Other tissue: blending of tissue with acetone followed by stirring with celite and filtration; residue extracted with hexane; volume reduction and combining of extracts, volume reduction; water removal. Partitioning with acetonitrile and clean up as for fat.	GC/FPD	Ethion: 0.002 ppm; ethion mono-oxon: 0.002 ppm; ethion dioxin: 0.005 ppm	Ethion: 86–100% ethion mono-oxon: 79–107% ethion dioxon: 63–98%	Ivey and Mann 1975
Chicken muscle and skin	Blending with acetonitrile followed by filtration; re-homogenization with acetonitrile-water; addition of zinc acetate, addition of sodium chloride and extraction with dichloromethane; removal of water from dichloromethane layer followed by extraction with hexane and cleanup using Florisil column.	GC/NPD	4.4–4.5 µg/kg	Mscle: 82±8.5% RSD at 39 µg/kg (ppb) skin: 91±8.5% RSD at 100 µg/kg (ppb)	Lino and da Silveira 1994

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Orange leaves (ethion, ethion monooxon, ethion dioxon)	Addition of wetting agent and extraction with methylene chloride, water removal, evaporation to dryness; re-dissolution in benzene.	GC/FPD, GC/ECD	No data	Ethion: 98±3% ethion monooxone: 95±2% ethion dioxon: 90±2%	Nigg et al. 1988
Wastewater	Extraction with 15% methylene chloride in hexane, water removal, solvent exchange to hexane. Clean-up using column chromatography if needed. (EPA Method 614.1)	GC/NPD, GC/MS	0.1 µg/L (ppb)	89±4.5% RSD at 1.8 mg/L (ppm)	EPA 1992
Water, soil, sediment, sludge	Less than 1% solids: continuous extraction with methylene chloride; Greater than 1% solids: If solids #30%, dilution with water and sonication prior to continuous extraction with methylene chloride. If solids >30%, extraction with methylene chloride:acetone or acetonitrile followed by methylene chloride using sonication; volume reduction followed by GPC of SPE cleanup. (EPA Method 1657)	GC/FPD	13 ng/L (ppt) with no interferences	47–149 (15% RSD)	EPA 1997c
Fatty foods (Ethion)	Extraction of fat and residues using sodium sulfate/petroleum ether; column clean-up if needed (FDA PAM Method 304).	GC/FPD	No data (sub-ppm)	>80%	FDA 1997

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Non-fatty foods (Ethion, ethion oxygen analog)	Extraction using acetone/water; filtration; extraction into organic solvent; column clean-up as needed depending on matrix (FDA PAM Method 302)	GC/FPD	No data (sub-ppm)	>80%	FDA 1997

FPD = flame photometric detector; GC = gas chromatography; GPC = gel permeation chromatography; ECD = electron capture detector; MS = mass spectrometry; NPD = nitrogen phosphorus detector; SPE = solid phase microextraction; SPE = solid phase extraction; TSD = thermionic selective detector

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 ethion is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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 ethion.

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. Methods exist for the determination of ethion in saliva (Nigg et al. 1993; LOD <1 ppm, 10% RSD) and urine (Singh et al. 1986; LOD <100 ng/mL). In the absence of a clear relationship between exposure concentrations and concentrations in biological samples, a definitive statement about the adequacy of the methods available is not possible. Methods for metabolites DEP, DETP, and DEDTP have also been described for saliva and urine (Nigg et al. 1993), but these compounds are not specific to ethion exposure. In addition, cholinesterase activity in plasma and red blood cells can be related to exposure to organophosphorus pesticides, but a decrease in activity is not unique to ethion effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for the determination of ethion in water have limits of detection of 0.1 µg/L (EPA 1992), 13 ng/L (EPA 1997c), and 10 ppt (0.01 µg/L; Termonia and Termonia 1997). Assuming a 2 L/day consumption of water, an exposure via drinking water of 0.02 µg/day could be quantified. The oral MRLs derived by ATSDR are 0.002 mg/kg/day for oral exposure in acute and intermediate conditions, and 0.0004 mg/kg/day for oral exposure in chronic conditions. These methods are adequate for determination of ethion at levels well below the MRL. Methods exist for the

determination of ethion in milk (DiMuccio et al. 1996; Erney et al. 1997, LOD=0.005 mg/kg), oranges, sweet potatoes, fruits and vegetables, strawberries, and meats (FDA 1997; Ferreira and Fernandes 1980; Ivey and Mann 1975; Lehotay and Valverde-García 1997; Lino and da Silveira 1994; Pearce et al. 1997; Torres et al. 1996). Sensitivities are as low as 4 µg/kg (ppb) (Lehotay and Valverde-García 1997). Assuming a total ingestion of 2 kg of food per day, potential exposures as low as 8 µg per day could be measured. Current methods are adequate to detect ethion in foods at the MRL levels. However, methods must be validated for other matrices before they can be used.

6.3.2 Ongoing Studies

No ongoing studies in which new methods for the measurement of ethion or its metabolites/breakdown products were identified.