7. ANALYTICAL METHODS

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

7.1 BIOLOGICAL MATERIALS

The biological half-life of guthion ranges from approximately 24 to 36 hours in humans (California EPA 2004; Loewenherz et al. 1997). As a consequence, monitoring human tissue for the parent compound only provides information regarding recent exposure or acute intoxication. Exposure to guthion is often measured by monitoring for dialkyl phosphate metabolites such as dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in the urine (Koch et al. 2002) or measuring cholinesterase activity in plasma, red blood cells, and whole blood (Vasilic et al. 1987). These methods are not specific to guthion because these metabolites are produced from the breakdown of other organophosphate compounds as well. Therefore, monitoring for DMP, DMTP, and DMDTP provide information regarding the potential exposure to organophosphate pesticides in general.

Quantification of the metabolites DMP, DMTP, and DMDTP in urine samples is typically accomplished using gas chromatography (GC) with nitrogen phosphate detection (NPD) or with flame photometric detection (FPD). Sample preparation usually includes solid-phase extraction, azeotropic distillation, and derivatization with pentafluorobenzylbromide (PFBB) in order to convert the dialkyl phosphate acids to esters (Loewenherz et al. 1997). Recoveries are usually around 90% and detection limits for the metabolites are in the parts per billion (ppb) range (Koch et al. 2002; Loewenherz et al. 1997).

GC with NPD or electron capture detection (ECD) has been used to quantify levels of guthion and other pesticides in human serum and urine (Pitarch et al. 2001). Mass spectroscopy (MS) in ion selective mode is used to confirm peak identity of the suspected compounds. These analyses require either solid-phase

extraction (SPE) with a C_{18} cartridge or liquid-liquid microextraction (LLME) procedure prior to quantification. In general, recoveries in both urine and serum were high for guthion (96%≥ depending upon the extraction procedure) and the detection limits are 1.7–6.0 µg/L for urine and 10 µg/L for serum (Pitarch et al. 2001). For human serum samples, the authors determined that the SPE extraction procedure was the preferred method since it was faster, less tedious, and avoided the formation of emulsions that were frequently encountered in the LLME procedure.

Organophosphates such as guthion cause toxic effects in humans primarily through the inhibition of acetylcholinesterase enzyme. Spectroscopic methods of measuring the depression of cholinesterase activity are based on the Ellman method (Ellman et al. 1961). Acetylthiocholine is hydrolyzed by acetylcholinesterase (AChE—also referred to as erythrocyte acetylcholinesterase or red blood cell [RBC] acetylcholinesterase) and plasma cholinesterase (PChE—also referred to as butrylcholinesterase, serum cholinesterase, or pseudocholinesterase), producing acetic acid and thiocholine. Thiocholine reacts with the Ellman reagent dithionitrobenzoic acid (DTNB) to produce the anion of 5-thio-2-nitrobenzoic acid, which forms a yellow color that is measured spectrophotometrically at 412 nm. The rate of color formation is proportional to the amount of either AChE or PChE. An adaptation of the Ellman assay is a microtiter assay method for AChE that has been developed by Doctor et al. (1987). The AChE samples to be assayed are added to microtiter plates and enzymatic hydrolysis is initiated by adding Ellman reaction mixture (DTNB). The hydrolysis reaction is terminated by the addition of an AChE inhibitor (1,5-bis(4-allyldimethylammoniumphenyl)-pentan-3-one dibromide. The absorbance of the microtiter is measured continuously at 405 nm.

An automated version of the Ellman assay has been implemented by the State of California to detect exposure to organophosphate pesticides in field workers (Knaack et al. 1978). Samples of whole blood and plasma are diluted with tris(hydroxymethyl)aminomethane (0.05 Molar) and sodium chloride (0.114 Molar) buffer adjusted to pH 7.7 with hydrogen chloride. The samples are centrifuged at 1,600 rpm for 4 minutes to separate red blood cells from plasma, which are then analyzed for esterase activity using a continuous flow Technicon Analyzer. Prediluted whole blood or plasma samples are passed through a 37 °C dry bath incubator for approximately 1 minute. The sample is then passed through a 12-inch dialyzer equipped with a Type C membrane and the released thiocholine is passed through a solution of DTNB. The thiocholine DTNB mixture is sent to a delay coil for color development prior to being passed through a 15x1.5 mm flow cell.

Methods for analyzing guthion in biological samples are shown in Table 7-1.

Table 7-1. Analytical Methods for Determining Guthion and Various Metabolites in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human blood	Solid-phase extraction (SPE) with C_{18} cartridge followed by elution with MTBE	GC/NPD	10 µg/L	119–121	Pitarch et al. 2001
Human blood	Collection of blood samples, addition of 0.1 M phosphate buffer (pH=8.0) and DTNB	UV absorbance (at 410–412 nm)	No data	No data	Ellman et al. 1961
Urine	Solid-phase extraction (SPE) with C_{18} cartridge followed by elution with MTBE	GC/NPD	1.7 µg/L	96–107	Pitarch et al. 2001
Urine	Liquid-liquid microextraction (LLME) using dichloromethane	GC/NPD	6.0 µg/L	98–109	Pitarch et al. 2001
Urine	Solid-phase extraction, followed by derivitization with PFBB	GC/FPD	7.4 µg/L (DMP) 1.1 µg/L (DMTP) 0.7 µg/L (DMDTP)	85–137	Koch et al. 2002
Urine	Solid-phase extraction, followed by derivitization with PFBB	GC/FPD	15 µg/L (DMTP) 13 µg/L(DMDTP)	47–116	Loewenherz et al. 1997

GC = gas chromatography; FPD = flame photometric detector; DMP = dimethyl phosphate; DMTP = dimethyl thiophosphate; DMDTP = dimethyl dithiophosphate; DTNB = dithionitrobenzoic acid (Ellman reagent); MTBE = methyl t-butyl ether; NPD = nitrogen phosphorous detector; PFBB = pentafluorobenzylbromide; UV = ultraviolet

7.2 ENVIRONMENTAL SAMPLES

The detection and analysis of guthion in environmental samples is routinely accomplished by GC/NPD, GC/FPD and GC/MS techniques. Organophosphate pesticides such as guthion may also be detected by the electron capture detector; however, the GC/ECD is not as specific as the NPD or FPD (EPA 2000b). Like most organophosphate pesticides, guthion is subject to hydrolysis under alkaline conditions; therefore, care must be exercised during the extraction and storage process in order to avoid hydrolytic degradation. Aqueous extraction is usually performed at neutral pH with methylene chloride using separatory funnel techniques such as EPA Method 3510 (EPA 1996a). Solid samples may be extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using Method 3540 (Soxhlet extraction) (EPA 1996b), Method 3541 (automated Soxhlet extraction) (EPA 1994a), Method 3545 (pressurized fluid extraction) (EPA 1998a), Method 3546 (microwave extraction) (EPA 2000a), or other appropriate technique. Method 3550 (ultrasonic extraction) is not as rigorous as other extraction methods for soils/solids, and EPA has not yet validated this technique for organophosphate pesticides (EPA 1996c). Storage is maintained under dark conditions at 4 °C in order to minimize biotic and abiotic degradation. Extraction is usually performed within 7 days of sample collection and analysis should begin within 40 days of extraction. Cleanup procedures using Florisil, silica gel, size exclusion chromatography, or some other appropriate method is usually required to remove various contaminants found in environmental matrices. Detection limits in water and soil are 0.10 μg/L and 5 μg/kg, respectively, using EPA Method 8141B (GC/FPD) (EPA 2000b). Method 8270D is a GC/MS method used for the detection of guthion in groundwater and has a detection limit of 100 μg/L (EPA 1998b). Air samples can be analyzed for the presence of guthion by GC/FPD as described by NIOSH Method 5600 (NIOSH 1994). The detection limit for this method is approximately 0.0012 mg/m^3.

Several analytical methods have been published in the open literature that summarize the analysis of guthion in environmental samples including fruits/foods/juices (Danis et al. 2002; Kyriakidis et al. 2001; Sheridan and Meola 1999). Using GC coupled with flame thermionic detectors (FTD) or MS detectors, Danis et al. (2002) demonstrated guthion detection limits in the low μg/kg range for fresh and canned peaches. Recoveries in spiked samples were essentially 100% using an SPE method with nonporous carbon-based packing (Danis et al. 2002). GC with ion trap tandem MS/MS was used to detect guthion and other pesticides at the parts per billion (ppb) levels in fruits, vegetables, and milk (Sheridan and Meola 1999). GC/NPD was used to detect guthion in peach and orange juice (Kyriakidis et al. 2001). Household or vehicular dust samples are analyzed for the presence of guthion using solvent extraction

followed by size exclusion chromatography and analysis by GC/MS (Moate et al. 2002; Simcox et al. 1995).

Methods for analyzing guthion in environmental samples are shown in Table 7-2.

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 guthion 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 guthion.

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. The most specific biomarkers for exposure to guthion are the parent compound itself and metabolites in body fluids. However, because guthion is rapidly metabolized and eliminated (see Section 3.4), the parent compound may only be found in cases of acute exposure to considerable amounts of the pesticide (Pitarch et al. 2001). Although an analytical method has been described for determining the level of guthion in blood and urine (Pitarch et al. 2001), exposure is usually analyzed by measuring the level of urinary metabolites DMP, DMTP, and DMDTP. Methods exist that can measure background levels as well as levels at which biological effects might occur for these metabolites in urine by GC or GC/MS (Koch et al. 2002; Loewenherz et al. 1997). These three metabolites are not specific to guthion, and may be present due to exposure to other organophosphates. A biomarker of exposure specific to guthion is needed.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on sorbent filter with a sampling flow rate of 0.2–1.0 L/minute. Extraction with toluene/acetone (9:1)	GC/FPD	0.0012 mg/m^3	97	NIOSH 1994
Air	Collection with high volume sampler followed by extraction with ethyl acetate/hexane	GC/MS SIM	No data	76	Foreman et al. 2000
Water	Sepratory funnel extraction with methylene chloride at neutral pH	GC/FPD	0.10 µg/L	101–126	EPA 2000b (Method 8141)
Groundwater	Sepratory funnel extraction with methylene chloride at neutral pH	GC/MS	100 µg/L	No data	EPA 1998b (Method 8270)
Soil	Extraction with hexane-acetone (1:1) or methylene chloride-acetone (1:1), cleanup with Florisil, silica gel, size exclusion chromato-graphy, or sulfur	GC/FPD	5 µg/kg	87–156	EPA 2000b (Method 8141)
Soil	Extraction with acetone/ dichloromethane (1:1)	GC/FPD	10 µg/kg	No data	Gamon et al. 2003
Soil	Ultrasonic sonication with acetone. Separation with hexane and water followed by drying with anhydrous sodium sulfate	GC/MS SIM	32 µg/kg	90	Simcox et al. 1995
Dust	Sieve samples to remove debris followed by acetone extraction and cleanup with size exclusion chromatography	GC/MS	55 µg/kg	62–81 (house dust); 81.4–106 (vehicle dust)	Moate et al. 2002
Dust	Collection with high volume surface sampler, sieve samples through mesh to remove debris, followed by extraction with acetone	GC/MS SIM	40 µg/kg	77	Simcox et al. 1995
Sediment	Soxhlet extraction in hexane/acetone	GC/MS SIM	14 µg/kg	70–100	Villa S et al. 2003
Sediment	Soxhlet extraction in acetone/dichloromethane	GC/ECD	0.20 µg/kg	96	Knuth et al. 2000

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fruit (peaches)	Homogenization followed by extraction with acetonitrile/toluene (3:1)	GC/FTD; GC/MS	8 µg/kg (GC/FTD); 12 µg/kg (GC/MS)	100–105	Danis et al. 2002
Fruit, vegetables, milk	Homogenization followed by extraction with acetonitrile/ethanol (95:5)	GC/MS/ MS	ppb range	No data	Sheridan and Meola 1999
Apples	Homogenization and extraction with acetone/hexane (5:1), followed by cleanup with gel permeation chromatography and Florisil	GC/MS	0.022 µg/kg	84	Rawn et al. 2006
Fruit juice	Extraction with ethyl acetate and sodium sulphate, followed by filtration with No. 1 Whatman filter paper	GC/NPD	0.004 mg/kg	87–110 (orange juice); 92–108 (peach juice)	Kyriakidis et al. 2001
Fish and macrophytes	Homogenized samples were extracted with acetone/dichloroethane	GC/FPD	0.20 µg/kg (fish); 0.22 µg/kg (macrophyte)	105 (fish); 86 (macro-phyte)	Knuth et al. 2000

GC = Gas chromatography; ECD = electron capture detector; FPD = flame photometric detector; FTD = Flame thermionic detector; MS = mass spectrometry; NPD = nitrogen phosphorous detector; SIM = selected ion monitoring

Effect. Guthion causes toxic effects in humans through the inhibition of acetylcholinesterase, thereby resulting in a buildup of acetylcholine at the neuromuscular junction and affecting neuromuscular transmission. Diagnosis of organophosphate poisoning, including guthion, can be made by the presence of characteristic clinical signs and measurements of serum (plasma) cholinesterase and RBC acetylcholinesterase activities. Enzyme inhibition, however, is not specific for organophosphates since exposure to carbamate insecticides also results in cholinesterase inhibition. Nonspecific cholinesterase (pseudocholinesterase, butyrylcholinesterase) is present in myelin, liver, and plasma, whereas acetylcholinesterase is present in the central and peripheral nervous systems and in RBC. A spectroscopic method exists which can measure the depression of cholinesterase activity (Ellman et al. 1961). Erythrocyte acetylcholinesterase or AChE and plasma butrylcholinesterase or PChE are both measured to diagnose exposure to organophosphates; however, it is believed that AChE is a more accurate test of synaptic acetylcholinesterase (Tafuri and Roberts 1987). The PChE measurement determines the pseudocholinesterase activity in the liver, which may be depressed by factors other than organophosphate exposure such as liver disease caused by cirrhosis or hepatitis. In addition, normal cholinesterase values vary widely in the human population, and a person with baseline activity near the upper limit of normal could be exposed to organophosphates and still have a reading within normal limits (Midtling et al. 1985; Tafuri and Roberts 1987). Thus, one data need is the development of markers specific to guthion, which enable early and reliable detection of systemic responses and health effects arising from such exposures.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining guthion levels in air (Foreman et al. 2000; NIOSH 1994), water (EPA 1998b; 2000b), soil (EPA 2000b; Gamon et al. 2003), sediment (Knuth et al. 2000; Villa et al. 2003), and various foods (Danis et al. 2002; Kyriakidis et al. 2001; Sheridan and Meola 1999) exist. These methods provide well-tested, reliable, and sensitive means for the analysis of guthion in environmental media. These methods are sensitive enough for measuring background levels and levels at which adverse health effects might occur. No additional analytical methods for determining low levels of guthion in environmental media are needed at this time.

7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2006) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs pertinent to the analysis of guthion in biological or environmental samples. Researchers at the University of Maine Laboratory for Surface

Science and Technology Center are developing an organophosphate pesticide vapor sensor and testing the feasibility of using this sensor to detect residues of two pesticides (guthion and phosmet) on blueberries.