7. ANALYTICAL METHODS

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

Atrazine can be detected in mammalian biological samples, as well as in foodstuffs related to human consumption. It has been detected in human saliva (Denovan et al. 2000), skin (Lioy et al. 2000; Lorberau and Pride 2000), plasma and organ tissues (Pommery et al. 1993), liver samples (Lang et al. 1996), and urine (Ikonen et al. 1988; MacIntosh et al. 1999) using gas chromatography (GC), high performance liquid chromatography (HPLC) (Buchholz et al. 1999), and enzyme-linked immunosorbent assay (ELISA) methods (Trochimowicz et al. 2001). A summary of various methods is supplied in Table 7-1.

Human tissue or other samples suspected of containing atrazine are usually extracted from the tissue or fluid sample prior to analysis. For urine analysis, urine samples can be extracted with diethyl ether. This solvent is recovered and combined with ethyl acetate. The ethyl acetate fraction is evaporated to a smaller volume and analyzed by GC (Ikonen et al. 1988). For liver tissue microsomes, the material is extracted with a solvent, such as dichloromethane (Lang et al. 1996), which is then evaporated. The residue containing the atrazine or its metabolites is dissolved in acetonitrile and analyzed by HPLC. For saliva samples, the material is simply centrifuged and then is used directly for ELISA analysis (Denovan et al. 2000).

Table 7-1. Analytical Methods for Determining Atrazine in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	The sample is first amended with saturating amounts of sodium chloride, then extracted with two volumes of diethyl ether; ether layer recovered and extracted with ethyl acetate (this was reduced to 1/5 its volume by evaporation)	GC	1 µg/L	Not reported	Ikonen et al. 1988
Plasma, organ tissues	Blood collected in heparinized tubes, centrifuged; plasma stored at -20 °C; atrazine was extracted from plasma with dichloromethane, evaporated to dryness under N_2, washed with acid and base, then dissolved in mobile phase (40% water; 60% methanol)	HPLC	14.25 ng/g	58–61%	Pommery et al. 1993
Saliva	Saliva collected on a cotton sampler (Salivette); the sampler is centrifuged, and cotton material removed, leaving the filtrate; sample used directly	ELISA	0.22 µg/L	Not reported	Denovan et al. 2000
Liver microsomes	The sample is extracted with dichloromethane, and then evaporated; the residues are dissolved in acetonitrile/aqueous KOH (5 mM)	HPLC-UV	2–5 pmol	96–103%	Lang et al. 1996
Food	EPA-approved method 4670 for drinking water that has been used for food; sample is minced or liquified, then filted and brought to neutral pH; then followed by proprietary ELISA method	ELISA	0.1 µg/L	Not reported	SDI 1999
Eggs	Supercritical fluid (carbon dioxide) extraction of eggs, followed by hexane and benzene in acetone elution, followed by GC-NPD analysis	HPLC	100 µg/kg	90.4%	Pensabene et al. 2000
Hand (dermal) contamination	Hand washed in 150 mL of isopropanol in a polyethylene bag for 30 seconds. Solution transferred to a glass jar; 10 mL removed from jar, derivatized in diazomethane derivitizing agent, silicic acid is added, followed by sample filtration and analysis by GC-ECD	GC-ECD	0.01 µg/mL	87.1–103%	NIOSH 1998b

ECD = electron capture detection; ELISA = enzyme-linked immunosorbent assay; EPA = Environmental Protection Agency; GC = gas chromatography; HPLC = high performance liquid chromatography; UV = ultraviolet

7.2 ENVIRONMENTAL SAMPLES

Atrazine can be determined in environmental samples using chromatographic, spectroscopic, and immunogenic methods. Standard EPA methods include Infrared spectroscopy, GC separation with flame ionization detection, and HPLC with detection at 254 nm (ultraviolet [UV]) (Stafford et al. 1992). Different GC methods have been used for atrazine detection and quantification and include GC coupled with a flame ionization detector (FID) (IARC 1999), GC coupled with an electron capture detector (ECD) (Albanis et al. 1998; Lopez-Avila et al. 1992; Trevisan et al. 1993; Walker and Porter 1990), GC coupled with a nitrogen-phosphorus detector (NPD) (Albanis et al. 1998; Amistadi et al. 1997; Ferrari et al. 1998; Mojasevic et al. 1996; Novak and Watts 1996; Sabik and Jeannot 1998; Trevisan et al. 1993), or GC coupled with a mass spectrometer (MS) detector (Albanis et al. 1998; Benfenati et al. 1990; de Almeida Azevedo et al. 2000; Hernandez et al. 2000; McLaughlin and Johnson 1997; Sabik and Jeannot 1998). Some GC methods have been refined into standard EPA methods for analysis of atrazine in drinking water and waste water. For GC-MS detection of atrazine, EPA methods 508.1 and 525.2 can be used. For detection of atrazine by GC-ECD, EPA methods 505 and 551.1 can be used, and for detection by GC-NPD, EPA methods 507 and 8141A can be used (IARC 1999).

HPLC methods generally use reverse-phase columns such as C-8, C-18, or octadecylsilane (ODS)-columns, and the sample constituents are resolved in different solvent systems. These have included acetonitrile/water gradients (Dankwardt et al. 1995), methanol/ammonium acetate gradient (Marcé et al. 1995), ammonium acetate/water gradients (Abián et al. 1993), or water/methanol gradients (Hogenboom et al. 1997). Detection of atrazine is done using a UV detector (Dankwardt et al. 1995), a diode array (Marcé et al. 1995) or MS (Abián et al. 1993; de Almeida Azevedo et al. 2000; Marcé et al. 1995) detection. Immunogenic methods are usually based on ELISA using sheep-based antibodies to atrazine (Amistadi et al. 1997; Dankwardt et al. 1995; Turiel et al. 1999). Other immunogenic methods have been developed in which the antibody is bound to a “dipstick”, and this is used to evaluate concentrations of atrazine in water or liquid food samples (Wittmann et al. 1996), while other sampling approaches have used immuno-affinity systems to concentrate atrazine prior to analysis by GC (Dallüge et al. 1999).

Aqueous samples suspected of containing atrazine may be concentrated and/or partly purified using solid phase extraction (SPE) or other approaches. Different matrices can be used for these SPE extractions, including XAD-2 resin-based columns (Baun and Nyholm 1996), or C_8 or C_{18} extraction columns

(commercially available as “Sep Pak”, “Bakerbond-SPE”, “Bondpac”, “Carbopak”, or others) (Albanis et al. 1998; Ferrari et al. 1998; Gaynor et al. 1995; McLaughlin and Johnson 1997; Mojasevic et al. 1996; Novak and Watts 1996), or combined solid phase columns. In the latter case, one combined solid phase columns consisted of 66.6% C-18 silica-bonded phase and 33.3% phenyl silica-bonded phase (Benfenati et al. 1990). Subsequent analysis of atrazine-containing samples by GC-MS analysis permitted a detection limit of atrazine of 0.002 μg/g (2 parts per trillion). The use of XAD-2 resins (Baun and Nyholm 1996) has been applied to bioassay of atrazine and the SEP-PAK preconcentration has been used prior to GC analysis (Mojasevic et al. 1996; Novak and Watts 1996). The other methods that can be used to improve extraction of the atrazine include microwave assisted extractions (Bouaid et al. 2000) and supercritical fluid extraction of atrazine from foodstuffs (Pensabene et al. 2000). A summary of methods of analysis of atrazine in environmental samples is supplied 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 atrazine 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 atrazine.

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. Atrazine can be detected in a number of human tissues including urine (MacIntosh et al. 1999), plasma (Trochimowicz et al. 2001), skin (Lioy et al. 2000), and saliva (Denovan et al. 2000). Detection limits are not uniformly characterized, but for urine, are likely to be 1 μg/L (McIntosh et al. 1999). There are needs for better and

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

Sample matrix	Preparation method	Analytical method	Sample detecton limit	Percent recovery	Reference
Water	Cleanup through immuno-affinity filter, desorbed by glycine buffer, dried, then dissolved in ethyl acetate	GC-NPD	1.5 ng/L (NPD)	88–96%	Dalluge et al. 1999
Water	Solid phase microextraction of samples prepared for method validation; samples desorbed from SPE material directly in the injection port of the GC by exposure for 5 minute at 240 °C	GC-NPD	7.4 ng/L	Not reported	Ferrari et al. 1998
Subsurface waters	SPE of water samples containing atrazine; analysis conducted by GC-NPD, GC-ECD, or GC-MS; 2-L volumes of water were filtered onto the SPE matrix; samples eluted using dichloromethane, then volumes reduced under a stream of nitrogen	GC-NPD GC-ECD GC-MS	2 ng/L	85–110% for spiked surrogates	Albanis et al. 1998.
Surface waters	1–20 L of river water extracted by liquid-liquid technique (dichloromethane-water) or by SPE; dichloromethane (pesticide containing fraction) was collected and evaporated to dryness and sample dissolved in ethyl acetate; SPE with carbon black (Carbopack B; 500–666 µm) was used as the SPE; samples eluted by ethyl acetate	GC-NPD HPLC	0.4 ng/L GC 0.6 ng/L (HPLC)	67–100%	Sabik and Jeannot 1998
Sediments, aquatic plants	Material ground in a Wiley-mill, extracted in ethyl acetate, then sonicated material treated again, and extracts combined, then concentrated under a stream of nitrogen; dried material was dissolved in hexane	GC-ECD	Not reported	90%	Bennett et al. 2000

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

Sample matrix	Preparation method	Analytical method	Sample detecton limit	Percent recovery	Reference
Water and soil	Leachates of water and soil used to compare GC to ELISA approaches to atrazine detection; SPE used to concentrate samples prior to GC analysis; the RaPID assay ELISA kit was used (Strategic Diagnostics, Newark, Delaware)	GC-NPD ELISA	Water: 100 ng/L GC 50 ng/L ELISA Soil: 1.0 µg/kg GC 200 ng/kg ELISA	Not reported	Amistadi et al. 1997
Water and soil	Solid Phase Microextraction of pesticides from water sample; samples extracted from soil using microwave assisted extraction into methanol	GC-MS	Water: 40 ng/L Soil: <3 µg/kg	Soil: >80%	Hernandez et al. 2000
Soil	EPA method 8081A tested on soil extracts using SPE extraction followed by hexane elution and GC analysis; discusses linearity of response and reproducibility	GC-ECD	Not reported	Not reported	Lopez-Avila et al. 1992
Water and soil	EPA approved method 4670 for drinking water; water sample is filtered and brought to neutral pH, followed by proprietary ELISA method	ELISA	0.1 µg/L	Not reported	SDI 1999
Household dust	Sample collected by two different types of samplers that mimic uptake of a chemical by a person's hand that is placed on dusty surfaces; samples washed from sampler by sonication in hexane, followed by GC	GC-ECD	0.21 ng/cm^2 EL 4.0 ng/cm^2 LWW	Not reported	Lioy et al. 2000
Water	AOAC method for analysis of pesticides in water, including dealkylated atrazine; sample is extracted in dichloro-methane, dried over anhydrous sodium sulfate, brought up in methanol and concentrated	HPLC-UV	5.0 µg/L	89.6%	AOAC 1993

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

Sample matrix	Preparation method	Analytical method	Sample detecton limit	Percent recovery	Reference
Air	NIOSH method 5602, air samples are collected in a filter/sorbent tube at a flow rate of 0.2–1 L/minute, for a total volume of 12–480 L; following collection, sample is derivitized with diazomethane-derivitizing agent, silicic acid is added, then the sample is filtered, and analyzed by GC-ECD	GC-ECD	0.2 µg/sample	Not specifically reported (all analytes tested ranged from 69 to 150%)	NIOSH 1998a
Hand contamination	Hand washed in 150 mL of isopropanol in a polyethylene bag for 30 seconds; solution transferred to a glass jar; 10 mL removed from jar, derivatized in diazomethane derivitizing agent, silicic acid is added, followed by sample filtration and analysis by GC-ECD	GC-ECD	0.01 µg/mL	87.1–103%	NIOSH 1998b

AOAC = Association of Official Analytical Chemists; ECD = electron capture detection; ELISA = enzyme-linked immunosorbent assay; EPA = Environmental Protection Agency; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; NIOSH = National Institute for Occupational Safety Chromatography; NPD = nitrogen-phosphorus detector; SPE = solid phase extraction; UV = ultraviolet

more uniform extraction methods for background levels of atrazine in the general population. Based on the analysis of atrazine in the saliva of pesticide applicators, levels in saliva appear to be at background levels at approximately 0.9 μg/L when sampled from the workers 1 month prior to the spraying season (Denovan et al. 2000). However, it was not known whether these reflected levels of background atrazine concentrations or metabolism of fat-stored atrazine in this population of pesticide workers. Therefore, more measures of salivary atrazine levels from the general population, or other potentially-exposed populations would be warranted to acquire a better understanding of atrazine background levels and background exposure levels. Other methods that attain a lower detection limit from other biological samples (e.g., urine analysis, blood analysis) may provide more sensitivity.

No data were located concerning methods of biological markers of atrazine effects. Atrazine has little toxicological effect, and does not produce uniform cancer-related effects (see Chapter 3) in laboratory animals. Therefore, at this time, it is not expected that accurate biomarkers would be found for atrazine.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for detection of atrazine in water, soil, sediments, food, household dust, subsurface samples, and air are based on GC, ELISA, and HPLC. The media considered to be of most concern for human exposure are food, water, and soil. As shown in Table 7-2, the most sensitive methodologies appear to be the ELISA based approaches and the GC-ECD, with detection limits of 0.4 ng/L for GC-NPD (Sabik and Jeannot 1998) and 2 ng/L for GC-NPD, GC-ECD, and GC-MS (Albanis et al. 1998). The ELISA assays will likely provide a much less expensive approach to environmental atrazine concentration determinations, with sensitivities approaching the levels of GC.

7.3.2 Ongoing Studies

The information in Table 7-3 was found as a result of a search of Federal Research in Progress (FEDRIP 2002) and Current Research Information System (CRIS 2002). These studies are being conducted to provide better means for food and environmental sample analysis. Most of the studies listed are examining the use of SPE approaches for better environmental sample stabilization prior to analysis.

Table 7-3. Ongoing Studies on the Development of Analytical Approaches to the Study of Atrazine

Investigator	Affiliation	Research description	Sponsor
Camper ND, Riley MB	Clemson University, Clemson, South Carolina	Evaluation of SPE approaches for improving extraction of and stabilization of pesticides from water samples. Sampling and approaches were tested, and stability of environmental samples was shown to be better when shipped in SPE matrices as compare to shipment of water samples. Should lead to better accuracy of determinations of pesticides in aquatic matrices.	Hatch award
Giesy J	CSREES, Michigan State University	Development of chemical fractionation techniques, instrumental analyses, and bioassays to screen for "estrogenic" compounds in animal tissues and human food stuffs. Development of the use of wildlife sentinels for the effects of these compounds. Monitor for ecological health effects. Exposure evaluations.	Hatch
Hatfield JL	CSREES, New Jersey	Develop new and improved methods to detect environmentally important compounds and assess agricultural impacts on water, soil, and air quality. Methods will be developed to extend analytical procedures from development to production scale application.	USDA
Leidy RB	North Carolina State University, Raleigh, North Carolina	Development of solid phase extraction approaches for collection and stabilization of pesticides from water samples. Research will try to demonstrate that the SPE disks improve stability of sample during transport (over shipment of water samples), and will result in less error between test labs.	Hatch
Leidy RB	North Carolina State University, Raleigh, North Carolina	Validate methods for analyses conducted with 3M Empore disc membranes for pesticides including atrazine.	Hatch
Montvaldo R et al.	University of Puerto Rico, Mayaguez, Puerto Rico	Evaluation of solid phase extraction approaches for sampling water for pesticides. Testing of sample showed excellent recoveries of test pesticides. Will lead to better analysis of pesticides in field samples by minimizing transportation and storage losses.	Hatch
Mueller TC	University of Tennessee, Knoxville, Tennessee	Evaluation of SPE approaches for stabilization of pesticides in water. Research demonstrated that the SPE approaches improved pesticide stability during transport.	Hatch

Table 7-3. Ongoing Studies on the Development of Analytical Approaches to the Study of Atrazine

Investigator	Affiliation	Research description	Sponsor
Sadowsky MJ, Wackett LP	CSREES, Minnesota	Investigation of the genetic regulation of the atrazine degradation genes by soil factors, such as the presence of nitrate and ammonia and triazine herbicides, and use these genes to design biosensors that can be used to detect triazine compounds in soils and water.	NRI

Source: CRIS 2002; FEDRIP 2002

CSREES = Cooperative State Research, Education, and Extension Service; NRI = National Research Institute; SPE = solid phase extraction; USDA = U.S. Department of Agriculture