6. ANALYTICAL METHODS

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

Several methods are available for analyzing disulfoton in biological media; some of the commonly used methods are reported in Table 6-l. A variety of detectors may be used for the gas chromatographic analysis of disulfoton, but flame photometric detectors are superior because of low background interference and good reproducibility (Holstege et al. 1991). Mass spectrometric detectors show high specificity (Kawasaki et al. 1992) and may also be used to confirm detection by other methods.

The stability of disulfoton must be considered at all stages of sample storage and analysis. Organophosphorus insecticides, including disulfoton, react with natural esterases in human tissue and may reduce the level of free organophosphates (Singh et al. 1986). Besides the esterases, phosphorylphosphatase in natural tissue hydrolyzes and inactivates organophosphorus compounds (Singh et al. 1986). As a result, the amount of organophosphates in blood and excreted in urine will be considerably less than the amount expected from its concentration at the time of exposure (Hattori et al. 1982; Singh et al. 1986).

As discussed in Section 2.5.1, the detection of certain thiophosphate esters in human urine may indicate exposure to disulfoton and/or other organophosphate insecticides. Several methods are

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Cow feces (disulfoton and 5 metabolites as total residue)	Extraction with chloroform; concentration then oxidation with m-chloroperbenzoic acid; clean-up by column chromatography	GC/FPD	1 µg/kg[a]	74	Bowman and Beroza 1969
Bovine liver and rumen (disulfoton only)	Extraction with methanol-methylene chloride; clean-up by column chromatography; concentration	Capillary GC/FPD	10–50 µg/kg	90–109	Holstege et al. 1991
Plasma and urine (disulfoton only)	Plasma: extraction with ethyl acetate; urine: adjustment to pH 7.4, centrifugation, extraction with ethyl acetate	Capillary GC/MS (SIM)	No data	>75 (urine) <10 (blood)	Singh et al. 1986
Blood, urine, and stomach content (disulfoton and its metabolites)	Dilution with 2% saline; fractionation by column chromatography; analysis; then oxidation with potassium permanganate; fractionation by column chromatography	Capillary GC/FPD; GC/MS	No data	101–105	Yashiki et al. 1990
Blood and urine	Homogenization of sample with acetonitrile; extraction with hexane; concentration; dissolution in acetonitrile	GC/MS (SIM)	No data	70–90	Hattori et al. 1982

[a]Instrumental detection limit

GC = gas chromatography; FPD = flame photometric detection; MS = mass spectrometry; SIM = selected ion monitoring

available for the quantitation of organophosphorus metabolites from urine (Bradway et al. 1981; Daughton et al. 1976; Lores and Bradway 1977; Shafik et al. 1973).

6.2 ENVIRONMENTAL SAMPLES

Analytical methods for determining disulfoton in environmental samples are reported in Table 6-2. The steps included in the methods are solvent extraction, purification and fractionation, and gas chromatographic analysis. Other analytical techniques, including capillary gas chromatography with mass selective detection (Stan 1989), high-performance liquid chromatography with either mass spectrometric (MS) or MS-MS detection (Betowski and Jones 1988), have been used to determine disulfoton in environmental samples.

Precautions should be taken to avoid disulfoton loss from stored water, soil, sediment, crop, and vegetable samples (Belisle and Swineford 1988; Miller et al. 1981; Munch and Frebis 1992; Szeto and Brown 1982). Disulfoton, disulfoton sulfone, and disulfoton sulfoxide were not recovered from spiked well water stored 14 days; however, sample extracts were stable for 14 days (84-92% recovery) (Munch and Frebis 1992). In most environmental samples, disulfoton will be present along with its environmental transformation products, disulfoton sulfone, disulfoton sulfoxide, disulfoton oxon, disulfoton oxon sulfone, and disulfoton oxon sulfoxide (Szeto and Brown 1982). Disulfoton and its oxon are very unstable, and they oxidize rapidly to the corresponding sulfoxides. The sulfoxides are relatively stable, but they oxidize slowly to their sulfones, which are most stable (Szeto and Brown 1982). Several methods for determining the metabolites of disulfoton in environmental samples are included in Table 6-2.

Few methods were located for determination of disulfoton in air. Ambient air monitoring data were reported for samples collected by ethylene glycol impinger samplers with subsequent gas chromatographic analysis; however, no performance data were reported (Kutz et al. 1976). A method for determination of organophosphorus pesticides, including disulfoton, in workplace air involves collection using a combined filter/XAD-2 sorbent sampler and gas chromatography/flame photometric (FPD) detection (Kennedy et al. 1994). Recovery is very good (>90%) and samples are stable for 30 days when stored cold.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Workplace air	Collection on filter/XAD-2 sorbent; desorption with solvent	capillary GC/FPD	0.07 µg/mL[a]	91-94	Kennedy et al. 1994
Water	Addition of bromine water; incubation with lyophilized bovine acetyl-cholinesterase; addition of indophenol acetate; incubation	Colorimetric (field screening method)	0.01–0.12 ppm for sulfur-containing compounds	No data[b]	Zweig and Devine 1969
Water	Extraction with petroleum ether; concentration	Dual column GC/thermoionic detection	0.04 µg/L	94	Zweig and Devine 1969
Water	Micro-extraction with hexane	GC/FPD	0.1 µg/L	97	Bourgeois et al. 1993
Water	Adjustment of pH to 6.0; SPE extraction	Capillary GC/NPD	≤20 ng/L	92–113	Borburgh and Hammers 1992
Drinking water	Extraction with methylene chloride; solvent exchange to methyl tert-butyl ether	Capillary GC/NPD (EPA method 507)	0.3 µg/L (disulfoton) 3.8 µg/L (sulfone) 0.38 µg/L (sulfoxide)	87–107 (disulfoton) 92–104 (sulfone) 54–95.1 (sulfoxide)	Edgell et al. 1991; EPA 1988c
Groundwater	Extraction with methylene chloride; clean-up by column chromatography if required	GC/FPD (EPA method 8140)	0.2 µg/L (disulfoton)	82	EPA 1986a
Waste water	Extraction with methylene chloride; clean-up by column chromatography if required	GC/FPD	0.2 µg/L (disulfoton)	111	Miller et al. 1981
Waste water	Extraction with methylene chloride; solvent removal; optional clean-up using GPC and/or SPE columns (Method 1657)	Capillary GC/FPD, confirmation using second GC column	32 ng/L	No data	EPA 1992c
Sediment	Extraction with acetone-methylene chloride; passage through anhydrous sodium sulfate; concentration	Capillary GC/FPD	≤0.1 mg/kg	95–100	Belisle and Swineford 1988

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, asparagus tissue	Extraction with ethyl acetate; clean-up by column chromatography; oxidation of fraction containing sulfoxide and oxon sulfoxide by potassium permanganate	GC/FPD	0.01 mg/kg	83.5–110 (for disulfoton and metabolites)	Szeto and Brown 1982
Cow milk (disulfoton and 5 metabolites as total residue)	Extraction with methylene chloride; dried residue oxidize with m-chloroperbenzoic acid; clean-up by column chromatography	GC/FPD	1 μg/kg[c]	80	Bowman and Beroza 1969
Various crops and processed foods	Extraction with suitable solvent; precipitation of pigments by addition of ammonium chloride-orthophosphoric acid; oxidation with potassium permanganate	GC/thermoionic detection	0.02 mg/kg	75–100	Thornton and Anderson 1968
Fresh fruits and vegetables	Extraction of homogenized sample with acetonitrile; partition with sodium chloride solution; concentration of extract	Capillary GC/MS	0.05 mg/kg	84	Liao et al. 1991
Rice, wheat, buckwheat, and dried beans	Extraction of powdered sample with n-hexane; clean-up by liquid-liquid partition and column chromatography	GC/FPD	0.3 μg/kg	68–75	Aoki et al. 1975
Tobacco plants	Extraction finely chopped samples with chloroform-methanol; concentration; separation into three fractions by column chromatography	GC/FPD	0.01–0.04 mg/kg	88–100	Bowman et al. 1969

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Produce	Homogenization; extraction with acetonitrile; concentration; solvent exchange	Dual capillary GC/AFID and FPD	0.1 mg/kg	76–118 (all organophosphates)	Hsu et al. 1991
Hair dyes	Extraction with acetone/-hexane; clean-up by GPC and silica gel chromatography	Dual capillary GC/ECD	0.01 mg/kg	No data	Cetinkaya 1993

[a]Instrumental detection limit; method detection limit ($\mu g/m^3$) will depend upon volume of air sampled.
[b]Test is qualitative: positive or negative
[c]Instrumental detection limit

AFID = alkali flame ionization detector; ECD = Electron capture detection; EPA = Environmental Protection Agency; FPD = flame photometric detection; GC = gas chromatography; GPC = gel permeation chromatography; HRGC = high resolution gas chromatography; NPD = nitrogen-phosphorus detection; SPE = solid phase extraction device

Overall recoveries are good (>80%) and detection limits are in the low to sub-parts-per-billion (ppb) range for determination of disulfoton in water (Borburgh and Hammers 1992; Bourgeois et al. 1993; EPA 1986a, 1988c). Methods for soil and sediment also provide good recovery (>80%) and detection limits are in the ppb range (Belisle and Swineford 1988; Szeto and Brown 1982). Methods for determination of disulfoton in food matrices generally provide acceptable recovery (≥75%) and detection limits in ppb range (Hsu et al. 1991; Liao et al. 1991; Szeto and Brown 1982).

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

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. Analytical methods are available to determine the concentrations of disulfoton and its metabolites in blood, urine, and other body tissue and fluids (Bowman and Beroza 1969; Brokopp et al. 1981; Hattori et al. 1982; Holstege et al. 1991; Singh et al. 1986; Yashiki et al. 1990). The accuracy of the methods in terms of percent recovery has been determined. A reasonably low detection limit can be obtained when flame photometric detection is used to determine disulfoton (Bowman and Beroza 1969; Holstege et al. 1991). When blood samples were spiked with 0.3 μg /g (ppm) of disulfoton, phosphorodithioate sulfone, and phosphorothiolate sulfone, and quantitated by gas-chromatography-flame photometric detection (GC/FPD) method, the recovery of disulfoton and two of its metabolites were in the range of

101.0-104.6% (Yashiki et al. 1990). In spiked urine samples, the detection limits of four metabolites of disulfoton (DEP, DETP, DEDPT, and DEPTh) were 0.01 ppm by a GC/FPD method (Brokopp et al. 1983). Due to the unavailability of data regarding disulfoton concentrations in tissues and body fluids of the background population, it is not known if the available analytical methods will be sensitive enough to determine these concentrations. Analytical methods are available for the detection of 4-hydroxy-3-methoxymandelic (HMMA), the major metabolite of catecholamine metabolism and a possible biomarker to characterize effects by disulfoton (Wysocka-Paruszewska 1971). It would be helpful to determine if the available analytical methods can measure disulfoton levels in body tissues and fluids of the background population. This will permit assessment of the severity of exposure of a highly exposed population.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Several methods are available for determining disulfoton and its degradation products in environmental samples such as contaminated water, food, and soil (Edge11 et al. 1991; EPA 1988c; Grant et al. 1969; Ruzicka et al. 1968; Szeto and Brown 1982; Zweig and Devine 1969). The specificity and accuracy of the methods are generally well established (see Table 6-2). In spiked water samples, the mean recoveries of disulfoton, disulfoton sulfone, and disulfoton sulfoxide were in the range of 86.5-104.0% (Edge11 et al. 1991). The recoveries of disulfoton and its metabolites in soil and asparagus tissue ranged from 83.5% to 110% (Szeto and Brown 1982). Consumption of contaminated food is probably the most important route of disulfoton exposure for the general population. Disulfoton has been found at much lower concentrations than the AD1 value (Winter 1992; Yess 1991). Developing sensitive methods for determining disulfoton in ambient air and for establishing background levels of disulfoton in environmental samples would be desirable.

6.3.2 Ongoing Studies

Drs. Singmaster and Acin-Diaz of the University of Puerto Rico are developing methods for determining disulfoton residue levels in food commodities. No other ongoing studies regarding the determination of disulfoton and its metabolite and degradation products in biological or environmental media were found.