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 white phosphorus, its metabolites, and other biomarkers of exposure and effect to white phosphorus. 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 MATERIALS

There are no standardized methods approved by federal agencies or organizations for determining elemental phosphorus in biological samples. In biological samples, phospholipids and other biogenic phosphorus-containing compounds may be present at levels that can contribute phosphorus far in excess of that arising from elemental phosphorus contamination (Idler et al. 1981). Interference also can occur from phosphine present in the sample. Therefore, the analytical methods for determining elemental phosphorus in biological samples must be able to separate these compounds before quantitation. Since elemental phosphorus can be lost from tissues stored in a cooler (3°C) or in a freezer (-40˚C), it is suggested that the stored tissues be immersed in benzene (Fletcher 1974). Table 6-l gives the methods used for determining elemental phosphorus in biological samples. The most suitable method available at the present time (in terms of sensitivity and ease of analysis) is the gas chromatographic method with phosphorus-sensitive detectors (Idler et al. 1981). Methods are also available for determining serum and urinary phosphate levels in humans and other animals (Harper 1969; Henry 1967). Although a thin layer chromatography (TLC) method was used to identify inorganic phosphates and an undefined organic phosphate as urinary metabolites in rats (Lee et al. 1975), more accurate methods for determining intermediate and final products of metabolism of white phosphorus in animal systems are lacking. It should be noted, however,

TABLE 6-1. Analytical Methods for Determining Elemental Phosphorus and Phosphine in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Biological tissues (phosphine, metal phosphide, and elemental phosphorus)	Pass nitrogen through ground sample to remove phosphine produced by putrefication; acidify and absorb phosphine in methanolic silver nitrate after removing hydrogen sulfide by passing through lead acetate; heat residual sample and absorb released gas in methanolic silver nitrate; the phosphine trapped as silver phosphide is oxidized to phosphine with chlorine gas	Neutron activation analysis	0.5 µg/kg in 20 g tissue	90–110	Krishnan and Gupta 1970
Biological tissues (elemental phosphorus)	Extract homogenized tissues with benzene; filter	GC-FPD	2 µg/kg in 10 g tissue	77–86	Addison and Ackman 1970; Fletcher 1974

FPD = flame photometric detector; GC = gas chromatography

that metabolite measurements for animal systems may not meet the needs of differentiating those species involved in biological effects (e.g., white phosphorus, linear and cyclic phosphorus, organicallycomplexed phosphorus).

6.2 ENVIRONMENTAL SAMPLES

Most of the analytical methods available in the literature for determining elemental phosphorus are based on older analytical techniques. Gorzny (1972) has discussed some of these methods for determining elemental phosphorus. Although a method for simultaneously determining phosphine, phosphide, and elemental phosphorus in water, soil, and sediment is not given in Table 6-2, the distillation method given by Ktishnan and Gupta (1970) can be used for this purpose. Krishnan and Gupta (1970) showed that phosphine can be removed from water or suspended solids by passing an inert gas through the sample before it is acidified. Reactions with acid liberate phosphine from metal phosphide. Elemental phosphorus, on the other hand, distills only after the sample is heated. As in biological samples, both gas chromatography with phosphorus-sensitive detectors or neutron activation analysis for determining elemental phosphorus in environmental samples are sensitive and accurate enough to meet the recommended or obligatory discharge standards (Idler et al. 1981; Lai and Rosenblatt 1977b).

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 white phosphorus 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 determine the health effects (and techniques for developing methods to determine such health effects) of white phosphorus.

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.

TABLE 6-2. Analytical Methods for Determining Elemental Phosphorus and Phosphine in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (elemental phosphorus	Trap elemental phosphorus in impingers containing xylene	GC-FPD	0.4 μg/m^3	No data	Bohl and Kaelble 1973
Air (elemental phosphorus)	Trap elemental phosphorus in Tenax®-GC tubes and extract with xylene	GC-FPD	0.5 μg/m^3 (for 100 L sample)	94–108 (at 10–370 μg/m^3)	Dillon et al. 1978; NIOSH 1987
Water (elemental phosphorus)	Extract with organic solvent; oxidize to phosphate	Spectrophotometric	1.5 μg/L	No data	Zitko et al. 1970; Idler et al. 1981
Water (elemental phosphorus)	Extract with organic solvent (benzene or isooctane)	GC-FPD	0.002 μg/L	74–78	Addison and Achman 1970; EPA 1991
Waste water (elemental phosphorus)	Extract with organic solvent, oxidize to phosphate and back-extract in water	Neutron activation analysis	0.01 μg/L	90–110	Lai and Rosenblatt 1977b
Soil and sediment (elemental phosphorus)	Extract with organic solvent; filter	GC-FPD	0.1 mg/kg	77–90	Addison and Ackman 1970; Idler et al. 1981
Soil and sediment (elemental phosphorus)	Extract with isooctane	Capillary Column GC-NPD	0.88 μg/kg	97.2	Walsh and Taylor 1992; Racine et al. 1993

TABLE 6-2. Analytical Methods for Determining Elemental Phosphorus and Phosphine in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediment (phosphine)	Trap gas in impingers containing toluene	GC-FPD, GC-NPD, or GC-MS	No data	No data	Devai et al. 1988
Water (elemental phosphorus)	Extract with isooctane or diethyl ether	GC-NPD	0.011 µg/L	107±12	Walsh 1995

FPD = flame photometric detector; GC = gas chromatography; MS = mass spectrometry; NPD = nitrogen-phosphorus detector

6. ANALYTICAL METHODS

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Elemental phosphorus, and inorganic and organic compounds containing phosphorus, are common in environmental and dietary material needed for the normal physiological functioning of living organisms. For these reasons, exposure to phosphorus is not unique. Human clinical and autopsy studies, where excess elemental phosphorus exposure has been documented, show apparent organ- and system-specific toxic effects. However, other than burn studies, there are few animal studies to quantitatively define these toxic effects, either acutely or long term. Moreover, the mechanisms of toxicity are not clearly understood. For example, elemental phosphorus is highly reactive in air and other environmental media. For this reason, its products (e.g., PH_2 phosphoric acid, and PH_3) could be significant contributors to toxicity. Minimizing formation of these reaction products may serve to prevent or alleviate the toxic effects of phosphorus.

Animal studies designed to quantitatively identify organ and system toxicity need to be carried out. Dosimetry studies to quantitatively identify levels of toxicant in blood or urine either as reaction products or elemental phosphorus need to be considered (see Section 25.1).

There are no specific effects that could be quantitatively related to phosphorus exposure (see Section 2.5.2).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Few methods are available for the simultaneous determination of different forms of phosphorus (phosphine, elemental phosphorus, and metal phosphides) in environmental samples, and the methods are based on older technology (Gorzny 1972). It would be helpful to develop methods based on modem techniques for this purpose. It would also be useful to develop a few standard methods for analyzing different forms of phosphorus found in environmental samples.

Several methods are available for determining the degradation products of elemental phosphorus (different phosphorus acids and organic phosphorus compounds) in environmental samples (EPA 1983). A liquid chromatographic method has been developed for determining polyphosphoric acids in phosphorus smokes (Braze11 et al. 1984). It appears that there is little need to develop analytical methods that determine degradation products of elemental phosphorus in phosphorus smokes.

6. ANALYTICAL METHODS

6.3.2 On-going Studies

No on-going studies were located for determining phosphorus or its degradation products/metabolites in environmental or biological samples.