6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring 1,2-diphenylhydrazine in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify 1,2-diphenylhydrazine. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect 1,2-diphenylhydrazine in environmental samples are the methods approved by federal agencies 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 a trade association such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

No adequate methods were located for the analysis of 1,2-diphenylhydrazine in biological materials. While thin-layer chromatography methods have been published, it is not clear if these methods would be capable of resolving 1,2-diphenylhydrazine from degradation products that appear rapidly in a sample upon standing (Bolton and Griffiths 1978; Dutkiewicz and Szymanska 1973). These products (some unidentified) are produced rapidly, and vary depending on the exact conditions (see below). In addition, none of the metabolites identified (e.g., benzidine, aniline) are suitable surrogates since they cannot be linked exclusively to 1,2-diphenylhydrazine exposure, but may result from exposure to other chemicals (and possibly drugs; see above).

6.2 ENVIRONMENTAL SAMPLES

Adequate analytical methods exist for the analysis of 1,2-diphenylhydrazine in environmental samples and are presented below. However, adequate methods are not available for the sampling, sample preservation, and sample preparation (extraction) of environmental media. Neither EPA nor NIOSH have standard methods for analyzing 1,2-diphenylhydrazine in any medium; 1,2-diphenylhydrazine is no longer on the Target Compound List (TCL) for the Contract Laboratory Program, but is identified as a semi-volatile compound (EPA 1987a). Riggin and Howard (1982) reported that 1,2-diphenylhydrazine at 100 μg/L in a municipal sewage effluent (after secondary treatment) had a time to 50% disappearance of 15 minutes (it degraded completely within 1 hour), but the half-life was extended to 60 minutes when the wastewater was deaerated. Also, the authors stated that 1,2-diphenylhydrazine "... analysis in wastewater is virtually meaningless, since the DPH level determined cannot be directly related to the DPH in the sample at the time of collection." This limitation may apply to all environmental media, depending on the exact conditions used to acquire and store the sample, as well as the sample itself. Even excellent

methods for the analysis of 1,2-diphenylhydrazine will not necessarily yield concentrations that are representative of the concentrations present in the medium sampled.

Extracting and concentrating the 1,2-diphenylhydrazine in an environmental sample may give poor results. Riggin and Howard (1979) reported that 1,2-diphenylhydrazine extraction from water was "irreproducible" because of instability when the extract was concentrated. An extraction efficiency of more than 50% was reported for chloroform extraction at pH 7. If stored for longer than 1 day, however, the extract contained less than 10% of the initial 1,2-diphenylhydrazine concentration. At different pH values, 1,2-diphenylhydrazine degraded into different products, not all of which were identifiable. This lack of clearly identifiable degradation products makes identification of a degradation product as a surrogate for 1,2-diphenylhydrazine difficult. Ahuja et al. (1988) found that extraction of 1,2-diphenylhydrazine in water containing THAM buffer (pH 9.2) showed only 0.9% loss over 30 minutes, although chromatography was performed within 30 minutes of extraction. Although this procedure was applied to pharmaceutical analysis, there is no apparent reason to believe it will not work for environmental analysis.

In addition, Riggin and Howard (1982) reported that analytical standards of 1,2-diphenylhydrazine prepared in benzene, methylene chloride, methanol, triethyl amine, acetonitrile, or acetic acid decomposed completely in 3 days or less. Matsui et al. (1983) reported that 1,2-diphenylhydrazine was oxidized to azobenzene in n-hexane solution at a rate of about 5%/hr; flushing the n-hexane with nitrogen reduced the conversion rate to about 4% in 2 hours. These authors also found that nitrogen flushed standards were stable over a l-hour period, based on the detector response factors during liquid chromatography. These limitations should be considered when interpreting analytical results.

Riggin and Howard (1982) reported that 1,2-diphenylhydrazine ". . . instantaneously decomposes to azobenzene in the GC injection port." The authors further stated that:

> "It is interesting to note that only one peak resulted from DPH injection. Occasionally, it was found that a second, later eluting peak was present . . . . but this was not always the case. This additional component may have been a solution decomposition product."

Because of this, GC, including GC/MS, does not appear to be an acceptable analytical tool for the analysis of 1,2-diphenylhydrazine in any sample.

Riggin and Howard (1979, 1982), Matsui et al. (1983), Fabre et al. (19841, and Ahuja et al. (1988) reported that High Performance Liquid Chromatography (HPLC) with UV or electrochemical detection is capable of analyzing 1,2-diphenylhydrazine. Reversed phase chromatographic columns

have been used most often (Ahuja et al. 1988; Fabre et al. 1984; Riggin and Howard 1979, 1982). Cyano-amino polar bonded phase columns also have been used (Matsui et al. 1983). Using a reversed phase and UV detection, the minimum amount detected (on column amounts) is approximately 6-7 ng and the minimum amount quantifiable is less than 1 µg (Ahuja et al. 1988; Fabre et al. 1984; Matsui et al. 1983).

In conclusion, HPLC is preferred over GC for analysis of 1,2-diphenylhydrazine. Sample preservation and extraction methods need improvement.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCIA, 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 1,2-diphenylhydrazine 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 1,2-diphenylhydrazine.

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 or eliminate the uncertainties of human health assessment. 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. No biomarker that can be associated quantitatively with exposure to 1,2-diphenylhydrazine has been identified (see Section 2.5). No adequate methods are available for the analysis of 1,2-diphenylhydrazine in biological materials. If this information were available, it would allow both investigators and reviewers to assess the accuracy and uncertainty of the methods used in toxicological studies. Furthermore, the ready availability of tested analytical methods, including sample preservation, would permit a standardized approach to the analysis of biological materials to assist in measuring human exposure and monitoring effects in humans. Adequate methods appear to be available for the analysis of 1,2-diphenylhydrazine metabolites in biological materials. Metabolites include azobenzene and aniline, both of which appear to be amenable to analysis by standard methods. 1,2-Diphenylhydrazine and its metabolites, however, have not been established as a quantitative biomarker of exposure to 1,2-diphenylhydrazine.

6. ANALYTICAL METHODS

No biomarker that can be associated quantitatively with effect has been identified (see Section 2.5). Thus, there are no analytical methods for the determination of biomarkers of effect for 1,2-diphenylhydrazine.

Methods for Determining Parent Compound and Degradation Products in Environmental Media. While analytical methods appear to be available for the analysis of 1,2-diphenylhydrazine, no methods were found for the preservation of 1,2-diphenylhydrazine in ambient air, water, or soil samples. Such methods would allow the development and analysis of a monitoring program designed to better assess the concentrations of 1,2-diphenylhydrazine in and around hazardous waste sites.
Methods for Determining Degradation Products in Environmental Media. Adequate methods are available for the analysis of some 1,2-diphenylhydrazine degradation products in environmental media; however, not all of the major degradation products have been identified. In addition, the number and nature of degradation products appear to change depending on conditions (e.g., pH). The development of adequate analytical methods for identifying degradation products would allow a monitoring program designed to assess the ambient concentrations of 1,2-diphenylhydrazine degradation products in environmental media to be established; this would provide information concerning both human and environmental exposure, since it would allow an estimation of the concentration of 1,2-diphenylhydrazine in environmental media to be established prior to degradation. The development of analytical methods, however, must be subsequent to generalized environmental fate studies that identify the degradation products. A standardized method could then be developed using spike recoveries from different media to determine the recovery efficiencies and precision and accuracy of the method.

6.3.2 On-going Studies

No studies were located regarding on-going analytical methods development for 1,2-diphenylhydrazine.