6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring 2,3-benzofuran 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 2,3-benzofuran. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. No methods approved by federal agencies or other groups specifically for detection of 2,3-benzofuran were located.

Environmental media or biological samples which contain 2,3-benzofuran are also likely to contain numerous other organic compounds with similar chemical and physical properties. Analysis of such samples generally proceeds by first extracting or concentrating some subset of the organic compounds and then separating and identifying them. Techniques for extraction of organic compounds from environmental media or biological samples include absorption onto a polymer and extraction with an organic solvent. Recovery is generally not complete, and so accurate quantification requires using matrix spikes (EPA 1986c). This has not been done in any studies for 2,3-benzofuran. Sensitive and selective techniques for identification of organic compounds in extracts are well established, using high-resolution gas chromatography (HRGC) to separate the compounds and mass spectrometry (MS) to identify them. HRGC achieves higher resolution than standard gas chromatography (GC) by using wall-coated capillary columns rather than packed columns for separation of compounds. Flame ionization detection is not specific enough for the analysis of 2,3-benzofuran in samples containing numerous other compounds, although it has been used to monitor the stability of 2,3-benzofuran in oil for animal feeding studies (NTP 1989). Accurate quantification of the concentration of chemicals in extracts can be achieved with GC/MS by daily calibration using actual and surrogate standards (EPA 1986c), although this has not been done specifically for 2,3-benzofuran.

6.1 BIOLOGICAL MATERIALS

2,3-Benzofuran has been detected, but not quantified, in samples of blood (Anderson and Harland 1980) and breast milk (Pellizzari et al. 1982). In both cases, volatile and semi-volatile organic compounds were purged from the biological fluids by bubbling with an inert gas at an elevated temperature. The compounds were trapped by adsorption onto a Tenax® cartridge. The percent recovery of 2,3-benzofuran by this purge-and-trap collection method was not examined. The Tenax® cartridge was heated to desorb the organic compounds directly into the inlet of the HRGC equipment. Mass spectrometry was used to identify the compounds, including 2,3-benzofuran, by mass fragmentation patterns. The percent recovery or concentration was not quantified in either study (Anderson and Harland 1980; Pellizzari et al. 1982).

Methods for detection of 2,3-benzofuran in biological.materials are summarized in Table 6-1.

TABLE 6-1. Analytical Methods for Determining 2,3-Benzofuran in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Nitrogen purging at 95°C, sorption on Tenax®, thermal desorption	HRGC/MS	No data	No data	Anderson and Harland 1980
Breast milk	Helium purging, sorption on Tenax®, thermal desorption	HRGC/MS	No data	No data	Pellizzari et al. 1982

HRGC = high-resolution gas chromatography; MS = mass spectrometry

6.2 ENVIRONMENTAL SAMPLES

2,3-Benzofuran can be trapped and concentrated from air samples by passing a large volume of air through a Tenax® (Erikson and Pellizzari 1978; Juttner 1986; van Netten et al. 1988) or Chromosorb (Seizinger and Dimitriades 1972) cartridge. The cartridge is then thermally desorbed into an HRGC/MS detection system similar to that used for biological samples. 2,3-Benzofuran can be concentrated from water samples using the purge-and-trap method (Pellizzari et al. 1979), or extraction with dichloromethane (Rostad et al. 1985), and analyzed by HRGC/MS. 2,3-Benzofuran can be extracted from particulate samples with dichloromethane and analyzed by HRGC/MS (Ferretti and Flanagan 1971; Hunt et al. 1982). The percent recovery of 2,3-benzofuran by these extraction methods has not been analyzed. The amount of 2,3-benzofuran in some environmental samples has been quantified (Pellizzari et al. 1979; Seizinger and Dimitriades 1972), but the precision of the quantification was not examined.

Methods for the determination of 2,3-benzofuran in environmental samples are summarized in Table 6-2.

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 2,3-benzofuran 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 2,3-benzofuran.

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 Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The only known biomarker of exposure to 2,3-benzofuran is its presence in blood (Anderson and Harland 1980) or breast milk (Pellizzari et al. 1982). 2,3-Benzofuran was not found in all samples of blood or. breast milk tested, but since existing methods for detection of 2,3-benzofuran in biological samples are not quantitative, it is not possible to assess whether those samples contained no 2,3-benzofuran or whether the method used was not sufficiently sensitive to measure background levels in the population. The levels at which human health effects occur are not known. Only the administered doses, not the target organ concentrations, are known for

TABLE 6-2. Analytical Methods for Determining 2,3-Benzofuran in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Forest air	Sorption on Tenax®, thermal desorption	HRGC/MS	No data	No data	Juttner 1986
Ambient air	Sorption on Tenax®, thermal desorption	HRGC/MS	No data	No data	Erikson and Pellizzari 1978
Indoor air	Sorption on Tenax®, thermal desorption	HRGC/MS	No data	No data	van Netten et al. 1988
Automobile exhaust	Sorption on Chromosorb, thermal desorption	HRGC/MS	0.1 ppm	No data	Seizinger and Dimitriades 1972
Groundwater	Extraction with dichloromethane	HRGC/MS	No data	No data	Rostad et al. 1985
Groundwater and process water	Helium purging, sorption on Tenax®, thermal desorption	HRGC/MS	0.1 ppb	No data	Pellizzari et al. 1979
Whey powder	Extraction with dichloromethane, vacuum distillation	GC/MS	No data	No data	Ferretti and Flanagan 1971
Baghouse filter ash from fluidized-bed coal combustion	Extraction with dichloromethane	HRGC/MS	No data	No data	Hunt et al. 1982

GC = gas chromatography; HRGC = high-resolution gas chromatography; MS = mass spectrometry

biological effects occurring in animals (NTP 1989), but these doses are relatively high (30 mg/kg/day or greater, see Chapter 2). Based on the general sensitivity of HRGC/MS methods, it is likely that levels of 2,3-benzofuran at which biological effects occur should be achievable with routine quantification procedures. The overall techniques of extraction followed by HRGC/MS analysis can be made precise, accurate, reliable, and specific, so that the opportunity exists to develop methods for sensitive quantitation of 2,3-benzofuran in biological samples. Refinement of existing purge-and-trap extraction techniques and investigation of alternative concentration techniques such as cryotrapping (Pankow and Rosen 1988) and supercritical fluid extraction (King 1989) would be useful. High-performance liquid chromatography as an alternative to HRGC and Fourier transform infrared spectroscopy and photodiode array detectors as alternatives to MS detection might offer advantages. Investigation of possible metabolites of 2,3-benzofuran as biomarkers of exposure would be most useful if accompanied by development of methods for their detection, such as immunoassay techniques and ^{32}P post-labelling for identifying macromolecular adducts.

No known biomarkers of effect were located in the literature. Investigation of biomarkers of effect of 2,3-benzofuran would be most useful if it were also to focus on developing precise, accurate, reliable, and specific methods for measuring background levels of the biomarker of effect in the population and also levels at which adverse effects occur.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The purpose of the analytical methods for 2,3-benzofuran is to identify contaminated areas and to determine if contaminant levels constitute a concern for human health. The media of most concern for human exposure to 2,3-benzofuran are drinking water, soil, and air. It is likely that 2,3-benzofuran exists in these media primarily adsorbed to organic-rich particulates (Hassett et al. 1983). 2,3-Benzofuran has been found relatively infrequently in environmental media, but most samples have excluded particulates. Insufficient work has been done on quantification of 2,3-benzofuran, particularly percent recovery, to determine whether the methods are sensitive enough to measure background levels in the environment (Erikson and Pellizzari 1978; Hunt et al. 1982; Juttner 1986; Pellizzari et al. 1979; Rostad et al. 1985; Seizinger and Dimitriades 1972). The levels of 2,3-benzofuran at which health effects occur in animals are equivalent to 400 ppm in the diet or more (NTP 1989). Existing methods have nominal detection limits of 0.1 ppb (Pellizzari et al. 1979; Seizinger and Dimitriades 1972), indicating that existing methods are probably sensitive enough to detect levels at which health effects occur. The basic techniques of HRGC/MS have the potential for excellent precision, accuracy, reliability, and specificity, with sufficient research and development. One novel technique which may be suitable for in situ monitoring of 2,3-benzofuran in water is surface-enhanced Raman spectroscopy using silver electrodes (Carrabba et al. 1987). No information is available concerning degradation products of 2,3-benzofuran; investigation of 2,3-benzofuran degradation would be most useful if it included development of reliable analytical methods.

6.3.2 On-going Studies

No information was located concerning studies directed towards improving methods for detection of 2,3-benzofuran specifically.