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-hexanone 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-hexanone. 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 2-hexanone 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 groups 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

In biological systems in which 2-hexanone may have been metabolized, or may itself be a metabolite, consideration must be given to possible binding of the analyte as a conjugate. In such cases, 2-hexanone may be released by hydrolysis with acid (Fedtke and Bolt 1986). Following pre-treatment, which varies with the sample and may include homogenization, centrifugation, and acidification, 2-hexanone can be released from biological samples by purging or perfusion and trapped on a sorbent, extracted with a solvent such as acetone, or extracted directly onto sorbent solids.

Sensitive and selective methods are available for the qualitative and quantitative measurement of 2-hexanone, after it is separated from its sample matrix. Gas chromatography using sensitive and highly specific mass spectrometry (MS) or highly sensitive flame ionization detection (FID) is the analytical method most commonly used. Capillary gas chromatography, also known broadly as high resolution gas chromatography (HRGC), has greatly facilitated the analysis of compounds such as 2-hexanone that can be measured by gas chromatography and has resulted in vast improvements in resolution and sensitivity. It has made the choice of a stationary phase much less crucial than is the case with the older method using packed columns. The instrumental capability to separate volatile analytes by HRGC is, for the most part, no longer the limiting factor in their analysis. High performance liquid chromatography (HPLC) may also be used and has the advantage of compatibility with the liquid matrix of biological samples.

Methods for detection of 2-hexanone in biological materials are summarized in Table 6-1.

TABLE 6-1. Analytical Methods for Determining 2-Hexanone in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Hydrolysis of metabolic conjugates with HCl, extraction on C18 cartridges, desorption	HRGC/MS	0.05-0.08 µg/mL	81±3.2%[a]	Fedtke and Bolt 1986
Biological samples (chicken plasma)[b]	Extraction with ether after addition of HCl and Na_2SO_4, concentrated under N_2	HRGC/FID	No data	78±4%[c]	Nomeir and Abou-Donia 1985
Biological samples (chicken plasma)[b]	Extraction with ether after addition of HCl and Na_2SO_4, concentrated under N_2	HPLC/UV	No data	No data	Nomeir and Abou-Donia 1985
Biological tissues, (blood, brain, kidney, liver)[b]	Homogenization with acetone, centrifugation, injection of acetone extract	GC/MS	No data	98±12%-110±16%[d]	White et al. 1979
Blood (human)[e]	Perfusion at 95°C, collection on Tenax®, release by heating	GC/MS	No data	No data	Anderson and Harland 1980

[a]Percent recovery for 2,5-hexanedione was 83±3.6%.
[b]This method was also used in the determination of 2,5-hexanedione, a metabolic product of 2-hexanone.
[c]Percent recovery for 2,5-hexanedione was 62±3%
[d]Percent recovery for 2,5-hexanedione was 96±13% to 110±16%
[e]$C_6H_{12}O$ ketone detected in blood of fire victims at necropsy

GC = gas chromatography; FID = flame ionization detector; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; RSD = relative standard deviation; UV = ultraviolet light

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6.2 ENVIRONMENTAL SAMPLES

For the determination of 2-hexanone in air, the analyte is usually trapped and concentrated from a large volume of air on a solid sorbent such as Tenax® or activated carbon from which it can be released thermally or eluted with a solvent such as carbon disulfide for subsequent measurement. For aqueous samples, 2-hexanone is purged with an inert gas and collected on a solid such as Tenax®, followed by thermal desorption and measurement. Cryogenic trapping has also been used for removal of 2-hexanone from water samples (Badings et al. 1985). Gas chromatography using sensitive and highly specific MS or highly sensitive FID is the analytical method of choice for the determination of 2-hexanone in environmental samples.

Methods for the determination of 2-hexanone 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-hexanone 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-hexanone.

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. As noted in Section 6.1, methods are available for the qualitative and quantitative measurement of 2-hexanone after it is separated from its sample matrix (Anderson and Harland 1980; Fedtke and Bolt 1986; Nomeir and Abdou-Donia 1985; White et al. 1979). High-resolution gas chromatography for 2-hexanone analysis has been developed to the point that the instrumental capability to separate volatile analytes by HRGC is, for the most part, no longer the limiting factor in their analysis. Flame ionization detection has enabled detection at very low levels and MS has assured specificity in measurement.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wastewater and spent oil shale	Collection on Tenax®, thermal desorption	HRGC/FID; GC/MS	No data	No data	Hawthorne et al. 1985
Air	Retention by activated carbon, elution with carbon disulfide	GC/FID	20 µg	No data	NIOSH 1984
Water, environmental samples	Purge, cryogenic trap	HRGC	<10 µg/kg	No data	Badings et al. 1985
Groundwater	Purge by helium, collection on solid, thermal desorption	GC/MS	50 µg/L	No data	EPA 1986
Solid waste	Purge by helium, collection on solid, thermal desorption	GC/MS	50 µg/kg	No data	EPA 1986

FID = flame ionization detector; GC = gas chromatography; HRGC = high-resolution gas chromatography; MS = mass spectrometry

6. ANALYTICAL METHODS

More specific methods to determine biomarkers of exposure to 2-hexanone would be helpful in detecting exposure to this compound before adverse morphological or clinical effects occur. Finding biological markers of exposure to 2-hexanone is complicated by the fact that this compound is itself a biological indicator of exposure to n-hexane (Fedtke and Bolt 1986). In addition, the presence of the metabolite, 2,5-hexanedione, may indicate exposure to 2-hexanone, but it is also a biological indicator of exposure to n-hexane (Fedtke and Bolt 1986). There is insufficient information in the literature to determine if methods for determining biomarkers of exposure and effect of 2-hexanone are sensitive enough to measure background levels in the population and levels at which biological effects occur. The precision, accuracy, reliability, and specificity of these methods are not sufficiently documented. This information would be valuable for interpreting monitoring data.

Refinement of existing purge-and-trap extraction techniques and investigation of alternative concentration methods such as cryotrapping (Pankow and Rosen 1988) and supercritical fluid extraction (King 1989) would be useful. In addition, several major challenges remain. One of these is to transfer analytes that have been isolated from a biological or environmental matrix quantitatively and in a narrow band to the HRGC. Another major challenge is to identify and accurately measure the quantity of compounds in the HRGC peaks. Mass spectrometric detection has been outstanding for identification, but other techniques, particularly Fourier transform infrared spectroscopy (FTIR), may offer some advantages (Wieboldt et al. 1988).

Metabolites of 2-hexanone in biological materials are difficult to determine in routine practice because of the lack of standardized methods for their measurement. As shown in Table 6-1, there are very few well characterized methods for the determination of metabolites of 2-hexanone in biological materials (Nomeir and Abou-Donia 1985; White et al. 1979). The precision, accuracy, reliability, and specificity of existing methods need to be evaluated, and the methods refined and adapted to routine practice.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The media of most concern for human exposure to 2-hexanone are drinking water (primarily from groundwater sources) and air. From the data presented in Table 6-2 (Badings et al. 1985; EPA 1986; Hawthorne et al. 1985; NIOSH 1984), it may be concluded that the methods available for the determination of 2-hexanone in water and air are not sensitive enough to determine background levels of this compound. Existing methods are satisfactory for measuring levels at which health effects occur.

The precision, accuracy, reliability, and specificity of methods to determine 2-hexanone in water and air are not well documented, and additional work is needed in this area.

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Methods for determining the parent compound, 2-hexanone, in water, air, and waste samples are available (Badings et al. 1985; EPA 1986; Hawthorne et al. 1985; NIOSH 1984). Sampling methodologies for compounds such as 2-hexanone continue to pose problems such as nonrepresentative samples, insufficient sample volumes, contamination, and labor-intensive, tedious extraction and purification procedures (Green and Le Pape 1987). It would be helpful to have means to measure organic compounds such as 2-hexanone in situ in water and other environmental media without the need for sampling and extraction procedures to isolate the analyte prior to analysis.

6.3.2 On-going Studies

There are no known on-going studies to improve methods of analysis for 2-hexanone or its metabolites in biological or environmental samples.