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27 ANALYSIS OF BIOLOGICAL MATERIALS AND XENOBIOTICS

27.1. INTRODUCTION

As defined in Chapter 22 a xenobiotic species is one that is foreign to living systems. Common examples include heavy metals, such as lead, which serve no physiologic function, and synthetic organic compounds that are not made in nature. Exposure of organisms to xenobiotic materials is a very important consideration in environmental and toxicological chemistry. Therefore, the determination of exposure by various analytical techniques is one of the more crucial aspects of environmental chemistry.

This chapter deals with the determination of xenobiotic substances in biological materials. Although such substances can be measured in a variety of tissues, the greatest concern is their presence in human tissues and other samples of human origin. Therefore, the methods described in this chapter apply primarily to exposed human subjects. They are essentially identical to methods used on other animals and, in fact, most were developed through animal studies. Significantly different techniques may be required for plant or microbiological samples.

The measurement of xenobiotic substances and their metabolites in blood, urine, breath, and other samples of biological origin to determine exposure to toxic substances is called biological monitoring. Comparison of the levels of analytes measured with the degree and type of exposure to foreign substances is a crucial aspect of toxicological chemistry. It is an area in which rapid advances are being made. For current information regarding this area, the reader is referred to excellent reviews of the topic, and several books on biological monitoring such as those by Angerer, Draper, Baselt, and Kneip and coauthors as listed in the back of this chapter under “Supplementary References,” are available as well.

The two main approaches to workplace monitoring of toxic chemicals are workplace monitoring, using samplers that sample xenobiotic substances from workplace air, and biological monitoring. Although the analyses are generally much more difficult, biological monitoring is a much better indicator of exposure because
it measures exposure to all routes—oral and dermal as well as inhalation—and it
gives an integrated value of exposure. Furthermore, biological monitoring is very
useful in determining the effectiveness of measures taken to prevent exposure, such
as protective clothing and hygienic measures.

27.2. INDICATORS OF EXPOSURE TO XENOBIOTICS

The two major considerations in determining exposure to xenobiotics are the
type of sample and the type of analyte. Both of these are influenced by what happens
to a xenobiotic material when it gets into the body. For some exposures, the entry
site composes the sample. This is the case, for example, in exposure to asbestos
fibers in the air, which is manifested by lesions to the lung. More commonly, the
analyte may appear at some distance from the site of exposure, such as lead in bone
that was originally taken in by the respiratory route. In other cases the original
xenobiotic is not even present in the analyte. An example of this is methemoglobin
in blood, the result of exposure to aniline absorbed through the skin.

The two major kinds of samples analyzed for xenobiotics exposure are blood and
urine. Both of these kinds of samples are analyzed for systemic xenobiotics, which
are those that are transported in the body and metabolized in various tissues. Xeno-
biotic substances, their metabolites, and their adducts are absorbed into the body and
transported through it in the bloodstream. Therefore, blood is of unique importance
as a sample for biological monitoring. Blood is not a simple sample to process, and
subjects often object to the process of taking it. Upon collection, blood may be
treated with an anticoagulant, usually a salt of ethylenediaminetetraacetic acid
(EDTA), and processed for analysis as whole blood. It may also be allowed to clot
and be centrifuged to remove solids; the liquid remaining is blood serum.

Recall from Chapter 22 that as the result of Phase 1 and Phase 2 reactions,
xenobiotics tend to be converted to more polar and water soluble metabolites. These
are eliminated with the urine, making urine a good sample to analyze as evidence of
exposure to xenobiotic substances. Urine has the advantage of being a simpler
matrix than blood and one that subjects more readily give for analysis. Other kinds
of samples that may be analyzed include breath (for volatile xenobiotics and volatile
metabolites), air or nails (for trace elements, such as selenium), adipose tissue (fat),
and milk (obviously limited to lactating females). Various kinds of organ tissue can
be analyzed in cadavers, which can be useful in trying to determine cause of death
by poisoning.

The choice of the analyte actually measured varies with the xenobiotic substance
to which the subject has been exposed. Therefore, it is convenient to divide
xenobiotic analysis on the basis of the type of chemical species determined. The
most straightforward analyte is, of course, the xenobiotic itself. This applies to
elemental xenobiotics, especially metals, which are almost always determined in the
elemental form. In a few cases organic xenobiotics can also be determined as the
parent compound. However, organic xenobiotics are commonly metabolized to other
products by Phase 1 and Phase 2 reactions. Commonly, the Phase 1 reaction product
is measured, often after it is hydrolyzed from the Phase 2 conjugate, using enzymes
or acid hydrolysis procedures. Thus, for example, trans,trans-muconic acid can be
measured as evidence of exposure to the parent compound benzene. In other cases a
Phase 2 reaction product is measured, for example, hippuric acid determined as evidence of exposure to toluene. Some xenobiotics or their metabolites form adducts with endogenous materials in the body, which are then measured as evidence of exposure. A simple example is the adduct formed between carbon monoxide and hemoglobin, carboxyhemoglobin. More complicated examples are the adducts formed by the carcinogenic Phase 1 reaction products of polycyclic aromatic hydrocarbons with DNA or hemoglobin. Another class of analytes consists of endogenous substances produced upon exposure to a xenobiotic material. Methemoglobin formed as a result of exposure to nitrobenzene, aniline, and related compounds is an example of such a substance which does not contain any of the original xenobiotic material. Another class of substance causes measurable alterations in enzyme activity. The most common example of this is the inhibition of acetylcholinesterase enzyme by organophosphates or carbamate insecticides.

27.3. DETERMINATION OF METALS

Direct Analysis of Metals

Several biologically important metals can be determined directly in body fluids, especially urine, by atomic absorption. In the simplest cases the urine is diluted with water or with acid and a portion analyzed directly by graphite furnace atomic absorption, taking advantage of the very high sensitivity of that technique for some metals. Metals that can be determined directly in urine by this approach include chromium, copper, lead, lithium, and zinc. Very low levels of metals can be measured using a graphite furnace atomic absorption technique, and Zeeman background correction with a graphite furnace enables measurement of metals in samples that contain enough biological material to cause significant amounts of “smoke” during the atomization process, so that ashing the samples is less necessary.

A method has been published for the determination of a variety of metals in diluted blood and serum using inductively coupled plasma atomization with mass spectrometric detection. Blood was diluted 10-fold and serum 5-fold with a solution containing ammonia, Triton X-100 surfactant, and EDTA. Detection limits adequate for measurement in blood or serum were found for cadmium, cobalt, copper, lead, rubidium, and zinc.

Metals in Wet-Ashed Blood and Urine

Several toxicologically important metals are readily determined from wet-ashed blood or urine using atomic spectroscopic techniques. The ashing procedure may vary, but always entails heating the sample with strong acid and oxidant to dryness and redissolving the residue in acid. A typical procedure is digestion of blood or urine for cadmium analysis, which consists of mixing the sample with a comparable volume of concentrated nitric acid, heating to a reduced volume, adding 30% hydrogen peroxide oxidant, heating to dryness, and dissolving in nitric acid prior to measurement by atomic absorption or emission. Mixtures of nitric, sulfuric, and perchloric acid are effective though somewhat hazardous media for digesting blood, urine, or tissue. Wet ashing followed by atomic absorption analysis can be used for...
the determination in blood or urine of cadmium, chromium, copper, lead, manganese, and zinc, among other metals. Although atomic absorption, especially highly sensitive graphite furnace atomic absorption, has long been favored for measuring metals in biological samples, the multielement capability and other advantages of inductively coupled plasma atomic spectroscopy has led to its use for determining metals in blood and urine samples.  

**Extraction of Metals for Atomic Absorption Analysis**

A number of procedures for the determination of metals and biological samples call for the extraction of the metal with an organic chelating agent in order to remove interferences and concentrate the metal to enable detection of low levels. The urine or blood sample may be first subjected to wet ashing to enable extraction of the metal. Beryllium from an acid-digested blood or urine sample may be extracted by acetylacetone into methylisobutyl ketone prior to atomic absorption analysis. Virtually all of the common metals can be determined by this approach using appropriate extractants.

The availability of strongly chelating extracts for a number of metals has lead to the development of procedures in which the metal is extracted from minimally treated blood or urine, then quantified by atomic absorption analysis. The metals for which such extractions can be used include cobalt, lead, and thallium extracted into organic solvent as the dithiocarbamate chelate, and nickel extracted into methylisobutyl ketone as a chelate formed with ammonium pyrrolidinedithiocarbamate.

Methods for several metals or metalloids involve conversion to a volatile form. Arsenic, antimony, and selenium can be reduced to their volatile hydrides, AsH₃, SbH₃, and H₂Se, respectively, which can be determined by atomic absorption or other means. Mercury is reduced to volatile mercury metal, which is evolved from solution and measured by cold vapor atomic absorption.

**27.4. DETERMINATION OF NONMETALS AND INORGANIC COMPOUNDS**

Relatively few nonmetals require determination in biological samples. One important example is fluoride, which occurs in biological fluids as the fluoride ion, F⁻. In some cases of occupational exposure or exposure through food or drinking water, excessive levels of fluoride in the body can be a health concern. Fluoride is readily determined potentiometrically with a fluoride ion-selective electrode. The sample is diluted with an appropriate buffer and the potential of the fluoride electrode measured very accurately vs. a reference electrode, with the concentration calculated from a calibration plot. Even more accurate values can be obtained by the use of standard addition in which the potential of the electrode system in a known volume of sample is read, a measured amount of standard fluoride is added, and the shift in potential is used to calculate the unknown concentration of fluoride.

Another nonmetal for which a method of determining biological exposure would be useful is white phosphorus, the most common and relatively toxic elemental form. Unfortunately, there is not a chemical method suitable for the determination of exposure to white phosphorus that would distinguish such exposure from relatively
high background levels of organic and inorganic phosphorus in body fluids and tissues.

Toxic cyanide can be isolated in a special device called a Conway microdiffusion cell by treatment with acid, followed by collection of the weakly acidic HCN gas that is evolved in a base solution. The cyanide released can be measured spectrophotometrically by formation of a colored species.

Carbon monoxide is readily determined in blood by virtue of the colored carboxyhemoglobin that it forms with hemoglobin. The procedure consists of measuring the absorbances at wavelengths of 414, 421, and 428 nm of the blood sample, a sample through which oxygen has been bubbled to change all the hemoglobin to the oxyhemoglobin form, and a sample through which carbon monoxide has been bubbled to change all the hemoglobin to carboxyhemoglobin. With the appropriate calculations, a percentage conversion to carboxyhemoglobin can be obtained.

27.5. DETERMINATION OF PARENT ORGANIC COMPOUNDS

A number of organic compounds can be measured as the unmetabolized compound in blood, urine, and breath. In some cases the sample can be injected along with its water content directly into a gas chromatograph. Direct injection is used for the measurement of acetone, $n$-butanol, dimethylformamide, cyclopropane, halothane, methoxyflurane, diethyl ether, isopropanol, methanol, methyl-$n$-butyl ketone, methyl chloride, methyl ethyl ketone, toluene, trichloroethane, and trichloroethylene.

For the determination of volatile compounds in blood or urine, a straightforward approach is to liberate the analyte at an elevated temperature allowing the volatile compound to accumulate in headspace above the sample followed by direct injection of headspace gas into a gas chromatograph. A reagent such as perchloric acid may be added to deproteinize the blood or urine sample and facilitate release of the volatile xenobiotic compound. Among the compounds determined by this approach are acetaldehyde, dichloromethane, chloroform, carbon tetrachloride, benzene, trichloroethylene, toluene, cyclohexane, and ethylene oxide. The use of multiple detectors for the gas chromatographic determination of analytes in headspace increases the versatility of this technique and enables the determination of a variety of physiologically important volatile organic compounds.5

Purge-and-trap techniques in which volatile analytes are evolved from blood or urine in a gas stream and collected on a trap for subsequent chromatographic analysis have been developed. Such a technique employing gas chromatographic separation and Fourier transform infrared detection has been described for a number of volatile organic compounds in blood.6

27.6. MEASUREMENT OF PHASE 1 AND PHASE 2 REACTION PRODUCTS

Phase 1 Reaction Products

For a number of organic compounds the most accurate indication of exposure is to be obtained by determining their Phase 1 reaction products. This is because many compounds are metabolized in the body and don't show up as the parent compound.
And those fractions of volatile organic compounds that are not metabolized may be readily eliminated with expired air from the lungs and may thus be missed. In cases where a significant fraction of the xenobiotic compound has undergone a Phase 2 reaction, the Phase 1 product may be regenerated by acid hydrolysis.

One of the compounds commonly determined as its Phase 1 metabolite is benzene, which undergoes the following reactions in the body (see Chapter 23, Section 23.4):

\[
\text{O} + \{\text{O}\} \xrightarrow{\text{Enzymatic epoxidation}} \text{Benzene epoxide} \xrightarrow{\text{Nonenzymatic rearrangement}} \text{Benzene oxepin}
\]

Therefore, exposure to benzene can be determined by analysis of urine for phenol. Although a very sensitive colorimetric method for phenol involving diazotized p-nitroaniline has long been available, gas chromatographic analysis is now favored. The urine sample is treated with perchloric acid to hydrolyze phenol conjugates and the phenol is extracted into diisopropyl ether for chromatographic analysis. Two other metabolic products of benzene, trans,trans-muconic acid and S-phenyl mercapturic acid, are now commonly measured as more specific biomarkers of benzene exposure.

Insecticidal carbaryl undergoes the following metabolic reaction:

\[
\text{O} \xrightarrow{\text{Enzymatic processes}} \text{Carbaryl} \xrightarrow{\text{+ other products}} \text{1-Naphthol}
\]

Therefore, the analysis of 1-naphthol in urine indicates exposure to carbaryl. The 1-naphthol that is conjugated by a Phase 2 reaction is liberated by acid hydrolysis, then determined spectrophotometrically or by chromatography.

In addition to the examples discussed above, a number of other xenobiotics are measured by their phase one reaction products. These compounds and their metabolites are listed in Table 27.1. These methods are for metabolites in urine. Normally the urine sample is acidified to release the Phase 1 metabolites from Phase 2 conjugates that they might have formed and, except where direct sample injection is
employed, the analyte is collected as vapor or extracted into an organic solvent. In some cases the analyte is reacted with a reagent that produces a volatile derivative that is readily separated and detected by gas chromatography.

**Phase 2 Reaction Products**

Hippuric acids, which are formed as Phase 2 metabolic products from toluene, the xylene, benzoic acid, ethylbenzene, and closely related compounds, can be determined as biological markers of exposure. The formation of hippuric acid from toluene is shown in Chapter 23, Figure 23.2, and the formation of 4-methylhippuric acid from \( p \)-xylene is shown below:

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{C} - \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{O} \\
\end{align*}
\]

\(+\{\text{O}\}, \text{Phase I oxidation}\)

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{C} - \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{O} \\
\end{align*}
\]

\(+2\{\text{O}\}, \text{enzymatic oxidation with loss of } \text{H}_2\text{O}\)

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{C} - \text{O} \\
\text{H} & \quad \text{H} \\
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{C} \\
\text{C} & \quad \text{OH} \\
\end{align*}
\]

(27.6.3)

Other metabolites that may be formed from aryl solvent precursors include mandelic acid and phenylglyoxylic acid.

Exposure to toluene can be detected by extracting hippuric acid from acidified urine into diethyl ether/isopropanol and direct ultraviolet absorbance measurement of the extracted acid at 230 nm. When the analysis is designed to detect the xylene, ethylbenzene, and related compounds, several metabolites related to hippuric acid may be formed and the ultraviolet spectrometric method does not give the required specificity. However, the various acids produced from these compounds can be extracted from acidified urine into ethyl acetate, derivatized to produce volatile species, and quantified by gas chromatography.

A disadvantage to measuring toluene exposure by hippuric acid is the production of this metabolite from natural sources, and the determination of tolulymercapturic acid is now favored as a biomarker of toluene exposure.\(^{10}\) An interesting sidelight is that dietary habits can cause uncertainties in the measurement of xenobiotic metabolites. An example of this is the measurement of worker exposure to 3-chloropropene by the production of allylmercapturic acid.\(^{11}\) This metabolite is also produced by garlic, and garlic consumption by workers was found to be a confounding factor in the method. Thiocyanate monitored as evidence of exposure to cyanide is increased markedly by the consumption of cooked cassava!

**Mercapturates**

Mercapturates are proving to be very useful Phase 2 reaction products for measuring exposure to xenobiotics, especially because of the sensitive determination of
**Table 27.1. Phase 1 Reaction Products of Xenobiotics Determined**

<table>
<thead>
<tr>
<th>Parent compound</th>
<th>Metabolite</th>
<th>Method of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclohexane</td>
<td>Cyclohexanol</td>
<td>Extraction of acidified, hydrolyzed urine with dichloromethane followed by gas chromatography</td>
</tr>
<tr>
<td>Diazinone</td>
<td>Organic phosphates</td>
<td>Colorimetric determination of phosphates</td>
</tr>
<tr>
<td>p-Dichlorobenzene</td>
<td>2,5-Dichlorophenol</td>
<td>Extraction into benzene, gas chromatographic analysis</td>
</tr>
<tr>
<td>Dimethylformamide</td>
<td>Methylformamide</td>
<td>Gas chromatography with direct sample introduction</td>
</tr>
<tr>
<td>Dioxane</td>
<td>β-hydroxyethoxyacetic acid</td>
<td>Formation of volatile methyl ester, gas chromatography</td>
</tr>
<tr>
<td>Ethybenzene</td>
<td>Mandelic acid and related aryl acids</td>
<td>Extraction of acids, formation of volatile derivatives, gas chromatography</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>Methoxyacetic acid</td>
<td>Extracted with dichloromethane, converted to volatile methyl derivative, gas chromatography</td>
</tr>
<tr>
<td>monomethyl ether</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Formic acid</td>
<td>Gas chromatography of volatile formic acid derivative</td>
</tr>
<tr>
<td>Hexane</td>
<td>2,5-Hexanedione</td>
<td>Gas chromatography after extraction with dichloromethane</td>
</tr>
<tr>
<td>n-heptane</td>
<td>2-Heptanone, valerolactone, 2,5-heptanedione</td>
<td>Measurement in urine by GC/MS</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>Acetone</td>
<td>Gas chromatography following extraction with methylethyl ketone</td>
</tr>
<tr>
<td>Malathion</td>
<td>Organic phosphates</td>
<td>Colorimetric determination of phosphates</td>
</tr>
<tr>
<td>Methanol</td>
<td>Formic acid</td>
<td>Gas chromatography of volatile formic acid derivative</td>
</tr>
<tr>
<td>Methyl bromide</td>
<td>Bromide ion</td>
<td>Formation of volatile organobromine compounds, gas chromatography</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>p-Nitrophenol</td>
<td>Gas chromatography of volatile derivative</td>
</tr>
<tr>
<td>Parathion</td>
<td>p-Nitrophenol</td>
<td>Gas chromatography of volatile derivative</td>
</tr>
<tr>
<td>Polycyclic aryl</td>
<td>1-Hydroxypyrene</td>
<td>HPLC of urine</td>
</tr>
<tr>
<td>hydrocarbons</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 27.1. (Cont.)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Acid</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Styrene</td>
<td>Mandelic acid</td>
<td>Extraction of acids, formation of volatile derivatives, gas chromatography</td>
</tr>
<tr>
<td>Tetrachloroethylene</td>
<td>Trichloroacetic acid</td>
<td>Extracted into Pyridine and measured colorimetrically</td>
</tr>
<tr>
<td>Trichloroethane</td>
<td>Trichloroacetic acid</td>
<td>Extracted into Pyridine and measured colorimetrically</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>Trichloroacetic acid</td>
<td>Extracted into Pyridine and measured colorimetrically</td>
</tr>
</tbody>
</table>

these substances by HPLC separation, and fluorescence detection of their $\alpha$-phthalaldehyde derivatives. In addition to toluene mentioned above, the xenobiotics for which mercapturates may be monitored include styrene, structurally similar to toluene; acrylonitrile; allyl chloride; atrazine; butadiene; and epichlorohydrin.

The formation of mercapturates or mercapturic acid derivatives by metabolism of xenobiotics is the result of a Phase 2 conjugation by glutathione. Glutathione (commonly abbreviated GSH) is a crucial conjugating agent in the body. This compound is a tripeptide, meaning that it is composed of three amino acids linked together. These amino acids and their abbreviations are glutamic acid (Glu), cysteine (Cys), and glycine (Gly). The formula of glutathione may be represented as illustrated in Figure 27.1, where the SH is shown specifically because of its crucial role in conjugation.

![Figure 27.1](image_url)

**Figure 27.1.** Glutathione conjugate of a xenobiotic species (HX-R) followed by formation of glutathione and cysteine conjugate intermediates (which may be excreted in bile) and acetylation to form readily excreted mercapturic acid conjugate.
role in forming the covalent link to a xenobiotic compound. Glutathione conjugate
may be excreted directly, although this is rare. More commonly, the GSH conjugate
undergoes further biochemical reactions that produce mercapturic acids (compounds
with N-acetylcysteine attached) or other species. The specific mercapturic acids can
be monitored as biological markers of exposure to the xenobiotic species that result
in their formation. The overall process for the production of mercapturic acids as
applied to a generic xenobiotic species, HX-R (see previous discussion), is illust-
trated in Figure 27.1.

27.7. DETERMINATION OF ADDUCTS

Determination of adducts is often a useful and elegant means of measuring
exposure to xenobiotics. Adducts, as the name implies, are substances produced
when xenobiotic substances add to endogenous chemical species. The measurement
of carbon monoxide from its hemoglobin adduct was discussed in Section 27.4. In
general, adducts are produced when a relatively simple xenobiotic molecule adds to
a large macromolecular biomolecule that is naturally present in the body. The fact
that adduct formation is a mode of toxic action, such as occurs in the methylation of
DNA during carcinogenesis (Chapter 22, Section 22.8), makes adduct measurement
as a means of biological monitoring even more pertinent.

Adducts to hemoglobin are perhaps the most useful means of biological monitor-
ing by adduct formation. Hemoglobin is, of course, present in blood, which is the
most accurate type of sample for biological monitoring. Adducts to blood plasma
albumin are also useful monitors and have been applied to the determination of
exposure to toluene diisocyanate, benzo(a)pyrene, styrene, styrene oxide, and
 aflatoxin B1. The DNA adduct of styrene oxide has been measured to indicate
exposure to carcinogenic styrene oxide.13

One disadvantage of biological monitoring by adduct formation can be the
relatively complicated procedures and expensive, specialized instruments required.
Lysing red blood cells may be required to release the hemoglobin adducts,
derivatization may be necessary, and the measurements of the final analyte species
can require relatively sophisticated instrumental techniques. Despite these complex-
ities, the measurement of hemoglobin adducts is emerging as a method of choice for
a number of xenobiotics including acrylamide, acrylonitrile, 1,3-butadiene, 3,3’
dichlorobenzidine, ethylene oxide, and hexahydrophthalic anhydride.

27.8. THE PROMISE OF IMMUNOLOGICAL METHODS

As discussed in Chapter 25, Section 25.5, immunoassay methods offer distinct
advantages in specificity, selectivity, simplicity, and costs. Although used in simple
test kits for blood glucose and pregnancy testing, immunoassay methods have been
limited in biological monitoring of xenobiotics, in part because of interferences in
complex biological systems. Because of their inherent advantages, however, it can
be anticipated that immunoassays will grow in importance for biological monitoring
of xenobiotics.14 As an example of such an application, polychlorinated biphenyls
(PCBs) have been measured in blood plasma by immunoassay.15

In addition to immunoassay measurement of xenobiotics and their metabolites,
immunological techniques can be used for the separation of analytes from complex
biological samples employing immobilized antibodies. This approach has been used to isolate aflatoxicol from urine and enable its determination along with aflatoxins B1, B2, G1, G2, M1, and Q1 using high-performance liquid chromatography and post-column derivatization/fluorescence detection. A monoclonal antibody reactive with S-phenylmercapturic acid, an important Phase 2 reaction product of benzene resulting from glutathione conjugation, has been generated from an appropriate hapten-protein conjugate. The immobilized antibody has been used in a column to enrich S-phenylmercapturic acid from the urine of workers exposed to benzene. Many more such applications can be anticipated in future years.

LITERATURE CITED


**SUPPLEMENTARY REFERENCES**


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QUESTIONS AND PROBLEMS

1. Personnel monitoring in the workplace is commonly practiced with vapor samplers that workers carry around. How does this differ from biological monitoring? In what respects is biological monitoring superior?

2. Why is blood arguably the best kind of sample for biological monitoring? What are some of the disadvantages of blood in terms of sampling and sample processing? What are some disadvantages of blood as a matrix for analysis? What are the advantages of urine? Discuss why urine might be the kind of sample most likely to show metabolites and least likely to show parent species?

3. Distinguish among the following kinds of analytes measured for biological monitoring: parent compound, Phase 1 reaction product, Phase 2 reaction product, adducts.

4. What is wet ashing? For what kinds of analytes is wet ashing of blood commonly performed? What kinds of reagents are used for wet ashing, and what are some of the special safety precautions that should be taken with the use of these kinds of reagents for wet ashing?

5. What species is commonly measured potentiometrically in biological monitoring?

6. Compare the analysis of Phase 1 and Phase 2 metabolic products for biological monitoring. How are Phase 2 products converted back to Phase 1 metabolites for analysis?

7. Which biomolecule is most commonly involved in the formation of adducts for biological monitoring? What is a problem with measuring adducts for biological monitoring?

8. What are two general uses of immunology in biological monitoring? What is a disadvantage of immunological techniques? Discuss the likelihood that immunological techniques will find increasing use in the future as a means of biological monitoring.

9. The determination of DNA adducts is a favored means of measuring exposure to carcinogens. Based upon what is known about the mechanism of carcinogenicity, why would this method be favored? What might be some limitations of measuring DNA adducts as evidence of exposure to carcinogens?

10. How are mercapturic acid conjugates formed? What special role do they play in biological monitoring? What advantage do they afford in terms of measurement?

11. For what kinds of xenobiotics is trichloroacetic acid measured? Suggest the
pathways by which these compounds might form trichloroacetic acid metabolically.

12. Match each xenobiotic species from the column on the left below with the analyte that is measured in its biological monitoring from the column on the right.

1. Methanol (a) Mandelic acid
2. Malathion (b) A diketone
3. Styrene (c) Organic phosphates
4. Nitrobenzene (d) Formic acid
5. n-Heptane (e) p-Nitrophenol