Traditionally, air sampling and analyses have been used to determine a worker's exposure to various airborne contaminants. Airborne Threshold Limit Values and permissible exposure levels have been developed for many contaminants. In certain situations, however, measurements of airborne concentrations are not always a reliable index of employee exposure. The determination of a chemical agent or its metabolite in a biological medium such as blood may provide more accurate information on exposure and the effects of exposure to hazardous substances. Perhaps the most common application for biological monitoring has been the determination of lead in blood. Analytical techniques have been developed for an additional parameter, zinc protoporphyrin, which, together with the blood-lead level, can give a more complete picture of lead absorption and metabolism. Information on blood-lead and zinc protoporphyrin monitoring as well as the relationship between the two parameters for a particular industry are discussed.

Blood as a matrix for biological monitoring

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introduction

As the field of industrial hygiene has progressed, air samples have been used as the primary means of evaluating potential health problems, and airborne guidelines, known as Threshold Limit Values, have been developed for a large number of contaminants. In many situations, however, measurements of airborne concentrations do not provide accurate information on exposure and the physiological effects of exposure to hazardous substances. Biological monitoring tests have been developed to aid in resolution of questions related to poor work practices, off-the-job exposures ("moonlighting"), or exposures to mixtures of chemicals. Several biological media have been investigated and suggested standards or "biological threshold limit values" have been proposed for many compounds in different biological media.[1,2]

The purpose of a biological monitoring program is generally twofold: to provide an indirect method of determining an exposure level to an airborne contaminant and to evaluate the significance of the airborne level of a pollutant. It is a fundamental tenet of such programs that each worker is a different biological system because of individual variability in human anatomical structure, physiological function and biochemical performance, and that each such system reacts in a slightly different manner from any other system. Air sampling programs are unable to address these differences and, therefore, such programs cannot yield useful information on the quantity of a hazardous substance which is absorbed or its potential health effects. However, as a WHO Expert Committee observed in 1973: "Using man as a biological monitor may or may not give a better estimate of magnitude of exposure than direct measurement of environmental concentration, depending on how well the measured internal concentration reflects the magnitude of the effective dose in critical organs or sites within the body."[3]

In the field of industrial hygiene, biological monitoring has been applied to a range of specimens and has been used successfully to obtain information in a number of situations in which air sampling is of little or no value. Examples of such situations include intermittent exposures, exposures to a complex mixture of chemicals, peculiar individual work practices (under this item, we would include the effectiveness of personal protective equipment), effects resulting from percutaneous or skin absorption and added exposures resulting from off-the-job activities.[4] A partial list of types of specimens that have been used includes whole blood, urine, feces, serum, breath, hair, nails and saliva.[1,2,5] Biologic monitoring most commonly involes the direct measurement of a hazardous agent in biologic media, for example, the determination of lead in blood or carbon monoxide in breath. Even for these supposedly simple cases, however, a great deal of controversy continues to rage over the significance of a given level. Certain types of organic compounds can undergo extensive biochemical transformation via metabolism in the liver and other organs. In these situations, biologic monitoring usually focuses on a metabolite which accumulates in biologic fluids in quantities proportional to the dose of the parent compound.[5] Examples include the determination of plasma trichloroacetic acid or trichloroethanol following trichloroethylene exposure, polynuclear aromatic hydrocarbon (PAH) metabolites in urine, or the measurement of hippuric or methylhippuric acid in urine following exposure to toluene or xylene, respectively. A third general form of biologic monitoring involves the quantitation of a unique biochemical change attributable to a toxic agent, *e.g.*, the inhibition of the enzyme cholinesterase by certain pesticides.

blood sampling and analysis

The ideal biologic monitoring method would permit the direct measurement of a toxic agent in the target organ. Since this would be impractical on a continuing basis, blood sampling is generally considered the most useful biologic

TABLE I
Suggested Blood Biological Threshold Limit Values

	Normal	BTLV
Lead	20 μg/dL	60 - 80 μg/dL
Manganese	-	10 μg/dL
Carbon Disulfide	none	15 μg/dL
Carbon Monoxide (as CoHb)	1%	10%
Arsenic	20 - 100 μg/dL	-
Beryllium	none	15 μg/dL
Cadmium	0.3 μg/dL	40 μg/dL
Copper	110 μg/dL (serum)	150 μg/dL
Mercury	0.2 - 0.5 μg/dL	20 μg/dL
Nickel	2 μg/dL (plasma)	-

monitoring technique since it provides an indirect measure of the level of a toxic agent in target organs or tissues. Blood has been recognized as an ideal biologic monitoring matrix since Dr. Kehoe's extensive studies which demonstrated a correlation between inorganic lead exposure and biologic levels of this metal.[6]

The advantages of blood as a biologic monitoring matrix are many. The absorption, excretion and retention of a number of compounds in blood is better understood than for other body fluids. Most industrial chemicals which might cause systemic effects are transported by the blood. Moreover, in most cases, blood levels accurately reflect dosage. Although obtaining blood is an invasive or intrusive mechanism, a 10-mL vacutainer sample is usually large enough to permit an initial and repeat analysis of several substances of interest from an industrial toxicological standpoint. Blood analysis has several applications to biological monitoring including insecticide exposure control, heavy metal studies, alcohol abuse, volatile solvent monitoring and carbon monoxide exposure. Examples of parameters that have been studied include lead, iron, nickel, copper, zinc, ZPP, δ-aminolevulinic acid, carboxy-hemoglobin (COHb), and serum protein changes in coal workers' pneumoconiosis.[7]

Some of the disadvantages of using blood as a biological matrix include difficulties in obtaining frequent specimens and the low concentrations of some of the parameters. This latter fact has led to problems in obtaining reproducible results even for as widely studied a parameter as lead in blood.[8,9]

The studies that have been performed to date have resulted in proposed biological threshold limit values for several substances. Some of these BTLV's are shown in Table I. Suggested BTLV's have been published for guidance although caution in application of these levels is suggested.[1,2,7]

Because of the decided advantages of blood as a biologic monitoring matrix, it is surprising that blood monitoring has not been used more frequently in industrial hygiene. As discussed earlier, the determination of lead in blood is undoubtedly the most common application of biological monitoring in occupational and environmental health and represents the most accurate measure of employee exposure to lead. Both the Federal Government (Department of Health and Human Services, CDC) and many of the states have instituted proficiency testing programs to monitor the quality of results produced by laboratories performing these analyses. Analytical techniques have been developed for an additional parameter, zinc protoporphyrin (ZPP) which together with the blood-lead level provide a more complete picture of lead intake, metabolism and its effect on the hematopoietic system. Bethlehem's Environmental Health Division Laboratory has performed additional studies of ZPP which extend the results of our August, 1979 report.[10]

methods and measurements

As suggested in the previous publication, a relationship appeared to exist between ZPP and blood-lead level for the population of workers studied:

$$\log \text{ZPP} = 0.023\,(\text{blood lead, } \mu g/dL) + 1.04$$

The ZPP value corresponding to a blood-lead of 60 μg/dL calculated from this equation is 270 μg/dL which was compared with relationships published by other researchers. We have continued to measure ZPP on all Bethlehem employees for which blood lead measurements are routinely performed. Accordingly, a sufficient data base has been developed to determine whether a constant relationship exists between blood-lead and ZPP and to examine the effects of such parameters as age and location. Of the large number of samples which have been collected in this study group of industrial workers engaged in a variety of occupations at several geographic locations, most of the blood-lead values have been well below 40 μg/dL.

Both blood-lead and ZPP are determined on whole venous blood collected by venipuncture in 10 mL low-lead heparinized vacutainers. Lead is determined by anodic stripping voltammetry using an ESA Model 3010A Trace Metals Analyzer and ZPP is measured using an ESA Model 4000 Hematofluorometer.

An examination of values without regard to location, age, occupation, or geographic location provided the following relationship:

$$\log \text{ZPP} = 0.018\,(\text{blood lead, } \mu g/dL) + 1.017$$

The correlation coefficient was calculated to be 0.735 and a ZPP value of 125 μg/dL is predicted for a blood-lead level of 60 μg/dL. It has been our observation as reported in our previous study that ZPP values are not significantly elevated

TABLE II
Distribution of Blood Lead Results from a Brass Foundry

Range of Blood-Lead Levels	Number
0-10 μg/dL	0
11-20 μg/dL	37
21-30 μg/dL	17
31-40 μg/dL	13
41-50 μg/dL	10
51-60 μg/dL	5

until the blood-lead values rise to 60 μg/dL or greater. Although it has been suggested that a linear relationship between these two variables cannot be established because of the difference in half-lives of those substances, workers in the various occupations examined are in a steady state situation. That is, certain subgroups of workers have chronic, intermittent exposures to lead which we believe permits comparisons between blood-lead and ZPP. As with any biological parameter, an individual ZPP value taken out of context and examined without regard to the blood-lead value or airborne lead level may have little or no meaning. Several observations can be made based on the comparable relationship for the larger study group:

1. The correlation coefficient for the predicted relationship is worse than for the original equation.
2. Because this relationship is significantly different from the predicted one, studies of this type may have to be conducted on an operation by operation basis.
3. The small number of blood-lead values of 40 μg/dL and above may not allow comparisons with ZPP due to our previous observations that a blood-lead of 60 μg/dL or higher is required to significantly affect ZPP concentrations.

Because of the questions raised by these differences, other factors which might affect blood-lead and/or ZPP concentrations were examined.

One of the factors examined was location. Our initial study contained a significant number of values from one geographic location — a brass foundry. If the blood Pb-ZPP relationship is now calculated for the eighty-two values from this single location the following equation results:

$$\log ZPP = 0.021 \text{ (blood lead, } \mu g/dL) + 0.901$$

The ZPP value corresponding to a blood-lead of 60 μg/dL is 150 μg/dL and the correlation coefficient for this relationship was found to be 0.688. Both the correlation coefficient and the ZPP value for the 60 μg/dL blood-lead level are considerably different from our previous findings. None of the blood-lead values were above 60 μg/dL and only five values were greater than 50 μg/dL. Blood-lead results for the brass foundry employees were distributed as shown in Table II. Most of these values fall into a range which would be considered normal.

An example of the distribution of values from a shipyard operation is shown in Table III. Again the range of blood-lead values falls within the normal range and the calculated relationship between log ZPP and blood-lead provides a correlation coefficient of 0.298 which is essentially no correlation. These blood-lead values are not unreasonable since most of the airborne lead levels at this operation were well below the OSHA permissible level of 50 μg/M^3 and action level of 30 μg M^3.

TABLE III
Distribution of Blood-Lead Results from a Shipyard

Range of Blood-Lead Levels	Number
0-10 μg/dL	15
11-20 μg/dL	18
21-30 μg/dL	5
31-40 μg/dL	0
41-50 μg/dL	1

TABLE IV
Blood-Lead and ZPP Values for Former Battery Company Employee

Date	Blood-Lead, μg/dL	ZPP, μg/dL
6-15-79	15	551
8-14-79	69	481
11-05-79	70	422
1-14-80	62	374
4-24-80	57	260
8-13-80	54	177
10-23-80	54	163
12-09-80	52	174
2-12-81	52	174

A similar examination of smoking habits and age has also shown little relationship between ZPP and blood-lead.

discussion

From the large amount of data collected to date it is our belief that for employees having little or no exposure to lead, the measurement of ZPP alone would be of little value. As with any biological measurement, if the measurement of ZPP is combined with information such as blood-lead values and airborne lead levels, interpretation of the significance of the values becomes possible. The measurement of ZPP as well as blood-lead has been of benefit in the clinical evaluation of several employees. For example, there have been two cases where workers have had elevated ZPP values (500 μg/dL) and no history of significant occupational lead exposure. A clinical investigation showed that these two workers had undiagnosed iron deficiency anemia. Also, periodic ZPP monitoring has identified a few employees with significant "off-the-job" exposures to lead. For example, the biologic monitoring program at one of our wire mills recently identified an employee with consistently elevated blood Pb and ZPP levels who worked in an area where airborne lead concentrations had been reduced years earlier due to various process changes. All other employees in this work area exhibited normal biologic values. Discussions with the affected employee revealed that he had previously worked and had been periodically "moonlighting" at a battery breaking operation. As shown in Table IV where the employee discontinued these outside activities, the ZPP and blood-lead levels were reduced to normal values.

Table V shows the improvement in biologic monitoring results over time for two employees whose airborne lead exposures have been reduced by a combination of engineering controls, work practices, and personal protective equipment. In the first case the blood-lead values have dropped

TABLE V
Blood-Lead and ZPP Values for Two Employees Over Time

Date	Blood-Lead, μg/dL	ZPP, μg/dL
Employee 1:		
7-16-76	50	178
8-03-76	46	195
4-20-77	44	137
6-19-79	46	228
8-22-79	46	203
10-10-79	45	188
12-14-79	50	161
4-08-80	57	100
6-09-80	49	133
9-22-80	50	131
11-18-80	36	90
2-18-81	36	74
Employee 2:		
1-15-79	54	88
3-03-79	59	144
5-08-79	54	162
12-04-79	64	73
1-25-80	58	65
4-29-80	48	62
10-16-80	41	35
12-17-80	41	34
2-12-81	36	34

below 40 μg/dL and ZPP values have declined from over 200 μg/dL. The second case, again, shows how both determinations can be used to observe the effectiveness of control measures.

conclusions

Blood is an important medium for biological monitoring studies of industrial populations, but results derived from such investigations must be considered in conjunction with airborne levels of the contaminants and knowledge of the workplace. Biological threshold limit values for several contaminants in blood have been suggested. Blood-lead levels have been examined extensively and in spite of the analytical problems are the single most useful measurement for evaluation of occupational lead exposure and the impact of such confounding factors as personal work habits and off-the-job exposures. Zinc protoporphyrin, a fairly recent addition to the battery of biochemical tests available for biologic lead monitoring, has been used successfully with other biological tests to evaluate the effectiveness of environmental controls at operations where workers are chronically exposed to lead.

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