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Environmental Biomonitoring

Exposure Assessment and Specimen Banking

EDITED BY
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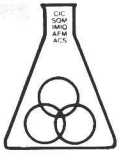
Developed from a symposium sponsored by the International Chemical Congress of Pacific Basin Societies and the ACS Division of Environmental Chemistry, Inc., at the 1995 International Chemical Congress of Pacific Basin Societies



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ACS Symposium Series

Foreword

THE ACS SYMPOSIUM SERIES was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of this series is to publish comprehensive books developed from symposia, which are usually "snapshots in time" of the current research being done on a topic, plus some review material on the topic. For this reason, it is necessary that the papers be published as quickly as possible.

Before a symposium-based book is put under contract, the proposed table of contents is reviewed for appropriateness to the topic and for comprehensiveness of the collection. Some papers are excluded at this point, and others are added to round out the scope of the volume. In addition, a draft of each paper is peer-reviewed prior to final acceptance or rejection. This anonymous review process is supervised by the organizer(s) of the symposium, who become the editor(s) of the book. The authors then revise their papers according to the recommendations of both the reviewers and the editors, prepare camera-ready copy, and submit the final papers to the editors, who check that all necessary revisions have been made.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previously published papers are not accepted.

ACS BOOKS DEPARTMENT

Preface

ENVIRONMENTAL BIOMONITORING has become an important tool for identifying and instituting remedies to the threat of contamination of the biosphere. The available information suggests that current pollutant levels have become stressful to a large number of sensitive flora and fauna, as well as to human beings. The deleterious impacts, both real and perceived, of natural and man-made pollutants have created the need to monitor their release and subsequent movement through the physical and biological components of the environment. It has become necessary to institute environmental surveillance systems to monitor noxious substances for early identification of, and response to, health and environmental problems caused by xenobiotic toxicants. In addition, increasing attention is being focused on the monitoring and assessment of human exposure to environmental contaminants throughout the world.

Environmental biomonitoring involves several aspects of environmental health, including the determination and identification of low levels of pollutants and their metabolites, exposure assessment, and measurement of biological markers of exposure. Another important facet of environmental biomonitoring is the emerging field of environmental specimen banking. A specimen bank acts as a bridge connecting real-time monitoring with future trend-monitoring activities.

This book is based on the symposium entitled "Environmental Biomonitoring and Specimen Banking", held at the 1995 International Chemical Congress of Pacific Basin Societies (Pacifichem '95) in Honolulu, Hawaii, on December 17–22, 1995. The purpose of the book is to assimilate current developments in the diverse field of environmental and human health. In particular, we have highlighted recent developments in monitoring strategies, exposure assessment, bioindicators (or biomarkers), and specimen banking.

The term "environmental biomonitoring", as used in this book, refers to the measurement and identification of pollutants and their metabolites in environmental and biological media. The term "media" refers to the environments in which the pollutants are present. The term "biological monitoring" is used when the concentration of a pollutant or its metabolite is monitored in a human indicator medium such as blood, urine, hair, saliva, or the placenta. Although the emphasis of the book is on monitoring the biosphere in the context of public health, monitoring efforts related to occupational exposure are also touched upon because of their

methodological relevance. A group of internationally renowned researchers was assembled to address these issues and to facilitate an assessment of the state-of-the-art of the field of environmental biomonitoring and specimen banking.

The book is organized into four sections: monitoring, exposure assessment, bioindicators, and specimen banking. Chapter 1 provides a comprehensive overview of the subject of environmental biomonitoring to set the tone of the book. Other chapters in the book have been developed from only 23 of the 60 papers presented at the symposium in order to avoid an incoherent collection of papers and to synchronize the flow of information from one chapter to the next. The mix of review papers, original research manuscripts, and reports of new work presented here best reflect the current state of the subject. This book represents a modest beginning of our efforts to achieve a comprehensive perspective of the field of environmental biomonitoring.

The contents of the book should appeal to scientists in the fields of analytical chemistry, clinical chemistry, ecology, medicine, and environmental science and health. We hope that the readers will find this book interesting and useful in their many and varied quests into the realm of environmental biomonitoring and specimen banking.

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Contents

Chapter 1

Environmental Biomonitoring and Specimen Banking

Bioanalytical Perspectives

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The health impact of toxic substances in the environment is a widely studied subject. The primary goal here is to develop reliable basis for risk assessment. In this context, recent advances in analytical chemistry have made it practical to measure very small quantities of chemicals in tissues, body-fluids, food and air. Consequently, it has been possible to quantify contaminants such as pesticides, other organic and inorganic constituents, metals, and solvent and drug residues in our environment. However, much emphasis during this developmental phase is placed on instrumentation, while factors affecting the "total quality" of a bioenvironmental investigation tend to be overlooked. Unless other tools for risk assessment such as sound biomonitoring programs (real-time and long-term) based on environmental epidemiological concepts are properly used in generating data, interpreting the significance of these findings will continue to be a challenge. Therefore, dedicated efforts are needed for (i) consolidating the biologic basis for selection of specimens for environmental surveillance, (ii) developing strategies for long-term preservation of sampled materials, (iii) accomplishing harmonization of analytical measurements, and (iv) recognizing the multidisciplinary expertise, for understanding the pollution trends and to provide a reliable basis for health risk assessment in exposure situations.

The concern about toxic substances in the biosphere and the need for developing strategies to eliminate or minimize the health hazards caused by these pollutants is well recognized. This is particularly relevant in the context of the estimated 60,000 or so chemicals of industrial consequence, of which only a handful are

currently being examined for their environmental impact *(1)*. For example, pesticides (chlorinated hydrocarbons and their degradation products) are ubiquitous in ecosystems. There is evidence in Finland that although the use of DDT and its derivatives ended in the early 1970s, these compounds are still encountered in trace amounts in fish *(2)*. Thus, contaminants such as pesticides, polycyclic aromatic hydrocarbons, halogenated hydrocarbons, heavy metals and other trace elements, enter the food chain through various sources (e.g. anthropogenic) in the biosphere. The organic pesticides are generally highly stable, lipophilic substances that tend to accumulate in adipose tissues over time, culminating in environmental toxicity and mutagenic changes.

The above mentioned events call for stringent measures for environmental surveillance, but the scenario is not easy to comprehend; there are too many chemicals and practically endless routes of exposure requiring comprehensive schemes for monitoring. Moreover, the state-of-the-art of analytical competence is not the same for all classes of pollutants. These problems warrant skillfully conceived multidisciplinary approaches including identification of appropriate specimens to assess human exposures to xenobiotic toxicants in the environment. However, any monitoring program should be designed to meet both real time pursuit (tracking short-term trends) and provisions for retrospective research (identifying long-term trends) as analytical capabilities improve. The ability to reevaluate data retrospectively extends the scope of data accumulation, and provides a sound basis for evaluation of the biological effects of various pollutants.

Therefore, dedicated efforts are needed for (i) consolidating the biologic basis for selection of appropriate specimens for environmental surveillance, (ii) developing strategies for long-term preservation of sampled materials, (iii) accomplishing harmonization of analytical measurements, and (iv) recognizing the multidiscilpinary expertise *(3)* to evaluate pollution trends. The benefits are: improvements in problem oriented analytical approaches, establishment of reliable baseline values for numerous chemical constituents in selected environmental media, and a proven experimental tool for assessment of the environmental health criteria. These aspects will be addressed in the following sections.

Chemicals in the Environment

Inorganic constituents: From the environmental pollution point of view, various anthropogenic activities, especially the burning of fossil fuels required in various industrial operations, are a major source of several toxic trace elements, including Se *(4)*. Among these, As, Cd, Hg, and Pb deserve special mention since they have profound effects on both domestic animals and human beings. Froslie and co-workers *(5)* have shown that heavy-metal contamination of natural surface soils from atmospheric deposition occurs even at very long distances from the major point source.

Several examples of human exposure to As, Cd, Hg, and Pb have been recorded in the scientific literature. It is known, for instance, that sections of

human populations from the south and far east Asia such as Japan, Taiwan and the Philippines have high levels of As in their blood, milk or hair *(6)*. The source of As is linked to the consumption of fish which is a significant component of an average diet. The As content of the soil also plays a role.

Among the innumerable sources of industrial pollution, gasoline is the major source of environmental Pb in those countries where unleaded petrol is not mandatory. In many urban populations blood-Pb levels have been shown to be well over 200 ng/mL *(6)*. This figure may be compared with the moderate or low levels of 90 ng/mL in Japan and Sweden, or even lower levels of 30 ng/mL observed in remote parts of Nepal and the interior regions of Venezuela *(6)*. Similarly, urban mothers have been shown to secrete more Pb into their milk than their corresponding rural controls *(7)*. A common problem is faced by young children who ingest several mg of soil (along with paint scrapings and house dust) per day resulting in significant extra-dietary exposure to not only Pb, but also Al, Si, Ti and V. This is a serious bioenvironmental problem faced in pediatric fields, since it is not easy to precisely estimate a child's daily intake of soil and other non-food sources. The ever widening understanding of Pb toxicity indicates that Pb intake by human populations has increased about one hundred-fold above the natural level based on analysis of samples of prehistoric human skeletons *(8)*.

It has also been reported that subjects residing in the vicinity of Cu smelters near Cluj-Napooa in Rumania excreted higher levels of As in their urine and hair than did controls. A five-fold increase in urinary As (6.4 vs 31 ng/mL) and an increase of up to 32 times in hair content (0.25 vs 8 ng/mL) was demonstrated *(9)*. Analysis of the body burden of As clearly indicated excessive exposure to this element resulting from Cu-ore smelting activities. Environmental air particulate analyses have shown a correlation between As and Cu near smelters.

Cadmium is a toxic industrial environmental pollutant. Tobacco smoking is a significant contributor to the high levels of Cd found in smokers. Blood levels of Cd in smokers are higher than in those in nonsmokers by a factor of 3 to 12 *(6)*. Excess exposure to Cd can cause renal tubular damage and obstructive lung disease. The amount of Cd transferred from soil to plant to animal or human can be of concern, especially where sewage sludge is used to fertilize the soils *(10,11)*. The degree of risk depends on several factors: type of food affected (e.g. commonly consumed foods and foods consumed in large quantities) by the application of the sludge, soil pH and the amount of Cd entering the soil.

Sea-food is a major source of Hg intake. For example, it has been shown that Alaskan Eskimo mothers have rather high concentrations of this element in placenta, hair, blood and milk *(12)*. It is also known that these concentrations of Hg are directly proportional to the quantity of seal meat consumed. Subjects who consumed seal meat daily had the highest levels of Hg *(12)*. This brings into focus considerations of infant nutrition since breast-fed babies of these mothers are potentially subjected to toxic doses of Hg.

Mineral oil contains about 0.2 μg/g of Se whereas coal contains as much as 3 μg/g, and in some exceptional cases levels can be much higher *(4,13,14)*.

Coal ash (especially the fine fraction of fly ash), which contains up to several hundred μg/g of Se is carried over long distances and distributed over soil surfaces. The availability of this Se to plants depends upon soil characteristics such as degree of alkalinity. A combination of soil and environmental pollution factors has been reported to be the cause of Se toxicity in the neighboring counties of selenium deficient areas in Northeastern China *(15)*. A mortality rate of about 50 percent was recorded in some villages. The source of this environmental Se was found to be a stony coal that contained an average of 300 μg/g of Se. The Se entered the soil and was readily taken up by plants (vegetables and maize) because of the traditional use of lime as fertilizer in that region, leading to an outbreak of human selenosis.

Organic Pollutants: The determination of halogenated aromatic and aliphatic compounds, aromatic amine compounds and other miscellaneous class of chemicals (e.g. benzene) and specific metallo-organic compounds such as methyl mercury and organotins in environmental, biological and food related matrices have been the focal point of several biomonitoring investigations. This is particularly relevant to marine monitoring programs because of increasing concerns about the effects of marine pollution on the health of marine mammals.

Geochemical: The High Altitude Context

Many interesting associations of I and Se with environment, high altitude and human and animal health are beginning to unfold *(16)*. Similarly, it may be postulated that if quantities of Pb in high-altitude environments are appreciable, or if Pb levels in foods consumed by these populations are high, Pb accumulation becomes a potential danger since more than 95 per cent of the absorbed fraction of Pb resides in blood, namely in erythrocytes *(17)*. In view of the elevated haematocrit in high altitude populations, the potential dangers are obvious. The erythrocyte-turnover cycle is 120 days, which means that accumulated Pb will be partially released and redeposited in bone in which lead has a long biological half-life. Therefore, for high altitude populations, it is imperative that environmental Pb levels be kept low.

There are some examples of altitude-related differences in Se levels from the animal world too. It has recently been shown in the USA that in rock squirrels captured at different elevations, ranging from grass-land to pinyon juniper ecosystems (elevations of 1600 to 2400 meters), the Se content of kidney and liver was highest in grass land animals and decreased with increasing elevation gradient *(18)*. As grass-land soil is alkaline, it would appear that the mobilization and uptake of Se by plants, and thereby also in animals, was the key contributory factor.

Bioanalytical Approaches

Harmonization of Environmental Measurements: The present day analytical techniques are capable of detecting extremely small quantities of chemical

substances in the biosphere and have eached the potential to serve as routine tools for ultra-trace measurements. Undoubtedly, the technological progress has greatly contributed to the advances in metrology (the science of measurements) of trace analysis of bioenvironmental systems. Similarly, analytical quality assurance brought about by the development and use of a variety of Standard Reference Materials (SRM) have further enhanced the metrological excellence. However, this capability is somewhat neutralized because of failure to observe proper procedures, analyzing inadequately prepared samples and generalizing the findings. Hence, in spite of the technological supremacy, inconsistencies in the quantitative data of environmental measurements are still prevailing. This is an indication that high detection capability and sensitivity of analytical techniques alone is not the solution to the problem of reliable data generation in bioenvironmental systems. Thus, there is much concern in the minds of the life sciences researchers that improved capability for quantification is not harmonized with appropriate analytical and biological perceptions. First of all, there is a lack of appreciation for multidisciplinary approaches, leading to poor planning of bioenvironmental studies.

"Bio"sources of analytical errors: Two major sources of errors need to be considered in this context: *conceptual* (arising from limited understanding of the "bio" dimension of the specimens such as biological variations that are identifiable but not always quantifiable, wrong statistical approach to data processing, wrong basis for expressing the data, and incompatible data interpretation); and *analytical* (stemming from sampling, sample preparation, calibration, matrix effects, procedural/instrumental, etc.). Of particular concern is the fact that a significant portion of the existing analytical information is derived from analyses of uncontrolled and often "spot" samples (e.g. hair, urine, soil and foods) with deficient sampling plans and inadequate AQA procedures. Yet, major decisions of public health concern are being made that are dependent on the analytical information obtained by monitoring and other biomedical studies. Apart from the analytical reliability expected of such studies, measures must be taken to ensure that the experimental design for obtaining such information is conceptually relevant *(19)*.

Analytical Quality Assurance

Bioenvironmental investigations require provisions for a "total" Analytical Quality Assurance (AQA) to yield meaningful results. These are measures that encompass all stages of an investigation such as experimental design, collection of analytically and biologically valid specimens, analytical measurement processes and proper evaluation of analytical data, including data interpretation. Further, one of the critical decisions that the analyst needs to make is the degree of quality (quality standard), or tolerance limits, required for the purpose of the investigation for which the analyses are being made. If the tolerance limits are set narrower than the investigation really requires or not feasible under practical laboratory conditions, it can cause unnecessary expense and loss of time.

In initiating an AQA program use of two or more independent analytical methods to verify the accuracy of an analytical finding is a crucial requirement. This is reflected through the development and certification of a wide variety of reference materials for many inorganic constituents *(20)*. Therefore, reasonably well-founded baseline data for trace elements in biomaterials have been generated by few selected laboratories around the world.

On the other hand, in the area of organic analysis procedures for AQA of many constituents are still evolving. Although there is ample awareness among the researchers concerning the role of AQA in generating reliable results, this perception has not translated into action other than the recognition of inconsistencies stemming from intercomparison trials. This is not swiftly followed up by identification of the sources of discrepancy, initiation of the remedy, and subsequent AQA exercises. One obvious reason for this slow progress is the paucity of funding for basic research, thus shifting a major fraction of the developmental work to a few institutions such as those dealing with Reference Materials Programs. In addition, the problem of lack of certified reference materials (CRM) for organic constituents has been existing for a long time. This is of course a reflection of genuine methodological and technical hurdles; by far the most challenging step being preparation of a natural matrix and extended preservation of the compositional integrity of the sampled material.

CRMs for Organic Constituents: NIST has developed several CRMs ranging from simple solutions for calibration of analytical instruments to complex natural matrix material, some fortified, for suitable for validation of methods adopted for biomonitoring and related programs. For example, the mussel tissue (Mytilus edulis; NIST SRM 1974) is certified for anthracene, benzo(b)fluoranthene, benzo(ghi)perylene, benzo(a)pyrene, fluoranthene, pyrene, perylene and phenanthrene. It also contains information values for a number of other PAHs, PCBs and chlorinated pesticides. Similarly, CRMs of organics in marine sediments (NIST SRM 1941) and cod liver oil (NIST SRM 1588), and PCBs in human serum (NIST SRM 1589) are available. Recently, frozen whale blubber (NIST SRM 1945) has been developed at the NIST for use as control material *(21)* for organic and inorganic contaminants including methyl mercury. This SRM has been analyzed for 30 polychlorinated biphenyl congeners and 16 chlorinated pesticides. Similarly, IAEA has released the shrimp homogenate (MA-A-3/OC) and lyophilized fish tissue (MA-B-3/OC) certified for chlorinated hydrocarbons. The Bureau of Community Reference Materials (BCR) has CRMs for pesticides in milk powder (BCR-150, BCR-51, BCR152), and for aflatoxins in milk powder (BCR-282, BCR-283, BCR-284, BCR-285). Concerning organo-metallic contaminants, besides NIST-SRM-1945 (whale blubber) certified for methyl mercury, fish tissue from the National Institute of Environmental Studies (NIES-11) has been certified for total Sn, tributyl and triphenyl Sn. The National Research Council of Canada has issued dogfish muscle (NRCC-DORM-1) and lobster hepatopancreas (NRCC-TORT-1) certifed for methyl mercury. Information on additional matrices can be found elsewhere *(22)*.

Database for Reference Concentrations

Baseline data in a well defined group of individuals reflect reference values. Obviously, factors such as age, sex, living environment and diet, among others, influence the concentration levels of certain trace elements, but some of these parameters can be well defined. In some cases, even the habits such as smoking tobacco (e.g. elevation of blood Cd) and consuming alcohol (e.g. elevation of blood Pb), should be considered and several reference sources are now available *(23-26)*.

In the inorganic area, over the last decade, there has been considerable progress in understanding the laboratory related analytical problems in improving the quality of trace element data generated *(26)*. With increased understanding of the sources of variations in elemental concentrations arising from physiological changes, pathological influences, and occupational and environmental exposures *(23,27)*, efforts to generate reliable reference data bases for elemental composition of human tissues and body fluids are showing signs of success.

In the organic area, the increasing emphasis on developing natural matrix reference materials has been an important step *(21,22)*. Data compilations developed by the U.S. EPA *(28)*, NOAA *(29)* and other sources *(22)* provide reference information on many organic substances in various media.

Bioenvironmental Surveillance

The role of bio-environmental monitoring (BEM) or surveillance is that of an early warning system to identify factors responsible for adverse health effects and measures to prevent them. However, BEM per se has different meanings and these should be understood properly. In the context of human health, the expression biological monitoring (BM) of health is commonly used and is aimed at the detection of biological effects arising from exposure to chemicals in the environment (e.g., exposure to metals resulting in proteinurea or perturbation of enzyme levels or other metabolites). BM is performed by measuring the concentrations of toxic agent and its metabolites on representative biological samples from the exposed organism. If appropriate indicator specimens, e.g. blood, urine, expired air and hair are used, the body burden of certain pollutants absorbed or retained in the organism during a specific time interval can be assessed, dose-response relationships can be established, and the data can be used for risk-assessment purposes.

Biostatistical considerations: The primary consideration for a viable Biomonitoring Program concerns the problem of sample size (number of samples needed for a meaningful study) and defining the target population. They should be randomly selected to preserve representativeness and to avoid selection bias. The sample size is related to the ability to detect differences between groups, and to some extent, it is also dictated by the nature of the problem studied. If very small differences between groups are to be differentiated, the burden rests on both the capability of the analytical methodology and a large enough sample size.