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INTERNATIONAL REVIEW OF ENVIRONMENTAL SPECIMEN BANKING

STEPHEN A. WISE AND
ROLF ZEISLER,
EDITORS

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International Review of Environmental Specimen Banking

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FOREWORD

Among the U.S. Environmental Protection Agency's (EPA) goals for the 1980's is to contribute to the definition and to the solution of global problems associated with the large number of potentially toxic chemicals which are produced, traded, and used throughout the world. Most of these substances were developed to serve the needs of mankind. To date, only a fraction of these substances have been investigated with respect to their toxic or ecologic effects. There are potential dangers that stem from the way society develops, produces, markets, transports, uses and disposes of these chemicals. Each Government should be made aware of the problems that exist at every stage in the life cycle of these substances to insure effective management of these potentially hazardous substances.

During the course of our industrial development, hazardous chemical wastes were placed for containment in landfills and above ground disposal sites. In many cases, these sites have proved to be ineffective in stopping the release and spread of these potentially toxic compounds into the environment. Some of these sites now pose a significant risk to health and to the environment.

Several major activities are required to adequately ensure the protection of human health and the environment from the often subtle, but sometimes serious, effects of chemicals in the environment. Toxicological research, ecological research, and control technology development, should be considered prior to the substance reaching the environment, while biological monitoring and specimen banking are required to estimate the spread of the substance once it had been dispersed into the environment.

There is no cost-effective way to test all of these potentially toxic chemicals. However, there are means that can effectively deal with the detection of specific hazardous substances in the environment and alert officials for the need to take appropriate action. The environmental monitoring and specimen banking concept is one such approach that can provide timely information on the distribution of chemicals in the environment. It can accomplish this goal by the systematic sampling and analyses of environmental scavengers, biological species that concentrate chemicals within themselves. Repetitive sampling and analyses of these environmental scavengers will provide trend data, informing use of either increasing or decreasing environmental threats from these potentially hazardous substances. If the measured levels of chemicals rise in these scavengers, we are alerted to a potential problem. Hazardous chemicals can be quickly isolated and dealt with in an effective and efficient manner. Environmental monitoring and specimen banking not only validate the other testing employed as specified by law but also serve as necessary back-up or fail-safe procedures to complement these other methods. In the absence of effective monitoring of environmental samples and specimen banking, the detection of serious environmental contamination and threats to human health from chemical pollutants might occur only after critical and possibly irreversible damage has been done.

The U.S. Environmental Protection Agency has played a pioneering role in environmental specimen banking. Ten years ago, in collaboration with the National Bureau of Standards, EPA initiated a long-term program of research with the aim of developing the necessary expertise to establish a National Environmental Specimen Bank, a systematic approach to environmental monitoring and specimen banking. This system would incorporate validated procedures for specimen sampling, analysis, and long-term storage. EPA also entered into an agreement with the Umweltbundesamt, Federal Environmental Agency, of the Federal Republic of Germany so that the resources and experience of both nations could be more effectively brought to bear upon the development of national and international systems of specimen banking to foster environmental protection.

Three international workshops were held, to define and elaborate on the state-of-the-art techniques for environmental banking and monitoring, and to determine how these technologies may play a role in exposure assessment. It is evident from the proceedings of these workshops that many of the analytical, technical and logistic requirements for successful environmental specimen banking have been convincingly resolved. We have a sound scientific basis for the application of specimen banking to the evaluation and management of chemicals in the environment. By integration of this concept into our overall approach we may both reduce the risk of and improve efficiency in averting an ecological disaster.

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ABSTRACT

In the past decade, the concept of an environmental specimen bank for archiving of biological and environmental samples for retrospective analysis has been recognized as an important component to systematic environmental monitoring. The long-term storage of carefully selected representative environmental samples in a specimen bank provides an important complement to the real-time monitoring of the environment. In 1975, the National Bureau of Standards (NBS), in conjunction with the U.S. Environmental Protection Agency (EPA), initiated research relating to environmental specimen banking. In that same year, the Federal Republic of Germany (FRG) and the U.S. agreed to cooperate in the study of environmental specimen banking activities and proposed the establishment of "pilot phase" specimen banks in both countries. In 1979 and 1981, specimen banking facilities were completed and pilot programs were initiated in the U.S. and FRG, respectively. Recently, a smaller pilot effort has been established in Japan. The Canadian Wildlife Service, which has an existing collection of more than 10,000 individual wildlife specimens dating back to the mid-1960s, is in the process of transforming this ad hoc storage operation into a viable specimen bank as an integral component of its national monitoring program for the toxic chemicals in wildlife. A similar specimen bank program with wildlife samples from the late 1960s is associated with the Environmental Monitoring Programme in Sweden and is located at the Swedish Musuem of Natural History.

In September 1983, the "8th U.S. - German Seminar of State and Planning on Environmental Specimen Banking" and the "International Review of Environmental Specimen Banking" were held at the National Bureau of Standards. At these meetings, the current status of Environmental Specimen Banking Program in the U.S., Federal Republic of Germany (FRG), and other countries was presented and discussed. This publication contains a brief summary of these meetings and separate contributions describing the specimen banking activities in Canada, FRG, Japan, Sweden and the U.S.

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THE EIGHTH U.S.-GERMAN SEMINAR OF STATE AND PLANNING ON ENVIRONMENTAL SPECIMEN BANKING AND THE INTERNATIONAL REVIEW OF ENVIRONMENTAL SPECIMEN BANKING

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I. Introduction

Researchers from the United States and the Federal Republic of Germany (FRG) have held annual bilateral meetings as part of activities in specimen banking that were initiated with an agreement on the environment between these two countries in 1975. These bilateral meetings have provided for the exchange of expertise and experience in specimen banking and have served to coordinate the research efforts of "pilot" environmental specimen banking programs in both countries. The U.S. pilot program for a National Environmental Specimen Bank (NESB) was implemented in late 1979 as a five-year study. In the Federal Republic of Germany a pilot program was started in 1976, followed by the dedication of a banking facility in 1981.

Since the pilot studies in both the U.S. and the FRG were reaching a final phase and since environmental specimen banking has become a research activity of international interest, the 8^{th} U.S.-German Bilateral Meeting was held with the intention of providing an international review of specimen banking. At this meeting the past experience from the U.S. and German pilot programs was summarized regarding the viability of long-term specimen banking. These results were re-enforced by the experiences of additional specimen banking activities in other countries. In the past, three international workshops, held in 1977, 1978, and 1982, have dealt with the concept of environmental specimen banking and have served as focal points for the research efforts in several countries. This international review brought together participants from the major environmental specimen banking programs in Canada, Germany, Japan, Sweden, and the United States. Review articles on these five programs and a summary of the bilateral meeting are included in these proceedings.

II. Meeting Summary

The 8^{th} U.S.-German Seminar of State and Planning on Environmental Specimen Banking was held on September 19-20, 1983, followed by the International Review of Environmental Specimen Banking on September 21, 1983. The meetings were held at the National Bureau of Standards, Gaithersburg, Maryland. The meeting schedule and a list of the participants are given in Appendices I and II.

The presentations in the "Working Sessions" focused on the significant progress in specimen banking and biomonitoring in the two pilot programs. The presentations included new approaches in the selection and sampling of specimens and in the preparation of samples for analysis. Emphasis was placed on advances in analytical methodology for both organic and inorganic trace constituents. These presentations demonstrated the existence of a solid scientific and technological base for specimen banking and conveyed the current status of specimen banking methodology.

The Workshop discussions developed around the five topics listed in Appendix I. The major points of the Workshop discussions are briefly described in the following section and the conclusions drawn from the discussions are summarized in the respective section below.

The experience gained and the problems encountered in the homogenization, storage, and stability of samples were discussed in detail. The U.S. and German pilot programs have pursued somewhat different approaches in sampling, sample preparation, homogenization, and storage, thereby allowing for a useful comparison of these different approaches and an exchange of information and experience which will benefit possible new specimen banking projects. Although neither the U.S. nor the German pilot programs have completed the evaluation of the stability of banked specimens, preliminary data indicate that the samples are stable with respect to the inorganic constituents.

Major needs for reference materials related to environmental specimen banking were made evident. For inorganic analyses, a compilation of data from organisms and materials used as biomonitors may help to develop reference materials which are sufficiently similar to the investigated matrices. Significant efforts are needed to develop reference materials for organic trace analysis.

Multidisciplinary collaborative efforts are the key to valid environmental sampling. Questions of quantity and frequency of sampling must be answered by considering both statistical requirements and specimen banking goals. The use of "pooled" samples may result in the loss of information on real health effects or actual concentration levels.

Potential uses of banked samples need to be further explored. Different scientific communities should be questioned to determine what information about the banked specimens is needed and what information might be available from these samples. Even though these samples are currently stored for future chemical analyses, they might also be used for other purposes, e.g., to obtain biochemical or histopathological information.

The International Review of Environmental Specimen Banking brought together a variety of participants including representatives of major environmental specimen banking projects from five countries, analytical chemists, environmental managers, and representatives of several U.S. government agencies (see List of Participants in Appendix II). The presentations reviewed the efforts of the existing banking programs and provided global perspectives on the role of specimen banking programs in helping to protect man and his environment. Details on the scope of the major specimen banking activities are presented in the specific contributions in this publication. The significant statements and discussions of the presentations in the international review are included in the conclusions below.

III. Conclusions

During this meeting, the usefulness of the concept of environmental specimen banks was re-enforced by the presentations and discussions. Specimen banks are an important component of systematic environmental monitoring. The archiving of carefully selected, representative biological and environmental samples provides opportunities far beyond the possibilities of real-time monitoring of the environment. Banked specimens are an undisputed alternative to the impractical task of following the trends of all new, potentially hazardous chemicals which may enter the environment. As analytical techniques improve or as concern for now unknown pollutants arises, the archived specimens will provide information on the pollutants of the past. The samples will serve as benchmarks against which pollution trends can be measured. These samples will also provide calibration points for changing monitoring strategies or analytical approaches. The existing pilot programs and other small-scale banking efforts have demonstrated the concept and indicated that it is both viable and necessary. The experience gained in these programs has demonstrated the technical feasibility of environmental specimen banking.

In particular, the concept of preservation of sample integrity from the time and point of sampling to the actual chemical analysis and the concept of long-term storage have been well established. With the use of appropriate sampling protocols and sampling implements to minimize contamination, the specimens are preserved in their in-vivo status as the samples are taken. The important factor in this preservation step for biological specimens is the immediate deep freezing of the sample and maintenance of these low temperatures throughout the sample processing and banking until the chemical analysis. Packaging the samples in Telfon containers, freezing them in liquid nitrogen, and shipping them in commercial liquid nitrogen vapor shippers have been demonstrated without problems in the U.S. EPA/NBS pilot program. Similarly, reliable long-term storage facilities have been established using liquid nitrogen vapor freezers in the German Umweltprobenbank and the EPA/NBS pilot programs. In addition, reliable storage has been demonstrated with large walk-in freezer rooms at -80 °C (Umweltprobenbank Münster) and at -25 °C (NIES, Japan), respectively.

State-of-the-art analytical methodology, which is required for the accurate determination of pollutant trace constituents, has made significant progress during the feasibility studies on specimen banking. Almost all known inorganic pollutant elements can be determined with routine analytical procedures at the levels that commonly occur in nature. New procedures have been developed which provide sufficient sensitivity for the determination of the relatively low levels of tin and the ultratrace levels of platinum. With the appropriate combination of techniques, 44 elements can be determined by sequential analysis in a single 300 mg sample. However, research efforts are needed to include some additional elements in the analytical procedures. At present rare elements such as beryllium and bismuth are not easily measured and some other more common elements such as aluminum, silicon, and vanadium impose difficulties in the analytical procedures. As in recent years, the cooperative efforts in the various specimen banking projects will accelerate the development of the analytical approaches needed and will make such state-of-the-art techniques available for similar programs worldwide. Similarly, though more complex, the determination of organic pollutants has made significant progress. In the German pilot bank, methods for the accurate determination of several groups of pollutants (chlorinated hydrocarbons, polychlorinated biphenyls, and polycyclic aromatic

hydrocarbons) have been developed. In turn, these methods and determinations have been adopted and verified by other programs, thereby providing for validation of measurements and data in the difficult field of organic trace analysis.

The value of the samples already banked in the various projects is indisputable. Even though the long-term stability of specimens has not been completely established for all classes of pollutants, specimen types, and possible storage conditions, preliminary results indicate that samples are stable at or below -80 °C with temperatures below -135 °C being the most favorable. Short-term studies have not shown evidence of changes in the composition under these conditions, whereas samples stored at -25 °C showed phase separation and recrystallization of ice; freeze-dried storage at room temperature is at least questionable for relatively volatile organic and inorganic constituents. Stable banked samples have been used recently as reference samples for analytical intercalibrations among the existing banking programs and within projects to provide control chart data points for evaluation of the stability of analytical results. In several instances, banked specimens have been put to use to answer monitoring questions and to establish previous baselines on newly investigated pollutants.

Recognizing the virtues of specimen banking in environmental monitoring, several environmental and human health agencies and programs have expressed interest in including specimen banking activities into their respective approaches. The pilot banking programs have proven the overall viability of specimen banking concepts as described above. It is now time to incorporate the experience gained in the pilot programs into actual monitoring programs. In the Federal Republic of Germany, specimen banking becomes part of the environmental monitoring system in 1985 utilizing the facilities and approaches developed during the pilot phase. This will be the first large-scale specimen banking activity. In Sweden and Japan, specimen banking is partially related to actual monitoring programs. In Canada, specimen banking has been integrated into existing wildlife monitoring programs. In the U.S., aspects of specimen banking will be incorporated into the National Marine Status and Trends Program of the National Oceanic and Atmospheric Administration. With the exception of Germany, the concept of specimen banking has not yet been fully adopted in the countries represented at this meeting. In spite of this fact, all of the existing specimen banking projects can be considered as valuable national resources which can be utilized as desired. The systematic specimen collections of biomonitors during the past five or more years will eventually prove their value by documenting long-term trends in environmental quality.

SECTION 2

SPECIMEN BANKING IN SUPPORT OF MONITORING FOR TOXIC CONTAMINANTS IN CANADIAN WILDLIFE

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I. Introduction

The Canadian Wildlife Service (CWS) operates an environmental specimen bank to support investigations of the levels of environmental contaminants in Canadian wildlife. CWS has conducted such investigations since the mid-1960's; some of the material collected during those earlier studies was preserved frozen at -25°C. By the early 1970's, frozen storage of a portion of specimens collected for specific research and for surveys of environmental contaminants in Canadian wildlife was carried out on a routine basis. In 1980, a Specimen Bank was established on a formal basis as an integral component of a CWS monitoring program for toxic chemicals in wildlife. Details regarding collection, handling and storage of specimens have been described previously [1].

This paper will outline the current CWS activities related to specimen banking. These include: 1) inventory and consolidation of old holdings; 2) investigations into stability of chemical residues in stored specimens; 3) recent developments in CWS monitoring programs; and 4) retrospective analysis projects being undertaken during 1984.

II. Results and Discussion

A. SPECIMEN BANK INVENTORY

The CWS specimen bank probably contains the largest collection of environmental material in Canada stored primarily for future analysis to measure environmental contaminants. However, many of the specimens were archived between 1966 and 1979, when the bank was basically an ad-hoc operation. Table 1 is a summary of the contents of this "Old Collection".

Table 1. Canadian Wildlife Service "old" tissue bank collection (1963-1979).

Class	Number of Species Represented	Number of Specimens In Storage	Collection Date Range
Birds	210	8,511	1963-1979
Mammals	43	1,180	1967-1979
Fish	50	467	1966-1977
Reptiles	11	35	1967-1976
Amphibians	6	27	1968-1976
Total	320	10,220	1963-1979

Under this earlier arrangement, workers had access to two large walk-in freezers maintained at about -25°C. A centralized inventory of holdings was available; however, some researchers preferred their own cataloguing systems. In 1980 with the formal establishment of the specimen bank, movement of material in and out of the freezers became better controlled. The task of sorting and cataloguing the old accumulated material began. The inventory has worked on the precept that no sample should be discarded unless it is clearly of little value due to physical deterioration or lack of reference information. Photographs have been taken to document cases of deterioration. The sample deterioration problems are of two main types. The first is simple desiccation of samples from incomplete sealing containers (Figure 1a). The second and more serious problem results from oxidation and associated deterioration of aluminum foil when in contact with animal tissue for long periods of time - even at low storage temperatures (Figure 1b). The photographs illustrate the importance of storing samples properly.

Figure 1a. Avian liver segment after storage for 12 years at about -25°C in an incompletely sealed glass jar. Desiccation of the sample is apparent.

Figure 1b. Franklin's Gull after storage for 13 years at -30°C in hexane rinsed aluminum foil. Foil layers have degraded and are adhering to feathers.

The specimen bank inventory is scheduled for completion early in 1985, at which time a catalogue will be produced describing the contents of the bank. The specimen bank catalogue will parallel an existing catalogue of data stored in the CWS National Registry of Toxic Chemical Residues [2].

B. STORAGE CONTAINER INVESTIGATIONS

A large portion of the CWS specimen collection is stored as 6.5 g aliquots of homogenized tissue. 20 mL scintillation vials of borosilicate glass were the standard containers for aliquots intended for organic analysis. Samples for trace element analysis are stored in polyethylene vials. The borosilicate glass vials were considered to be ideal for specimen bank purposes because of their low cost, suitable glass quality, and wide neck opening. However, these vials exhibited a tendency to crack upon removal from -40°C storage. Large numbers of cracked vials have also been discovered while still in freezers. The fracturing occurs at the bottom rim of the vial and is observed most often in vials containing samples with high moisture content (i.e., > 70%) such as egg homogenate. The pressures created during the expansion of freezing water appear to crack the vial at the bottom rim causing the bottom of the vial to fall out as a neat disc. Breakage could not be ascribed to any single batch purchased from a single supplier.

Tests were conducted to determine the influence of the positioning and rate of cooling on the tolerance of glass scintillation vials to freezing and thawing. As a result of the tests, it was concluded that such vials are not suitable for specimen banking. In order to find a suitable replacement, five commercially available glass vials and one vial with a custom-blown round bottom were tested for their tolerance to freezing and thawing. On the basis of these tests, a 15 mL French Square flint glass sample vial (Johns Scientific, Toronto) was chosen as the new standard vial type for the specimen bank. This vial did not crack in any of the tests, is readily available, relatively inexpensive, has a screw cap which can accept a teflon liner and a neck opening large enough to get sample material in and out without too much difficulty.

C. STABILITY OF CHEMICAL RESIDUES IN STORED SPECIMENS

Specimen banking is dependent on the assumption that chemical residues will not change or degrade significantly in stored specimens over extended time periods. The specimens in the CWS bank were stored for many years at temperatures of -20°C to -25°C and more recently at -30°C to -40°C with a small portion at -80°C. The -40°C temperature was choosen as the lowest affordable for storage of the large existing collection; -80°C was selected as the lowest practical temperature for standard chest freezers and also as a temperature at which enzymatic activity should be virtually arrested and any sample modification further reduced. Evidence that some organochlorine residues remain stable in egg samples is presented in Figure 2. This material consists of two large pools of Great Lakes Herring Gull egg contents stored as individual aliquots of 6.5 g at -30°C to -40°C since 1979 and 1980.

The analyses were conducted by the Ontario Research Foundation of Mississauga, Ontario. There is some scatter in the results ascribed mainly to variation in the laboratory's performance from batch to batch; however, there are no significant trends in the data for any of the chemicals analyzed. Some of this data together with the results of other studies on sample storage have recently been discussed in detail [3].

D. MONITORING ACTIVITIES

The bulk of the samples in the CWS specimen bank were collected for specific investigations involving threats to wildlife health from toxic chemicals. A brief review of past studies is available [1] as is a more extensive review of earlier work [4]. These projects include investigations of reproductive failure for certain colonial fish-eating bird species in the Great Lakes [5], investigations of population decline in some birds of prey [6] and seabirds [7] and large scale acute poisoning of song birds as a result of forest spraying actions [8].

Material to be added to the specimen bank is now selected more carefully. Specimens come from three main sources which are loosely defined in Table 2.

The long-term monitoring projects constitute the major source of new material. CWS toxic chemical monitoring is now confined to periodic measurement of organochlorine residues in avian species with evidence of a negative effect of such chemicals on wildlife populations. The wildlife monitoring data may also be used to indicate contamination of the particular ecosystem. The best example of such monitoring is the use of the Herring Gull, Larus argentatus, as an indicator of contamination in the Great Lakes. Details regarding this project and its integration into the International Joint Commission Surveillance Program are available [5].

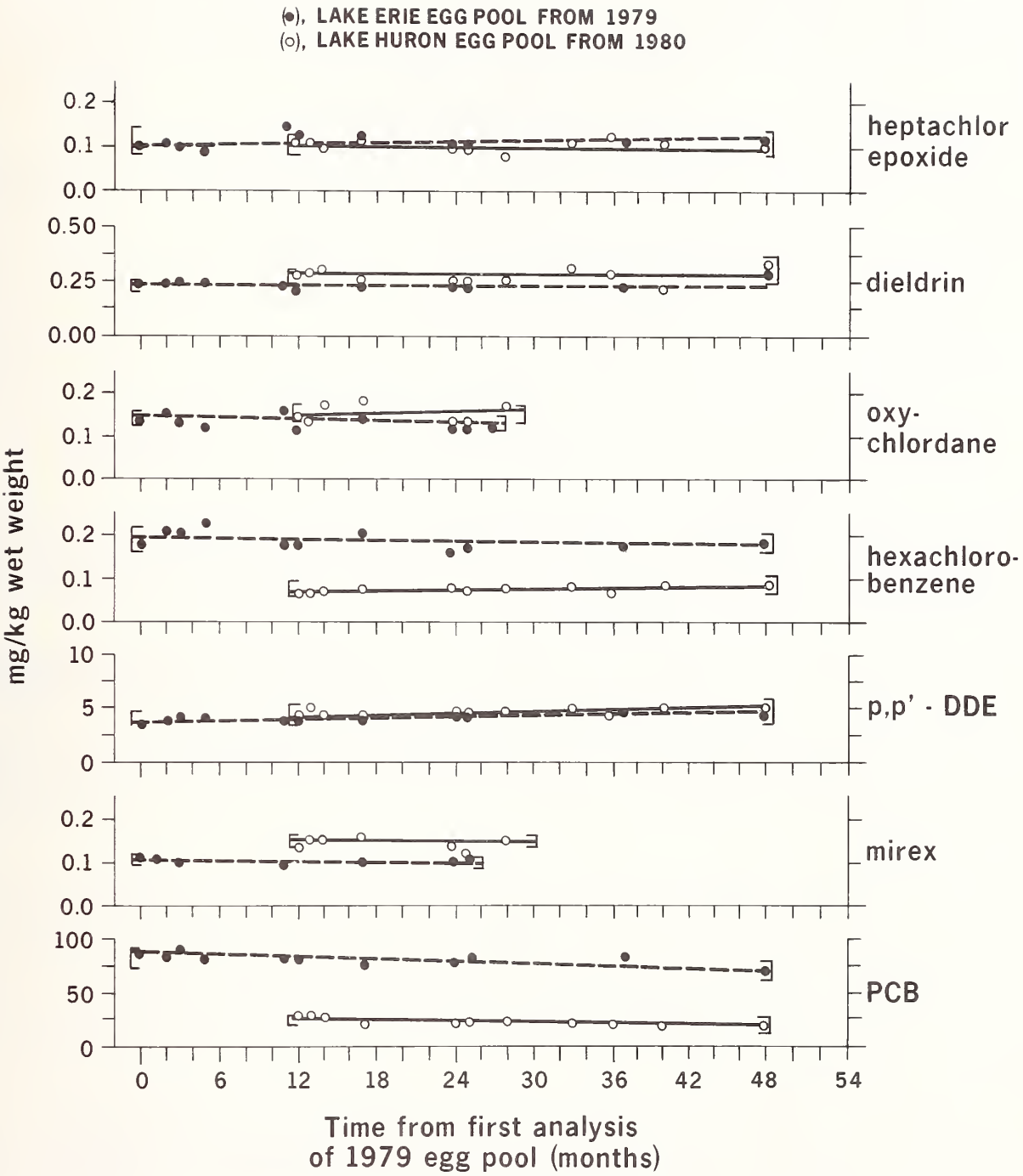

Figure 2. Re-analysis of herring gull egg homogenate stored at -30°C to -40°C.

Table 2. Sources of samples for CWS National Specimen Bank.

1. Long-term Monitoring Projects

   A) Atlantic Coast - Seabirds
   B) Eastern Forest - Woodcock Wings
   C) Great Lakes - Herring Gulls
   D) Prairie Provinces - Prairie Falcons
                        - Peregrine Falcons
   E) Pacific Coast
      Fraser Estuary - Great Blue Heron

2. Short-term Investigations

   A) Specific Surveys
   B) Response to Emergencies

3. "Piggy-backing"

   A) Arctic and Marine Projects
   B) Wildlife Autopsy Specimens

For this program, CWS collects 10 Herring Gull eggs from each of two monitor colonies from each of the Great Lakes. In addition, 10 eggs from other colonies are obtained on an annual rotating basis from investigations of individual lakes. The portions of the egg material remaining after analysis are placed in the Specimen Bank according to the scheme indicated in the Figure 3. This diagram also shows how the eggs are processed.

This sampling strategy is presently under review. A statistical evaluation of the feasibility to use more pooled analyses is in progress in an effort to reduce both cost and sample variance.

Short-term studies are also an important source of samples. These studies are initially of one or two years duration and are designed to assess local wildlife contamination from chemical spills, waste dumps or pesticide spray programs. Specimens from such investigation are often archived as the project may later be expanded or the samples may prove useful for background data or other purposes in the future.

The third source of samples are various "piggy-back" style operations. Material collected for other biological purposes or harvested by hunters or trappers can provide a cost effective source of specimens for monitoring. Piggy-backing on expeditions to the Canadian Arctic or other isolated areas provides specimens for studying the background levels and distribution of contaminants in regions remote from pollution sources. By establishing links with veterinary medical centers and museums, tissue samples can be obtained from animals brought in for autopsy or for museum collections. In particular, samples from endangered species or from species with small or otherwise inaccessible populations may be obtained. Subsequent analysis may yield valuable information not only about the possible role of toxic chemicals as the cause of death, but also about the dynamics of contaminants in various food chains.

The usefulness of samples obtained by this means of collecting has been documented for both Britain [9] and the U.S. [10,11]. A pilot study is now underway by CWS workers in British Columbia to determine the feasibility of this approach in Canada. Sharp-shinned Hawks (*Accipiter striatus*) and Barn Owls (*Tyto alba*) are being obtained as available from road kills or other sources and analyzed for organochlorine and mercury contamination.

E. USE OF SPECIMEN BANK SAMPLES

In a general sense the CWS specimen bank is used in support of surveillance and monitoring of wildlife for exposure to toxic chemicals. These uses can be broken down into four areas: 1) to fill in gaps in temporal or spatial trend analyses; 2) to augment previous sample sizes; 3) to verify previous results through re-analysis of samples using modern methodology; and 4) to measure for the presence of chemicals of current interest in samples collected in the past.

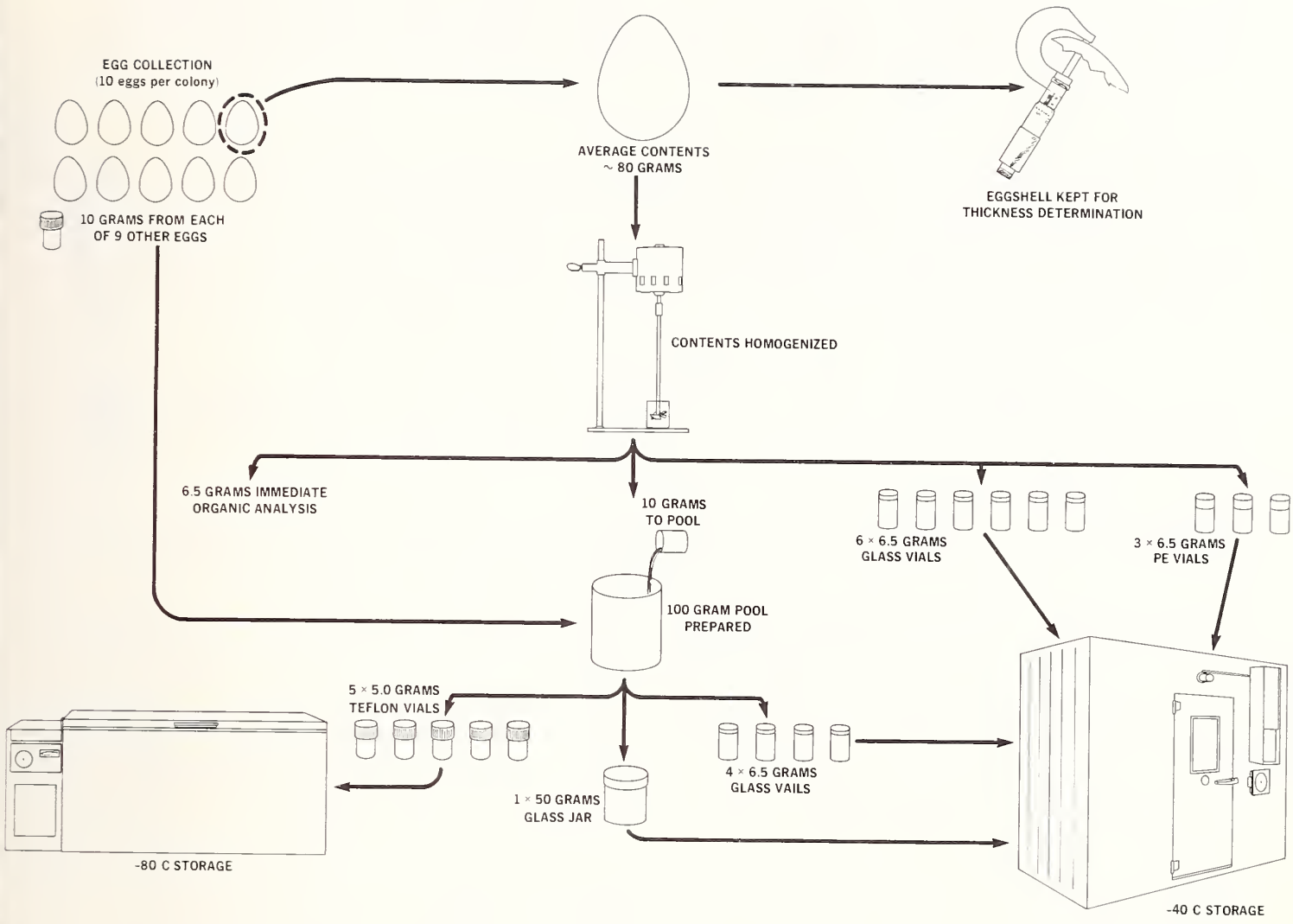

Figure 3. Typical allocation scheme for bird eggs collected by CWS for toxic chemical monitoring and specimen banking.

Table 3 outlines the use of specimen bank samples in some recent CWS projects. In example 1, after the discovery of dioxin residues in the eggs of Great Lakes Herring Gulls collected in 1981 [12], a series of eggs collected from 1971 to 1980 at Scotch Bonnet Island, Lake Ontario and stored in the CWS Specimen Bank were analyzed for the 2,3,7,8-TCDD isomer [13]. The results indicated a declining trend for this dioxin isomer. In example 2, Herring Gull eggs from colonies in Lake Ontario and Michigan are being re-analyzed for the compounds listed in the Table 3. Temporal trends and inter-lake differences for organochlorines have been determined using these retrospective data.

Examples 3 and 4 are from a project to review CWS data on contaminants in marine birds. The Gannet, Morus bassanus, is a large seabird, related to the pelicans, which breeds in colonies on the east coast of Canada. During the period, 1969-1973, there was a decline in the breeding population at Bonaventure Island (48°31' North, 64°9 West) in the Gulf of St. Lawrence. This decline has been linked to levels of toxic chemicals [14,15]. A series of eggs collected at Bonaventure Island from 1968 to 1976 and stored in the CWS Specimen Bank have been analyzed with current methods for organochlorine compounds, many of which could not be measured at the time of collection. The samples in example 4 are from a study of a seabird colony in the Canadian Arctic, Prince Leopold Island (74°2'N, 90°0'W). Previously unanalyzed samples from the specimen bank are being used to increase the sample size and to provide data on residue levels in various tissues.

Table 3. Retrospective usage of samples stored in the CWS National Specimen Bank.

Example	Project	Species	Tissues	Geographical Area	Original Collection Dates	Chemicals Analyzed[a]	Project Status
1	Great Lakes Monitoring	Herring Gull	Egg	Lake Ontario - Scotch Bonnet Island	71-80	dioxin	complete
2	Great Lakes Monitoring	Herring Gull	Egg	Lake Ontario - Snake Island Lake Michigan - Big Sister Island	71-81	OCs, PCBs, PCPs, dioxins, furans, Hg	partially complete
3	Seabird Monitoring	Gannet	Egg	Bonaventure Island, Quebec	68-70, 73,74, 76	OCs, PCBs	complete
4	Seabird Monitoring	Northern Fulmar Black-legged Kittiwake Thick-billed Murre	Egg Liver	Price Leopold Island, Northwest Territories	75-77	OCs, PCBs	partially complete
5	Monitoring of Arctic Wildlife	Polar Bear	Liver	Canadian Arctic	68-71	OCs, PCBs dioxins	preliminary analyses
6	Retrospective Analysis of Migratory Birds Collected in Surinam	Black-crowned Night Heron Purple Gallinule Common Egret Snail Kite	Liver	Wageningen, Surinam	71	dioxins	samples prepared

[a]OCS = organochlorine compounds (pesticide)
PCBs = polychlorinated biphenyls

Examples 5 involves retrospective analysis of stored Polar Bear samples collected between 1968 and 1971 from the Canadian Arctic. A 1982 survey of organochlorine and heavy metal levels in Canadian Polar Bears revealed unexpectedly high residues of metabolites related to the insecticide chlordane. Data from reanalysis of stored samples will aid in determining the source of the contamination.

The samples in example 6 are from waterbirds and their prey collected in 1971 in the vicinity of rice fields in Surinam. The samples were found to be heavily contaminated with sodium pentachlorophenol (PCP), used as a molluscicide. High mortality and illness in kites, egrets and herons were associated with this PCP contamination [16]. The samples were analyzed for PCP and other pesticides in Canada and the remains placed in the CWS specimen bank. Exposure of the poisoned birds to dioxin contaminants in the PCP was suspected at the time; however, the necessary analytical capability was not available. Contamination of PCP by dioxins has since been reported [17]. A set of pooled specimens from this Surinam collection are now being analyzed for dioxins using current methodology.

III. Acknowledgments

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SECTION 3

THE ENVIRONMENTAL SPECIMEN BANKING PROJECT OF THE FEDERAL REPUBLIC OF GERMANY

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I. General Considerations

The manufacture of chemicals began with the onset of the industrial revolution. Today, more than 80,000 individual substances are being traded world-wide. As many as 1,000 new substances may reach the market every year. We know very little concerning the behavior of the majority of these substances in the environment. For all the benefits derived from their use, several questions remain unanswered: what long-term effects do they have on man and the environment; where do they remain and accumulate; into what new chemicals and how rapidly are they transformed or are they transformed into other stable compounds that may be toxic? Surely, a large number of these substances degrade as water, oxygen, sunlight and, above all, biological systems act upon them; a considerable number of them, however, degrade not at all or very slowly. Many substances (or their transformation products) have proved to be harmful for man and the environment only after having been used over years or even decades, so that we must attempt to reduce this potential threat.

II. Objectives

In order to at least be able to determine, in retrospect, pollution trends in the future, German and American scientists and scientists of other countries, have been investigating the feasibility of establishing permanent environmental specimen banks in their countries [1,2].

Samples of certain biological species and soil types, selected for their ability to accumulate pollutants, would be obtained and stored under conditions which make it possible to get a picture of the environmental damage by pollutants at the time of sampling. In years to come, these samples would then be used as follows [3]:

- for the determination of the environmental concentrations of those substances, which at the time of storage were not recognized to be hazardous or which at that time could not be analyzed with adequate accuracy (retrospective monitoring);
- as reference samples for the documentation of the improvement of analytical efficiency and for the verification of previously obtained monitoring results;
- as authentic material from the past, these samples would make it possible to perform trend analyses on chemicals of interest. Such analyses are indispensable when assessing the hazard posed by a substance or checking up on the success of imposed bans or restrictions;
- to verify the exposure estimates made for new substances prior to their being placed on the market;
- to contribute to the selection of existing chemicals of environmental relevance within the meaning of the German Chemicals Act which is based on the 6th Amendment (1979) of the 1967 directive on dangerous substances (79/831/EEC);
- as an approach for monitoring pollutants throughout the Federal Republic of Germany.

III. Approach

Before initiating the archiving of such specimens in a permanent bank, extensive investigations had to be undertaken. It was, for example, essential to determine and test the conditions necessary for long-term storage; the specimen types suitable from both a biological and chemical-analytical point of view; and the manner of organizing such an undertaking. These questions could only be solved, and some are still in the process of being solved, in an interdisciplinary fashion; chemists, biologists, soil scientists, statisticians, low-temperature and clean-room engineers, all have to work cooperatively.

The majority of the problems have been treated and solved in the past few years, whether it be on the part of the National Bureau of Standard under the project leadership of the United States Environmental Protection Agency [4,5] or, on the German side, by the Federal Minister for Research and Technology and the Federal Minister of the Interior under the project leadership of the Federal Environmental Agency [6].

At the beginning of this joint effort in 1975, a memorandum of understanding pertaining to the joint U.S. - German Environmental Specimen Bank Program was implemented within the framework of the U.S. - German Agreement on Cooperation in Environmental Affairs [7]. In 1979 the German pilot program was initiated following a few screening projects in 1977 and in 1978.

IV. International Exchange of Information and Experience

In 1977, 1978, and 1982 international workshops were held with the aim to arrive at coordinated programs for establishing specimen banks to monitor the pollutant burden in soil and biological specimens. The first workshop, which took place in Luxembourg in 1977, was entitled "The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants" [8]. One of the main conclusions of this workshop was that limiting the investigation of the burden on our environment to human tissue would not be sufficient and that, therefore, other environmental specimens must be included in these investigations. The following workshop in Berlin in 1978, "Monitoring Environmental Materials and Specimen Banking" [9], dealt primarily with the selection of specimens and the conditions for long-term storage of biological specimens from the aquatic and terrestrial spheres. The last workshop in Saarbrücken in 1982, "Environmental Specimen Banking and Monitoring as Related to Banking" [10], gave a review of all specimen banking activities world-wide and gave recommendations as to the minimum size of a permanent bank.

V. Specimen Selection

After an intensive discussion, in the presence of the American colleagues, thirteen different types of specimens were selected for the project of the Federal Republic of Germany. One of the most important requirements of the pilot phase was the need for diversity of the samples. The specimens should represent different matrices in order to study the sampling, homogenization, storage and analytical techniques to be applied to each type. The specimens employed are as follows (each accompanied by a short explanation):

For the human sphere:

(1) Human blood

Blood is an indicator of the actual burden of hazardous substances. There are several reasons favoring the use of blood:

As a body fluid, it represents a special matrix type. It is readily available and inexpensive. It can be drawn from living persons and repeatedly from the same person over a period of years and even decades without harm.

(2) Human liver

The liver is an organ in which many inorganic and organic hazardous substances metabolize and accumulate. Sufficient sample material can be obtained from one individual specimen. According to American data, the distribution of trace elements in the liver is more homogeneous than in the kidney.

(3) Human adipose tissue

Human adipose tissue is the principal depot for lipophilic compounds. It is available in sufficient quantities and shows a homogeneous distribution of hazardous substances.

For the aquatic sphere:

(4) Carp

Fish are an important link in the food web. The carp was chosen because Japanese law specified Cyprinus carpio as a test fish for chemicals (Law Concerning the Examination and Regulations of Manufacture, etc. of Chemical Substances, 1973, Law No. 117). In addition, this species can be easily bred and maintained in the laboratory.

(5) Zebra mussel

This freshwater mussel (Dreissena polymorpha) is widespread and available in large numbers. It is clearly definable in the genetic sense, so that exact genetic identification can be established.

(6) Marine macroalgae

As the freshwater sphere was already represented by a fish and a mussel species, marine macroalgae (Fucus vesiculosus) were selected for the marine sphere. They are known as accumulators of heavy metals and organohalogen compounds. By choosing this type of sample, new problems for storage and analysis were introduced.

(7) Sewage sludge

Sewage sludge is a reservoir of anthropogenic waste and is one of the accumulators in the aqueous phase. In sewage sludge, polycyclic aromatic hydrocarbons (PAHs) are surprisingly constant in their concentrations and in their relative abundances, irrespective of origin and season, as investigations in Germany have shown [11].

For the terrestrial sphere:

(8) Wheat

Wheat is grown world-wide and is one of the basic foods of man. It is an indicator of both air and soil contamination.

(9) Cow's milk

Cow's milk is an essential part of the cows' and human food web. It is an interesting matrix with unknown problems in long-term storage. Fat and aqueous phases can be tested separately for lipophilic and hydrophilic substances.

(10) Soil

Dust fallouts settle on the soil, rain passes through it, and it is inhabited by microorganisms. Soil has filter, accumulation, and degradative functions. As there are various types of soil, the selection is particularly difficult. Two studies entitled "Criteria for the Selection of Soils for the Environmental Specimen Bank" and "Regionally Representative Selection of Soil Samples for an Environmental Specimen Bank" have been completed.

(11) Earth worm

Both the earth worm and the carabid beetle (listed below) are pollution indicators for soil. The earth worm is very common. It can store, for example, fairly large quantities of lead in its chlorogogue tissue. Lumbricus rubellus was chosen as the species for the pilot bank.

(12) Carabid beetle

The carabid lives on, rather than in, the soil, and reflects the contact surface air/soil. Its matrix is of a different nature than that of the earth worm and requires a different analytical technique. Carabus auratus (the brass beetle/gold-smith) was the species of choice for the pilot bank. All carabid species have now fallen under the Species Protection Act, and the carabid has therefore been dropped from the program.

(13) Grass

It would have been desirable to use moss and lichen because of their highly sensitive reaction. However, due to this sensitivity, they are no longer common in all areas. Thus, cities are virtual lichen deserts.

On the other hand there is no pure grass culture in nature. Therefore, a standardized grass exposure method, using Lolium multifloruum LAM spp. as the exposed species, has been developed for the determination of the toxic effects of pollutants. This method has been published as a guideline of the Verein Deutscher Ingenieure, VDI [12].

Additional specimen types were added in the course of the program, such as human urine (sample type no. 14) and leaves of poplar trees (Populus nigra italica) (sample type no. 15). The collection of air constituents by means of suitable sorbents was also included in the pilot program.

VI. Analysis

Analysis and specimen preservation are closely connected. The analytical program has two objectives. First, to determine how long the different substances persist in the various specimens and to which compounds they metabolize. The spectrum should cover a range from substances sensitive to oxygen, temperature, sunlight (e.g., ascorbic acid) to insensitive substances, such as organohalogen compounds. Using the analytical techniques presently available, it is determined which storage method is the best. Second, analytical methods have to be further improved to allow the utilization of smaller sample sizes. This will allow for a greater storage capacity of the future bank. For this, the homogeneity of each sub-sample is prerequisite. To achieve homogeneity is a task of particular importance in sample portioning and analysis. Once the optimal storage method is established, it can be assumed that determination of substances yet unknown will later become possible. While stability is of the greatest importance for organic substances, the possibility of migration from the container into the specimen and vice versa must be considered for inorganic substances.

The pollutants for which each specimen type is analyzed are summarized in Table 1.

VII. Organization and Results

For the tasks of sampling, analysis, loading the containers, storage, and collecting the results in a data bank, the efforts of ten different research groups were coordinated by the Federal Environmental Agency (Figure 1). Participants are universities, research centers, and laboratories of other federal agencies. These laboratories are distributed throughout Germany (Figure 2).

The Federal Environmental Agency functions as a Charge d'affaires for the Federal Ministry for Research and Technology. The project is sponsored primarily by this ministry and partially by the Federal Ministry of the Interior. The Federal Environmental Agency is under the authority of the latter.

In the pilot project two different deep-freezing techniques were developed and tested. The first technique, set up at the University of Munster, uses a walk-in freezer for storage at about minus 85°C. The second technique, which is set up at the Kernforschungsanlage (Nuclear Research Center) in Julich, is a technique in which the specimens are stored in containers over liquid nitrogen vapor at about minus 140°C and lower [13,14]. The Munster facility has a capacity of 650,000 samples at 20 mL each and the Julich facility will accommodate 150,000 samples. So far both techniques of low-temperature storage have proved to be successful. Within the time of storage (two years or longer), no change was observed in the investigated constituents.

The pilot project in the Federal Republic of Germany will come to an end in 1984. On the basis of interim reports provided by each research appointee [15], the evaluation of the whole project is currently in progress. The main result can be anticipated: A specimen bank is both technically and financially feasible. Enough progress has been made to warrant the institutionalization of the project.

VIII. Conclusions

The Government for the Federal Republic of Germany intends to establish a permanent Environmental Specimen Program in 1985 upon conclusion of the pilot phase. The Federal Minister of the Interior stated, on September 15, 1983 before the Lower House of Parliament: "It is planned to set up an environmental specimen bank to safely store important and conclusive samples on a long-term basis which will be kept available for comparative investigations on changes in the environment". (Non-official translation of the German original.)

The Federal Minister of the Interior must be able to fall back on an instrument such as the specimen bank in order to fulfill his executive functions. Such a bank is essential in order to establish a systematic picture of the burden of harmful substances. There are often differing scientific hypotheses regarding these burdens, but the correct one can only be reached step by step. The storage of additional types of specimens is being considered for the future. The Government of the Federal Republic of Germany is convinced that a tool is being created here for future generations which acknowledges our responsibility for the protection of man and the environment.

Table 1. Pilot Environmental Specimen Bank: Distribution of analytical work.

Specimen Types	Substance Class (Identification No.)									
	Halogenated Hydrocarbons (1)	Pesticides (2)	PAHs (3)	Aromatic Amines (4)	Phenolic Compounds (penta-, trichlorophenols) (5)	Unsaturated Fatty Acids (6)	Hormones and Steroids (7)	Ascorbic Acids (8)	Toxic Metals and Compounds (Pb,Cd,Hg) (9)	Metals in non-Homogenized Tissues (0)
1) Human Blood	GSF, Ulm Munster, Kiel		Ahrensburg		Ulm		KFA	Kiel	KFA	Bochum
2) Human Liver	GSF, Ulm Munster, Kiel		Ahrensburg		Ulm	Kiel	KFA	Kiel	KFA, Kiel Munster	Bochum
3) Human Adipose Tissue	GSF, Ulm Munster		Ahrensburg		Ulm	Kiel	KFA		KFA	Bochum
4) Carp	GSF, Ulm Kiel		Ahrensburg		Ulm	Kiel		Kiel	KFA, Kiel	
5) Zebra Mussel	GSF, Ulm		Ahrensburg		Ulm				KFA	
6) Sewage Sludge	GSF, Ulm		Ahrensburg	Ahrensburg	Ulm				KFA	
7) Wheat		BBA								
8) Cow's Milk	GSF, Ulm				Ulm	Kiel	KFA	Kiel	KFA, Kiel	
9) Soil a) Julich b) Berlin	GSF, Ulm	 BBA	Ahrensburg		Ulm				KFA	

Table 1. (Continued)

Specimen Types	Substance Class (Identification No.)									
	Halogenated Hydrocarbons (1)	Pesticides (2)	PAHs (3)	Aromatic Amines (4)	Phenolic Compounds (penta-, trichlorophenols) (5)	Unsaturated Fatty Acids (6)	Hormones and Steroids (7)	Ascorbic Acids (8)	Toxic Metals and Compounds (Pb,Cd,Hg) (9)	Metals in non-Homogenized Tissues (0)
10) Earth Worm	GSF, Ulm		Ahrensburg		Ulm				KFA	
11) Carabid	GSF, Ulm		Ahrensburg		Ulm				KFA	
12) Grass a) Julich b) Berlin		BBA	Ahrensburg		Ulm				KFA	
13) Marine Marco-algae	GSF, Ulm		Ahrensburg		Ulm				KFA	
14) Human Urine							KFA			
15) Poplar Leaves			Ahrensburg						KFA	

LEGEND:

Ahrensburg:	Biochemisches Institut für Umweltcarcinogene.
BBA:	Biologische Bundesanstalt fur Land- und Forstwirtschaft, Berlin.
Bochum:	Ruhr-Universität Bochum.
GSF:	Gesellschaft fur Strahlen- und Umweltforschung mbH, München.
KFA:	Kernforschungsanlage Julich GmbH.
Kiel:	Institut für Hygiene der Bundesanstalt für Milchforschung.
Munster:	Institut für Pharmakologie und Toxikologie der Westfalischen Wilhelms-Universität.
Ulm:	Universitat Ulm.

Figure 1. Distribution of tasks within the Pilot Environmental Specimen Bank Program of the Federal Republic of Germany.

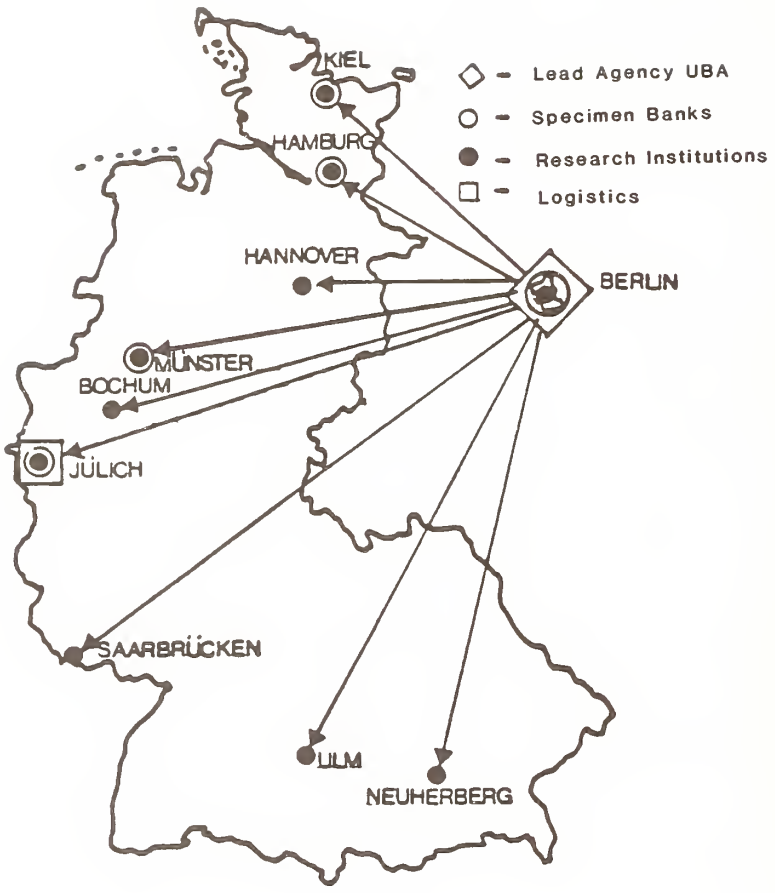

Figure 2. Research centers and banking facilities within the Pilot Environmental Specimen Bank Program of the Federal Republic of Germany.

IX. Acknowledgments

A major driving force in carrying through the pilot project successfully has been the excellent cooperation with the American colleagues over the years. In particular, the activities of the United States Environmental Protection Agency and the National Bureau of Standards have led to fruitful discussions and cooperation.

Thanks are also expressed to the Federal Minister for Research and Technology for laying the foundations for this important work. He is credited with recognizing the significance of such a tool and with funding the preliminary work which will lead to the establishment of a permanent specimen bank in the Federal Republic of Germany starting in 1985.

It is hoped that the work carried out on both sides of the Altantic, which has proved beneficial for both sides, will be continued and intensified in the future.

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SECTION 4

AN OVERVIEW OF THE RESEARCH ACTIVITIES RELATING TO ENVIRONMENTAL SPECIMEN BANKING IN JAPAN

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I. Introduction

In Japan, the importance and the usefulness of specimen banking for the long-term environmental monitoring of toxic chemicals are becoming more and more widely recognized by environmental scientists and administrative officials. At present, however, no concrete program has been made to establish a large-scale environmental specimen bank and only a few small specimen banks are in operation.

Several years ago some research activities relating to the environmental specimen bank were initiated by a few government and university laboratories. Among these activities, the program of National Institute for Environmental Studies (NIES) is the most significant. The specimen banking activities at NIES have been summarized previously [1].

II. The Research Activities at NIES

A preliminary study of the problems of specimen banking started with the cooperation of university laboratories to obtain the information necessary to prepare for the future establishment of a full-scale specimen bank. As initial studies on sample banking, several experiments were designed to investigate the stability of chemicals in various types of environmental samples.

Aliquots of each lot of each sample type were subjected to different conditions of pretreatment and storage (temperature and containers) and each of the samples will be analyzed at yearly intervals to examine changes during storage. The sample types and chemical substances tested are as follows:

a) Atmospheric particle samples collected on quartz glass fiber filters were stored in stainless steel boxes. Four conditions of storage were tested: the container boxes were filled with argon gas or with air, and the temperatures examined were 20°C and -20°C. Benzo[a]pyrene was monitored to detect changes in its concentration during storage. After two years storage, reductions in its concentration of approximately 20% and 8% were observed at 20°C and -20°C, respectively. The effect of the different storage gases was not significant. The preliminary results of this study have been reported [2].

b) Synthetic detergent in natural water was examined for its stability during storage. Polyoxyethylenealkylphenylether was spiked in lake water samples and stored in glass bottles in the 5°C and -20°C storage rooms. While in the frozen condition, no change was found in the concentration of the solute after two years of storage; a significant degradation of the compound took place in the 5°C room.

c) Lake sediment samples were stored at -20°C and -85°C freezers for determining the stability of the natural levels of polycyclic aromatic hydrocarbons (PAH) during storage. The freeze-drying method for storage of sediment samples was also examined.

d) Human blood serum samples were stored in the 20°C room after freeze drying treatment. The changes in elemental composition and halogenated organic compounds during the treatment process and storage period were examined.

It became clear that some of halogenated organics with low boiling points may escape during the process of freeze drying, but no significant losses appeared for the substances with high boiling points such as polychlorinated biphenyls (PCB). As for the trace metal composition, no changes occurred during the freeze-drying process except for mercury.

e) An experiment on the preservation of mussel tissue was designed to evaluate the changes of chemicals artificially spiked into the mussel tissue homogenate under different storage conditions.

Glass bottles, glass ampoules, and polyethylene bottles were examined as storage containers; the temperatures tested were -20°C, -85°C and liquid nitrogen vapor temperature (-140°C). Forty different chemicals, which are thought to be important in environmental pollution research such as PCBs, BHC, phthalate esters, hydrocarbons, etc., were added as a mixture solution in toluene into the mussel tissue homogenate samples. The samples stored under each conditions were programmed to be taken out once every year for distribution to the analytical laboratories.

Some interesting results have been obtained from these experiments up to the present time. In the case of dimethylnitrosoamine in the mussel tissue sample, the effect of storage temperature was clearly observed. After two years storage at -20°C, the concentration of dimethylnitrosoamine decreased by nearly 50%, but no detectable change occurred at the temperature below -85°C. Chlorinated organic substances, on the other hand, showed no significant change during storage in the glass containers at the temperatures tested, but a slight reduction in concentration was observed in the plastic container. These storage experiments are now still in progress and the final results will be obtained after a few years.

III. A Pilot Sample Bank Program at NIES

In conjunction with the above mentioned studies on the preservability of samples, a small-scale pilot sample bank has been operated using the storage facilities at NIES to examine problems relating to specimen banking such as the registration system of samples, maintenance of storage facilities, countermeasures against accidents (e.g., earthquakes), which are expected to occur in the practical operation of the bank in the future. The storage facility used for this sample banking program at NIES consists of 20°C, 5°C, and -20°C storage rooms; -85°C and -115°C freezers, and liquid nitrogen freezers as shown in the Table 1. The storage conditions applied in this sample bank, although perhaps not ideal, are those which are readily available and the most suitable method, at present, as selected for each sample taking into consideration the capacity of the storage rooms and the freezers.

Table 1. Storage facilities at NIES.

Storage Rooms		
No. 1	20°C	$20m^2$ x 1
No. 2	5°C	$20m^2$ x 1
No. 3,4 and 5	-20°C	$40m^2$ x 3
Freezers		
No. 1	-80°C	500 Liter x 1
No. 2	-115°C	500 Liter x 1
Liquid Nitrogen Freezers		
No. 1,2 and 3	-196°C	70 Liter x 3

The collection of the samples has not been systematic; several types of environmental samples, collected arbitrarily during various environmental programs and other surveys, have been stored considering that these samples will be valuable and useful for retrospective analyses or for the solution of other environmental problems in the future. At present the samples stored consist of biological samples and those relating to atmosphere and water pollution as listed in Table 2.

The number of the samples is increasing every year; however, since the capacity of the facility is limited, the samples are programmed to be replaced by new, more valuable ones when such samples appear.

Table 2. Environmental samples stored at the NIES Pilot Sample Bank.

	Approximate number of samples
Atmospheric Samples	200
Atmospheric particles collected on filters	
Rain water	
Water Samples	200
Lake water	
Sediment and Soil Samples	50
Lake sediment	
Biological Samples	400
Plant leaves	
Moss and lichens	
Fish	
Shellfish	
Birds	
Human hair	
Human blood	
Certified Reference Materials	
Pepperbush	
Pond sediment	
Blood serum	
Mussel meat	
Chlorella	
Human hair	
Tea leaves	

IV. Other Related Activities at NIES

NIES has recently initiated a certified reference material program aiming to prepare several types of environmental standard samples for measurement of trace elements. These reference materials will play an important role in assuring reliable analyses in the environmental specimen bank program.

The certified reference materials prepared by NIES are "pepperbush", "pond sediment", "chlorella", "human serum", "human hair", "mussel tissue" and "tea leaves".

V. Sample Banking at the Other Laboratories in Japan

In addition to sample banking at NIES, environmental samples are collected and stored for future retrospective analysis at several universities and other laboratories. For example, Ehime University (Prof. Tatsukawa) has collected a number of fish and bird samples to store in a -20°C room for the future determination of chlorinated organic compounds. At the laboratory of Tokushima University (Prof. Shimomura), human hair samples from a major part of the Shikoku region, were collected for the measurement of the mercury content; some portions of these samples have been preserved in several ways.

VI. References

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[2] Ambe, Y., Mukai, H, Yasuhara, A., and Yokouchi, Y., "A Preliminary Study on the Preservability of Benzo[a]pyrene in the Stored Atmospheric Particulate Matter Samples," In: Studies on the Method for Long-Term Environmental Monitoring, Y. Ambe and K. Fuwa, Eds., Research Report No. 79, National Institute for Environmental Studies, Japan, 1985, p. 75-81.

SECTION 5

THE ENVIRONMENTAL SPECIMEN BANK AT THE SWEDISH MUSEUM OF NATURAL HISTORY*

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I. Introduction

The Environmental Specimen Bank at the Swedish Museum of Natural History started its activities in the middle of the 1960's and has, since 1978, been financed by the Swedish Environmental Monitoring Program.

I wish to give an account of the goals of the Environmental Specimen Bank at the Swedish Museum of Natural History and its methods by answering some questions of concern within the field of chemical monitoring of the environment.

II. What is an Environmental Specimen?

Every sample of material from a specific environment can be said to represent that environment. In order to make use of the sample, however, one must place certain demands on definitions and descriptions of samples. One can, for example, say that certain wildlife preserves are giant environmental samples which certainly cannot be collected, but which on site contain samples of a specific environment and from which one can collect more or less well-defined samples, such as soil samples, air and water samples, and samples of biota in the form of whole colonies, organisms or organs. Another extreme case is found in the outflow area of a sewage tube which also represents a specific environment. Air, water, soil and biota are here defined, besides the description of composition, species, etc., also as types of pollutants, volumes of pollutants, and times of discharges polluting the recipient.

An environmental specimen's usefulness is determined, to a large extent, by the documentation which is connected with the specimen, regardless of whether the specimen represents an ecosystem, a colony, a population, an organism or an organ.

III. What is an Environmental Specimen Bank?

In order that environmental specimens can be available, but at the same time stored in a secure and suitable fashion, it is practical that some form of centralization of storage of the specimens take place. Thus far the collection of environmental samples does not differ from other collections of natural samples such as museum collections. On one important point, however, there is a difference between these two sample types. A specimen from a normal museum collection should be studied carefully, with as little destruction as possible, and thereafter returned in its original form to the collection. An environmental specimen, on the other hand, should generally (although not always) be used for chemical analysis and parts of or all of the specimen will be destroyed or transformed in connection with the chemical analysis. This difference is one reason why a collection of environmental samples is called an environmental specimen bank and not a museum collection. All important aspects, however, such as registration, cataloguing, etc., should be in agreement with normal museum operations, which makes it appropriate to station environmental specimen banks at museums.

IV. Why Do We Have an Environmental Specimen Bank?

The reason why one has an environmental specimen bank is to measure changes in conditions in environments. If no changes took place, one would be able, at any given moment, to collect representative environmental specimens and measure desired parameters. No need to measure older material would exist. Assuming a changing environment exists, specimens should be stored for future times series analyses. We will also estimate the size of the change, something which demands statistically satisfactory amounts of material.

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*This contribution was translated from the Swedish article published by M. Olsson in Memoranda Soc. Fauna Flora Fennica, 59: 93-100 (1983).

In many cases when one knows the parameters one wishes to study, it is important to store information, not in the form of specimens but rather analysis data. This applies, for example, to data within the traditional water chemistry. Experience has taught us, however, that tomorrow's problems are not known and can seldom be predicted. One can also allege that even today's problems are not known. In such a situation it is important to preserve information in the form of specimens on which newly discovered analytical techniques can be applied, whether the analysis consists of chemical, morphometric, or ocular methods. It is important here to maintain that if the analysis data is stored in a data bank, a comprehensive documentation on the analysis technique, apparatus, and personnel used, must also be stored. This is in order to ensure that a later utilization of data will be possible or facilitated.

In the beginning of the 1960's, when Johnels and Westermark studied mercury pollution in the Swedish environment, it was a touch of genius to make use of the available bird skins at Swedish museums and schools to judge the development of mercury pollution (see Figure 1). Here it was a question of pollution which was bound to proteins in bird feathers and where one could, generally speaking, ignore artifacts in the form of postmortem mercury contaminants of impurities in the feathers. If on the other hand arsenic had been the problem, the museum collections could not have been used, since arsenic soap has been used for a long time by taxidermists. Studies of mercury content in bird feathers became the start of the present operations in Sweden at the Environmental Specimen Bank.

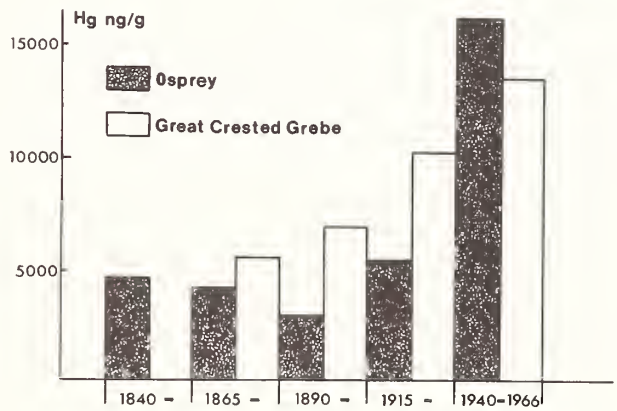

Figure 1. Mercury levels in Osprey and Great Crested Grebe feathers during the period of 1840 to 1966. From A. G. Johnels, C. Edelsteam, M. Olsson, T. Westermark. Fauna och Flora, 5, 1968.

It did not take longer than up until the end of the 1960's before it became clear that the next big environmental threat could not be studied with the help of traditional museum collections. Studies of DDT and PCB problems or studies of chlorinated hydrocarbons demanded material that could not normally be found in museums. First and foremost, these substances are fat soluble or lipophilic and fat is a substance which is carefully eliminated before a specimen becomes a dry preparation. Not even the alcohol fixation wet preparations could be used since alcohol also releases fat with its lipophilic pollutants.

When we, at the end of the 1960's, put an advertisement on the first page of the two largest daily newspapers in Sweden asking for old seal oil, we were desperate in our attempts to obtain such historical material--a material which would give us the possibility to use times series studies as an instrument in our analyses of the pollution problems in the Baltic Sea (see Table 1). Seal oil has been used earlier as painting oil by Swedish fishermen. The Environmental Specimen Bank can be said to have had its real start in 1964, but the need for it became even more apparent with consciousness

of nonpolar or fat soluble bioaccumulating substances in our environment. During the course of the past years, large amounts of specimens have been taken care of and are now preserved in the Specimen Bank at the Swedish Museum of Natural History.

Table 1. Levels of DDT and PCB substances (mg/kg) in seal oil from Åland Sea in the Baltic Sea. From M. Olsson, A.G. Johnels and R. Vaz. Swedish Environment Protection Board, PM serie 591: 43-65, 1975.

Period	n	sDDT	sPCB
1940-1945	1	0	0
1950-1955	1	10	5.2
1968-1970	27	270 (68-850)	100 (20-320)

n = number of sample s = sum

V. Why are Times Series Analyses so Important?

When evaluating a pollutant in the environment and its effects, one should bear in mind that there will always be difficulties in interpreting the effect of a given pollution content in the environment. This is because the ecosystem is extremely complex with different communities living under different conditions. How serious a certain amount of one environmental poison is in an individual population in a certain ecological situation (for example, winter, saltwater) should be considered a nearly impossible question to answer. Today, we are building up very expensive test systems for chemical substances which include different kinds of organisms, communities, and ecosystems as well. These test systems are necessary, but a warning is also necessary about over confidence in a test system's capacity and competence. In the future we probably will still have to live with the situation that an environmental poison has slipped out into nature. The environmental poison may emanate from an economically or occupationally important process in society. Great demands on the precision of evaluations of a specific substance's ecological effect will be made before often times expensive measures limiting its discharge can be taken. Perhaps one can quite often only prove the presence of a substance or a more minor biological disturbance.

If, on the other hand, with the help of a times series one can ascertain that the concentration of a substance in an environment is increasing, this is in itself an argument which should stop or limit its discharge. Even if one cannot point directly at the ecological consequences or effects of the discharge in question, one can verify that a continued increasing of it will sooner or later definitely lead to ecological consequences.

In the case of DDT and PCB research, we had to wait approximately 10 years before a significant trend in the Baltic Sea could be proven (see Figures 2 and 3). This is a long period of time when one considers the complexity of the present discharges into our environment. Today, we have the ability, to study in retrospect, the trends of many environmental contaminants in the Baltic Sea in the material which we have preserved during the last 15 years.

VI. What is Measured in the Environmental Specimens?

The measurements which can be called for are chemical, morphometric and ocular. The Environmental Specimen Bank at the Swedish Museum of Natural History presently aims primarily at providing for the needs of chemical measurements, even if we partly try to provide for a future need of histological samples for historical analysis, as well as the need of material for morphometrical studies of thickness and quality changes in eggshell, bones, and teeth. For examples, we can, now that it is too late, verify that histological samples suitable for histo-pathological studies of top predators in the Baltic Sea kept stored from periods when there was less human influence in the area, would have been an invaluable asset today. We try today to provide ourselves with such material, but as can easily be understood, this material has been markedly affected by human activity and most material has to be collected from environments other than the Swedish ones.

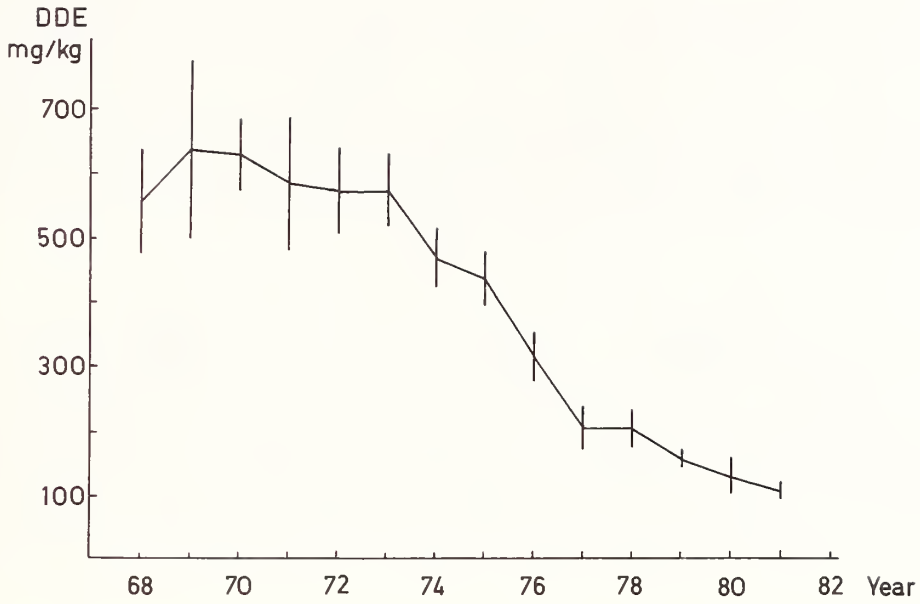

Figure 2. Mean levels of DDE and 95% confidence interval in eggs of Guillemot Uria aalgae collected at Stora Karlsö in the Baltic Sea proper during 1968-1981. From M. Olsson & L. Reutergårdh, Kommitten för Bottniska Viken. Årsrapport nr 10 (1982)

Figure 3. Mean levels of PCB and 95% confidence interval in eggs of Guillemot Uria aalgae collected at Stora Karlsö in the Baltic Sea proper during 1968-1981. From M. Olsson & L. Reutergårdh, Kommitten för Bottniska Viken. Årsrapport nr 10 (1982)

The chemical analyses which we feel that we can carry out on the Specimen Bank's material are all in the category of bioaccumulative substances. The reason for this limitation is as follows. If a substance is not bioaccumulative, its concentration will be highest in the media and not in the organisms. The risk of contamination of older media samples, whether it is through dishwater, containers, or dust or air, is incredibly great. A specimen bank for such samples is very difficult to operate and is therefore not a realistic possibility today. The largest amounts of bioaccumulative substances are found in biota and the risk for contamination of the organism by media of the type water, dust and air is thereby much less even if the risk is obvious and requires special precautionary measures. The result of this reasoning is that the samples in the Swedish Museum of Natural History's Specimen Bank are mostly organisms.

VII. What does a Suitable Specimen Look Like?

The specimen which is selected for preservation for trend studies must be well-defined but also representative for its environment. Furthermore, it should also be easy to collect so that the same sort of samples can be collected year after year for comparison between years, but without damage to the natural environment. If the environment is complex, the difficulty in getting a well-defined sample will be considerable. For this reason the collection of biota is mainly carried out in clearly limited, ecologically homogeneous environments in order to prevent the statistical variation in the samples from becoming too great. As has been pointed out earlier, the Specimen Bank consists mainly of organisms. There are arguments which speak for this arrangement which have been mentioned earlier, but one further argument is that the organisms or their organs are well-defined statistical units.

One important question is what the sample should represent. Some times the monitoring program has not always considered the question if one wishes to study the burden of an environmental pollutant in a population or if one wants to study pollution burdens in an environment. In my opinion, the pollution burden in a population should be studied only in a situation where the population is directly threatened by an environmental poison. A study of a population requires a large number of analyses because it should, in a statistically satisfactory manner, cover all of the variations in the population caused by size, age, sex, habitat, migration, etc.

The pollution burden of an environment, on the other hand, is measured in biota because the biota collects environmental pollutants. Thus, biota has an advantage as an object of analysis as compared to media. But at the same time physiological and ecological processes in the biota can influence the concentration of a pollutant, which is why the fraction that is selected for analysis should be as comparable as possible within a certain year as well as over a period of several years. Thus, not only geographical position but even biological, measured variables such as species, age, size, sex, etc., shall be considered with the goal of collecting that part of the population which represents a stationary part of the population representative of the specific environment to be studied. Furthermore, periods during the year when dramatic changes in the environment occur, such as spring flow, shall be avoided as well as periods when dramatic physiological alterations occur (e.g., lactation periods for female mammals and breeding periods for birds), since these processes may cause variations in body burden of pollutants and the demand for precision in tissue sampling will increase and cause practical difficulties. If these factors are considered, the statistical variation will decrease and the precision of the trend studies will increase. It becomes apparent that there is a certain difference between the average museum collection and a specimen bank to the extent that a museum collection strives to keep a certain breadth where both a population's composition and its geographical distribution are concerned, whereas a specimen bank is considerably limited in both of these aspects.

VIII. How is an Environmental Specimen Stored in the Specimen Bank?

Scores of conservation methods have been discussed during the course of the years. The simplest method, drying out after cleaning, can be appropriate for bones, feathers and hair, on the condition that a substance to be studied at a future date is bound to this material. Another method is to add a conserving preparation. Regardless of which preserving preparation is used, a solution is added to the tissue with a composition and properties that relatively speaking, mean that the possible existence of an unknown substance cannot be excluded today. Preserving preparations are only used in long-range histological studies at the Swedish Specimen Bank today. This kind of treatment is also generally limited to top predators.

Freeze drying has also been practiced at the Specimen Bank. For metal analyses this method seems to work well, whereas analyses of lipophilic substances after freeze drying have not worked satisfactorily. Freeze drying is used mainly by us today for sample preparation, for example homogenization, before chemical analysis.

Deep freezing is the method which is most often used. To freeze materials down to -30°C has certain advantages. It is inexpensive and simple and, perhaps most important, it requires a minimum of human handling. One does not have to add any preserving preparations and one can favorably freeze whole organisms so that the risk of contamination is decreased. The method does, however, have disadvantages, such as the fact that chemical decomposition takes place even at that low a temperature. One way to lessen this process is to lower the temperature to -60°C to -70°C. At present the Specimen's Bank material is frozen to -30°C. We are forced to accept the decomposition which takes place for practical reasons. To freeze large amounts of material to -70°C is not economically realistic in Sweden when one takes into consideration the volume of samples we feel we must keep.

Experiences from the last 15 years' environmental pollutant studies show that the amounts of samples required for a chemical analysis are generally between 0.1g and 50g whereas, most methods require 2-10g sample material. Furthermore, samples which are too small can cause poor representativity, which means that methods using small amounts of samples are not always preferable. When one considers that every collected sample, which is provided with data on collecting technique and time, age, size, sex, etc., has to be used in several analyses, the need for space for an individual sample becomes apparent. We are quite conscious of the fact that certain chemical analyses may not be possible in the future with our present preserving techniques, but we have still opted for deep freezing as our main method.

IX. What is Stored in the Specimen Bank?

The cost of storing samples in the bank is considerable, and is spread out over collection, preparation, documentation, data registration, storage, and handling at the bank. This means that a selection of material must take place. One can generally say that storage in the bank follows the guidelines given in the Swedish Environmental Monitoring Program. Operations within the scope of the environmental monitoring program are carried out at the Swedish Museum of Natural History. The following material is stored.

A. WITHIN THE MONITORING PROGRAM

Samples are collected from well-defined, ecologically homogeneous areas representing three different types of ecosystems: terrestrial, fresh water, and marine. Each ecosystem is represented by about 10 areas from which biological material is collected yearly and kept in the Specimen Bank (see Figure 4). About 50 individuals of each species are collected in each habitat.

The following species have been chosen where different areas are represented by different species:

Terrestrial: rabbit, reindeer, moose, fox, starling yearlings.

Fresh water: perch, roach, char, pike.

Marine: Blue mussels, flounder, cod, young Baltic herring (< 4 years), guillemot.

A total of 3500 samples are collected yearly.

B. THE REFERENCE MATERIAL PROGRAM

Every fifth year the material is collected from areas where local complex contaminations can be expected to influence the area. The samples are intended to serve as reference data over a period of time. In the future such material can give us information about when an environmental pollutant was first introduced. Here the material consists of stationary fish species and yearlings of seabirds.

C. THE PROGRAM FOR ENDANGERED SPECIES

In this program material is collected from organisms where the populations are directly threatened by environmental pollutants. One can here mention such species as the white-tailed sea-eagle, peregrine falcon, osprey, otter, grey seal, common seal, and ringed seal. In addition, material is secured for future studies from all of the dead wild game animals which accrue to the Crown (the State) according to paragraphs 18 of the Swedish Hunting Statute. The material consists of a

◇ marine areas with annual collection
◆ archipelago areas with annual collection
● agricultural areas with annuan collection
■ forest areas with annual collection
▲ alpine areas with annual collection
□ recipients with complex pollution with annual collection
○ trend series started in late 60th

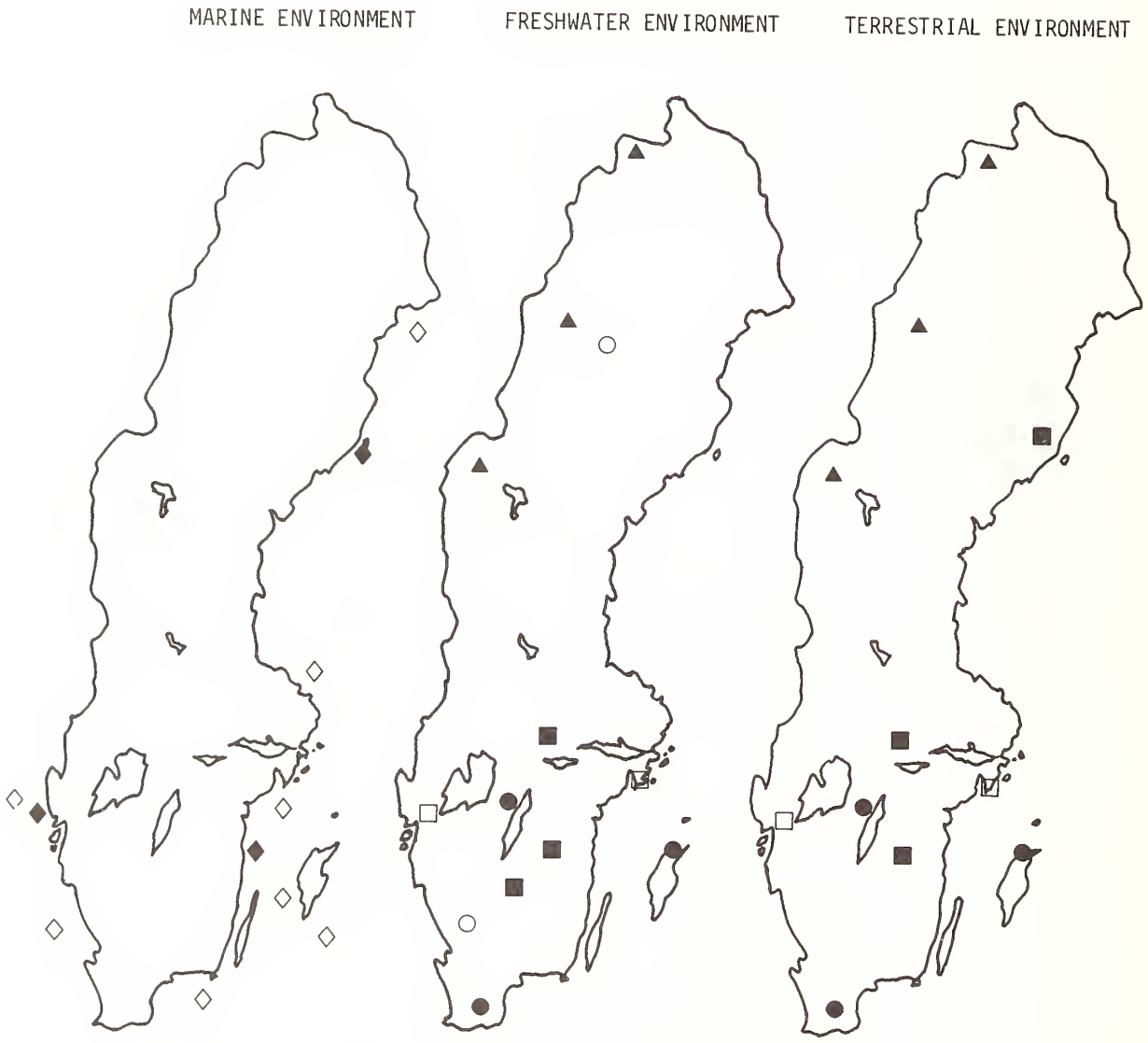

Figure 4. Collection areas within the Environmental Monitoring Program. The map shows the location in the three environments: marine, freshwater, and terrestrial environment. The collection started during the 1970's.

long series of rare species. Although these organisms could never be actively collected on account of their low number, such material is secured for future studies. The yearly collection amounts to about 700 samples.

D. LARGER, SCIENTIFICALLY VALUABLE SERIES

Certain larger series of well-documented material is kept for future studies. At present moss samples are taken care of. The yearly accession amounts to about 1000 samples.

X. How Large can the Specimen Bank Become?

The yearly accessions to the volume of the Specimen Bank continually increase and their cost will therefore soon reach an unacceptable level. It is obvious that an elimination of some material should take place. The following reasoning will probably be used to decide which material will be eliminated. At every point in time when a times series is to be evaluated, the nearest preceding period is probably the most interesting. There can of course be deviations from this, but this reasoning should be acceptable in most cases. For this reason the material in the Specimen Bank for the last 10-15 years should be represented by annual collections. The interval of 15-25 years can be represented with material from every third year. The period 25-50 years could be represented by samples collected at 5-year intervals.

Appropriate intervals and frequencies can and should be discussed, but it is absolutely clear that a continuous elimination process must be carried out. The eliminated material can eventually be used as exchange material in contacts with other specimen banks.

SECTION 6

THE U.S. PILOT ENVIRONMENTAL SPECIMEN BANK PROGRAM

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I. Introduction

Many hazardous chemicals are produced each year and to some extent enter the environment. To monitor the environment for all of these hazardous materials is an impossible task. Thus, monitoring programs generally focus on the measurement of specific chemical species which are recognized as hazardous or which are of particular interest to a specific study. If measurable levels of a hazardous compound are found in the environment, questions arise such as: When did it first enter the environment? Where did it come from? Is it increasing or decreasing in concentration and at what rate is the concentration changing? In addition, are hazardous chemicals present in the environment which are currently unknown (i.e., "new pollutants")? Many of these questions could be answered successfully if carefully collected and preserved environmental specimens from the past were available. However, there are few, if any, environmental samples from the past which have been collected and stored without change or contamination to serve as benchmarks against which contemporary levels of pollutants can be compared.

The concept of an environmental specimen bank for archiving of biological and environmental samples for retrospective analysis has been recently recognized as an important component of systematic environmental monitoring [1-3]. The long-term storage of carefully selected, representative environmental samples in an environmental specimen bank provides an important complement to the real-time monitoring of the environment. The availability of environmental specimens collected at the present time and archived for future analyses would serve the following functions:

(1) These specimens would provide a bank of well-preserved and documented samples for retrospective analyses in future years as analytical techniques improve or as concerns for new (as yet unidentified) pollutants arise.

(2) These specimens would serve as reference samples to document improvements in analytical techniques and to verify previous monitoring results.

(3) These specimens could be used to detect changes in the environment (trend monitoring) by comparison of samples collected at various times and analyzed using comparable analytical techniques, thereby providing verification of the effectiveness of restrictions, regulations, or management practices employed in the manufacture and/or use of toxic chemicals.

Environmental samples archived as part of monitoring programs have already proven useful in specific studies. For example, in the case of kepone in the James River in Virginia, banked samples were used to help establish when this chemical first entered the river [4]. In the Great Lakes, retrospective analysis of Herring Gull eggs collected in 1971 and stored in a specimen bank until 1980 was used to confirm a decrease in the concentration of 2,3,7,8-tetrachlorodibenzo-p-dioxin [5]. Both of these examples refer to "new pollutants" (i.e., kepone and dioxin) which have become national concerns in recent years. At the time the above mentioned specimens were collected, these pollutants were not recognized as problems.

II. Development of the Specimen Bank

The development during the 1970's of the concept of a National Environmental Specimen Bank (NESB) in the United States has been reviewed previously [6,7] and is summarized in Table 1. Several workshops held in the early 1970's identified the need for a systematic collection, storage, and analysis of environmental specimens and finally, in 1973 the U.S. Environmental Protection Agency (EPA) proposed the establishment of a NESB system.

TABLE 1. Development of the Environmental Specimen Bank Program in the U.S.

1972	NAS/NRC[a] Workshop - Identified Shortcomings of Uncoordinated Sampling, Storage, and Analysis of Environmental Specimens
1973	GERHD/NAS/NRC[a] - Identified the need for National Environmental Specimen Bank (NESB)
	EPA Workshop - Proposed NESB Concept
1974	EPA/NBS - Interagency Agreement for Evaluation of Research Methodology for NESB
1975	Established Bilateral Agreement with Federal Republic of Germany for Collaborative Research in Specimen Banking
1976	EPA/NBS Workshop - Recommendations for the Design of a Pilot Program to Study the Feasibility of NESB
1977	International Workshop on "The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants" held in Luxembourg
1978	International Workshop on "Monitoring Environmental Materials and Specimen Banking" held in Berlin, Germany
1979	U.S. Pilot Environmental Specimen Bank Facility at NBS Completed
1980	Collection and Analysis of Human Liver Samples Initiated
1982	International Workshop on "Environmental Specimen Banking and Monitoring as Related to Banking" held in Saarbrücken, Germany
	Collection and Analysis of Mussel Samples Initiated

[a]National Academy of Science/National Research Council (NAS/NRC), Geochemical Environment in Relation to Health and Disease (GERHD).

Since 1975 the National Bureau of Standards (NBS), in conjunction with EPA, has been involved in research relating to environmental specimen banking. The initial plans and preliminary results of the joint EPA/NBS research and evaluation of methodology for the NESB have been described previously [8-13]. Also in 1975 the Federal Republic of Germany (FRG) and the U.S. agreed to cooperate in the study of environmental specimen banking activities and proposed the establishment of "pilot phase" specimen banks in both countries. At an EPA/NBS Workshop on "Recommendations and Conclusions on the National Environmental Specimen Bank" held in 1976 [8], a "pilot" NESB program was outlined for the U.S. This pilot program was designed to evaluate the feasibility of a national program by providing actual working experience in all aspects of specimen banking, i.e., specimen collection, processing, storage, and analysis.

The major goals of this pilot study were: to develop analytical protocols for sampling, processing, and storage of four types of environmental accumulators; to evaluate and improve analytical methods for the determination of both trace element and organic pollutants in biological matrices; to establish baseline data on selected environmental specimens; to evaluate the feasibility of long-term storage at various conditions; and to evaluate specimen banking as a means of storing samples for pollutant trend monitoring and of permitting retrospective analyses as concerns for new pollutants arise and as new analytical techniques are developed.

III. The EPA/NBS Pilot Environmental Specimen Bank

Four types of environmental specimens, which represent environmental accumulators, were selected for inclusion in the EPA/NBS pilot program (6), i.e., (1) human soft tissue - liver, (2) marine accumulator - marine mussels (Mytilus edulis), (3) food accumulator, (4) air pollutant accumulator. Human liver was selected as the first sample type for inclusion in the pilot specimen banking program. Reasons for the selection of the liver as the human soft tissue include the following: the liver is a filter organ in which both inorganic and organic species accumulate; the macroscopic pathology of the liver is relatively homogeneous as compared to other human tissues; and sufficient

sample can be obtained from one individual specimen. As each new sample type is incorporated into the pilot program, collection, storage, and analysis of the previous sample types(s) will continue. The number of sample types and samples has been minimized so that the pilot program would not be overwhelmed with samples or analyses, but could focus on the protocol development and research aspects necessary to provide reliable analytical data.

Since 1980, NBS has been involved in the sampling, processing, storage, and analysis of the first sample type, human liver. In this article the experience gained during this pilot program relating to sample collection, processing, storage, and analysis of human liver samples is described. The findings of this and the other pilot studies will be used to evaluate the feasibility of specimen banking activities on a much larger scale. In addition, some of the significant results from this pilot phase will be discussed which illustrate the potential advantages of such activities.

A. SAMPLE COLLECTION AND STORAGE

Due to the extremely low levels of trace element and trace organic pollutants found in most environmental samples, extreme caution must be exercised during sample collection and processing to avoid contamination. A detailed sampling protocol, designed to provide samples suitable for trace element and trace organic analyses, was developed and implemented for the collection of human liver samples [19]. The liver sampling protocol was developed in conjunction with individuals performing the autopsies, and implementation of the protocol required periods of education and close cooperation to achieve a suitable protocol within the bounds of practicality.

The sampling protocol was designed to avoid possible contamination of the sample by either inorganic or organic constituents [19]. Teflon® materials (e.g., sheets, bags, and storage jars) were selected as the most suitable material for non-contamination of the sample with respect to both inorganic and organic constituents and for low diffusion rates of water [9,17]. The protocol specifies the use of such non-contaminating items as non-talced, vinyl gloves; pre-cleaned, dust-free Teflon® sheets and bags; high-purity water; and a titanium/Teflon® knife. These items were provided to each collection site by NBS to ensure uniformity in sampling and container materials. The titanium-bladed knife with a Teflon® handle was designed and constructed at NBS to bisect the liver specimen after removal from the donor. These special knives are used to avoid trace element contamination by various constituents associated with a regular stainless-steel scalpel/knife (e.g., Ni and Cr) and to limit the possible contamination to an element of little environmental interest, namely titanium. The liver samples are sealed in Teflon® bags, frozen in liquid nitrogen (LN_2), and shipped to NBS in a biological shipper at LN_2 vapor temperature.

A data form, sent to NBS with each liver sample, contains information about the donor and specimen: date of birth, sex, residence, ethnic group, height, weight, smoking history, occupation (if known), previous disease history (if known), date and time of death and autopsy, diagnosis of autopsy, and liver specimen weight.

The sampling protocol for human livers developed for the NBS pilot specimen bank program was recently used as a model for collection of human tissue at a workshop entitled "Protocols Mineral/Element Analysis of Human Tissues" held in St. Louis, MO [20]. The goal of this workshop was to prepare specific protocols for sample selection, collection, preservation, and analysis of human tissues. The St. Louis workshop was organized as a follow-up meeting to the "Workshop on Research Needed to Improve Data on Mineral Content of Human Tissue" [21].

To minimize possible sample contamination during sample processing at NBS, all sample handling procedures are performed in a specially designed Class 100 "clean laboratory" to reduce the potential for contamination from the air. In addition, a cryogenic homogenization procedure was developed which minimizes contamination generally encountered in conventional sample homogenization procedures.

The storage scheme for the liver samples illustrates the approach used in the NBS pilot program. This scheme is designed to evaluate the question of appropriate temperature for storage of biological samples as well as to provide a bank of well-characterized reference samples. The liver samples are received at NBS as duplicate sections of the left lobe, identified as Sections "A" and "B". All of the "A" sections are placed in long-term storage at LN_2 vapor temperature, and the "B" sections are used for the storage evaluation. Approximately 30 of the "B" sections per year are homogenized using the cryogenic homogenization technique (see discussion below) to provide about 20 aliquots of 6-8 g each per sample. The sample remains frozen during grinding, and the sample aliquots are transferred to the Teflon® storage jars inside a cold nitrogen atmosphere glove box to minimize water condensation on the frozen samples.

To investigate the question of the appropriate temperature for long-term storage, the sample aliquots are stored under four different conditions: room temperature after freeze-drying, frozen at -25°C, frozen at -80°C, and frozen at liquid nitrogen vapor (temperature -125°C to -190°C). These aliquots will be reanalyzed at various time intervals during the pilot study and the results will be compared to the data from "real-time" analysis (i.e., analyses performed soon after homogenization) to determine if changes in the concentration of trace elements or trace organics (e.g., organochlorine pesticide residues) have occurred. The "A" sections are reference samples which may be used to reevaluate results obtained for a particular "B" section. At the end of the pilot program, the "A" sections will represent a valuable bank of well-characterized, documented samples available to the scientific community. In addition, a large quantity of analytical data from the analyses of the "B" sections will be available for many of these liver samples. During the first year of sample collection 100 liver samples were obtained form each of three locations: Baltimore, MD, Minneapolis, MN; and Seattle, WA. During the past two years samples have been obtained only from Seattle at a rate of approximately 100/year. At present, about 500 different liver specimens are stored in the pilot bank.

Collection of the second sample type, mussels (Mytilus edulis) , was initiated in late 1982. The specific specimens for the food accumulator and air pollutant accumulator have not been selected. However, suggested specimens for the food accumulator include milk, grain, a total diet food composite, or specific foods. Moss, lichen, or air particulate filters are possible candidates for the air pollutant accumulator specimens. In this article, we will focus only on the experience gained in the EPA/NBS pilot program with the human liver specimens.

B. HOMOGENIZATION OF BIOLOGICAL TISSUES

Sampling for trace analysis is a major concern when accurate and representative analytical results are required [22]. The reduction of a bulk sample to a laboratory sample (test portion) suitable for the analytical technique employed often introduces errors due to contamination and/or sample inhomogeneity. These errors may become the limiting factor in achieving precise and accurate analytical results. An efficient and contamination-free homogenization procedure allows the characterization of an inhomogeneous bulk sample when only one or a few test portions are analyzed.

Despite the macroscopic homogeneous appearance of human liver, large inhomogeneities within a single liver have been reported when using one gram analytical test portions [23]. To evaluate storage conditions and to completely characterize the bulk liver sample, analytical results from different subsamples of the same liver must be compared. Since the bulk liver sample must be homogenized to provide equivalent analytical test portions, a major research effort of the pilot environmental specimen bank program has been directed towards the development and evaluation of a system for contamination-free homogenization of biological tissues.

A cryogenic homogenization procedure has been developed and evaluated using Teflon® ball mills and disk mills. Iyengar and Kasperek [24] first reported the technique of cryogenic homogenization (brittle fracture technique) using Teflon® ball mills for relatively small freeze-dried biological samples (5-15 g). Initial studies in the EPA/NBS pilot program used Teflon® ball mills with a 150-g capacity. After performance evaluations of the ball mills (see discussion below), a new design using a Teflon® disk mill was developed. Details of the design and performance evaluation of these two types of mills for homogenization are described elsewhere [25,26].

The liver samples are received frozen at LN_2 temperature; the samples are prefractured, placed in the disk mill, and homogenized at cryogenic temperatures. After homogenization, the liver homogenate is handled at cryogenic temperatures and maintains a particulate appearance. Assuming a particulate homogenate, the performance of the two types of mills, i.e., ball and disk, was investigated. Ingamells [27,28] developed a practical model to assess the sampling of particulate materials. Ingamell's proposed sampling constant, K_s, was used for direct comparison of the performance of the different cryogenic homogenization systems. To determine K_s experimentally, the particulate homogenate was subsampled at different sample sizes and analyzed. If the analytical error is sufficiently small, then K_s can be determined using the equation $K_s = wR^2$, where R is the observed relative standard deviation of a set of subsamples and w is the mass of the respective subsamples. The sampling constant corresponds to the weight of the sample required to limit the sampling uncertainty to one percent with 68 percent confidence.

The sampling constant, K_s, was determined with radiotracer experiments using ^{24}Na, i.e., a 5-g subsample of liver tissue was irradiated in a nuclear reactor and then homogenized with the bulk material. From these experiments sampling diagrams were generated. The comparison of the ball mill with a disk mill (100-g capacity) is shown in Figure 1. Using the ball mill, a sampling constant K_s = 32 g is obtained. Based on this result for the ball mill, it was concluded that the material was

not sufficiently homogeneous. Thus, for a typical 1-g analytical test portion, sample inhomogeneity could be the source of errors which exceed the precision of the analytical technique. The inadequate performance of the ball mill resulted in the design of a disk mill which yielded a K_s = 0.95 g. Thus, by using the disk mill, the analytical data from a 1-g sample would depend equally on the precision of the analytical techniques and on the uncertainty of sampling.

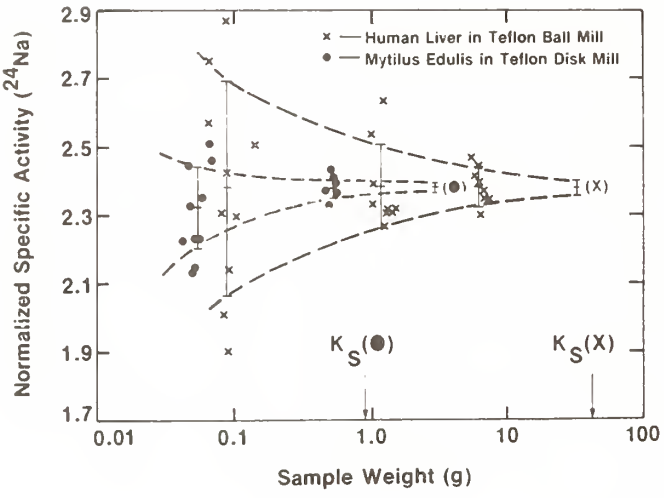

Figure 1. Sampling diagram of ^{24}Na in human liver and mussel homogenate. Specific activity (cts $g^{-1}s^{-1}\cdot10^{-2}$) scale is for the liver homogenate; values for mussel homogenate are normalized to the liver values for comparison

The performance of the mills with respect to particle size reduction was also evaluated by sieving experiments [25]. A direct comparison of the particle size distribution for the ball mill and the disk mill indicated that the disk mill was more effective in producing a small particle homogenate. With the disk mill, virtually all of the material passed through a 40 mesh sieve, i.e., the particles are less than 0.46 mm in diameter. In addition, data supporting the homogeneity of the material was obtained by elemental analysis of the homogenate. For the majority of the elements, the standard deviation for a set of 10 1-g test portions of a single liver was observed to be smaller than 5 percent and less than 2 percent for several of the essential trace elements for which large differences (e.g., a factor for 1000) in 1-g test portions were reported previously [23].

As a result of the above performance evaluations and operational experience in the pilot specimen bank program, the Teflon® disk mill is recommended as an effective, contamination-free device for size reduction and homogenization of biological tissue. Operation at cryogenic temperatures reduces loss of volatile components and possible changes in composition during the size reduction step. A disk mill has been constructed to accommodate samples as large as 1000 g. The quality and quantity of samples produced using this technique indicate its usefulness for sample preparation of biological tissues for analysis.

C. INORGANIC ANALYTICAL SCHEME

An important part of the pilot specimen bank program has been the development and implementation of an analytical scheme of known and documented accuracy for the chemical characterization (both inorganic and selected organic constituents) of these specimens. The first priority in the development of an inorganic analytical scheme has been the determination of as many elements of interest as possible. The elements of interest were: (1) trace elements of environmental concern [2] and (2) minerals and trace elements of biological importance which may be of interest for the assessment of nutrition and health-related aspects of the individuals (see Figure 2). With the measurement of as many elements as possible from these two groups, a large data set is obtained which has the same analytical history (i.e., sampling, processing, and measurement procedures), thereby possibly providing important information about interelement relationships, the relationships of elements to health and nutrition, and the impact of pollutant elements on the biological system.

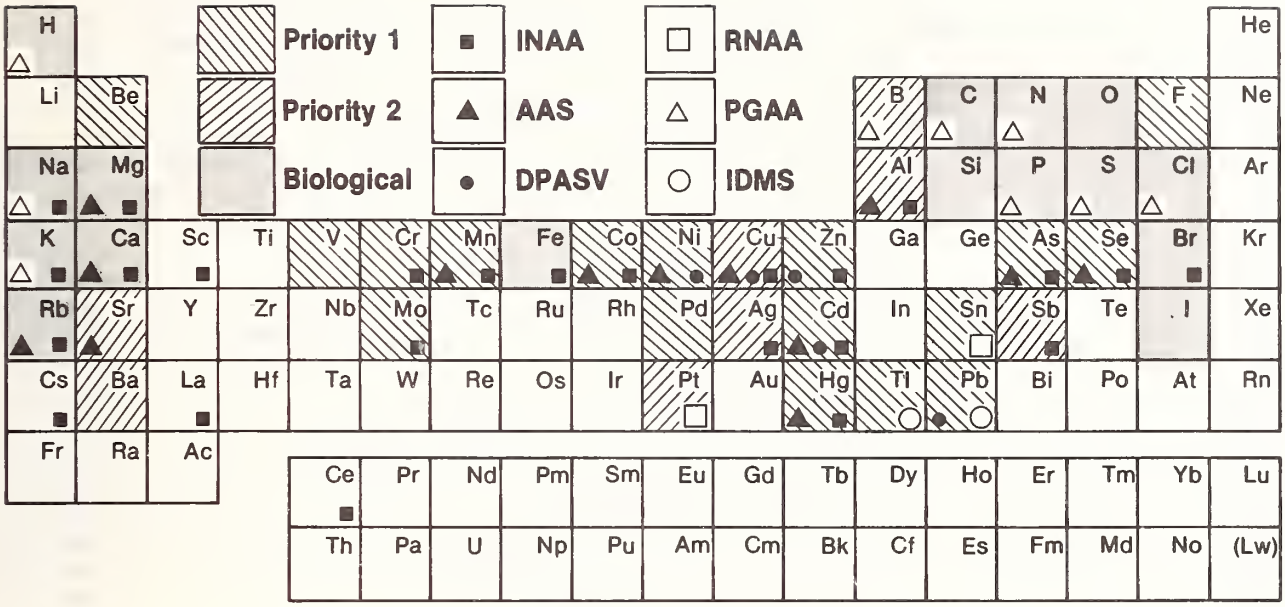

Figure 2. Elements of interest and analytical techniques used for the determination of trace elements in human liver specimens. Priority 1 elements are from ref. 2; priority 2 elements are additional elements of environmental concern; biological elements refers to elements of biological interest. (INAA = instrumental neutron activation analysis, AAS = atomic absorption spectroscopy, VOLT - voltammetry, PGAA prompt gamma activation analysis, RNAA = radiochemical neutron activation analysis, and IDMS = isotope dilution mass spectrometry.)

The elements determined and the analytical techniques used in the analysis of 36 human liver samples collected during the first year of the pilot program are summarized in Figure 2. Four analytical techniques were used for analyses of these samples: atomic absorption spectroscopy (AAS), isotope dilution mass spectrometry (IDMS), instrumental neutron activation analysis (INAA), and voltammetry (VOLT). Using these techniques, nearly complete coverage of the elements of interest (31 elements) was achieved. In addition to the four techniques which were used routinely for these samples, two new radiochemical NAA procedures for Sn and Pt were developed and evaluated on selected liver specimens [29,30]. Complete details concerning the experimental procedures and results from each technique have been published [26,31]. The analyses of 30 additional liver samples from the second-year collection have been completed recently using only two techniques, INAA and AAS.

The second priority in the development of the inorganic analytical scheme was the implementation of two procedures to assure the quality of the chemical analyses: (1) the simultaneous analysis of Standard Reference Materials (SRM's) with the same or similar sample matrix and (2) the determination of constituents by two or more independent analytical techniques. Due to the limited number of analytical test portions for each liver sample, a three-fold approach was incorporated into the analytical system as an additional means of quality assurance. First, the multielement technique of INAA was selected for analysis of each homogenate in duplicate because of the small sample size (1g fresh weight) used in this technique. Second, as many elements were determined as economically possible using different techniques (see Figure 2). Third, sufficient sample homogenate was banked (as well as the complete "A" section of each liver sample) to repeat an analysis if a result appeared to be questionable.

Using the analytical techniques summarized in Figure 2, 31 trace elements have been determined in a total of 66 human liver samples collected during the first two years of the pilot program. The results of these analyses are summarized in Figure 3. This figure contains a plot of the more than 2000 data points obtained from the analyses of the human liver samples and it also includes the trace element concentration ranges reported for human livers in the compilation by Iyengar et al. [32]. Conclusions on environmental impacts, health effects, etc. are not readily available due to the small

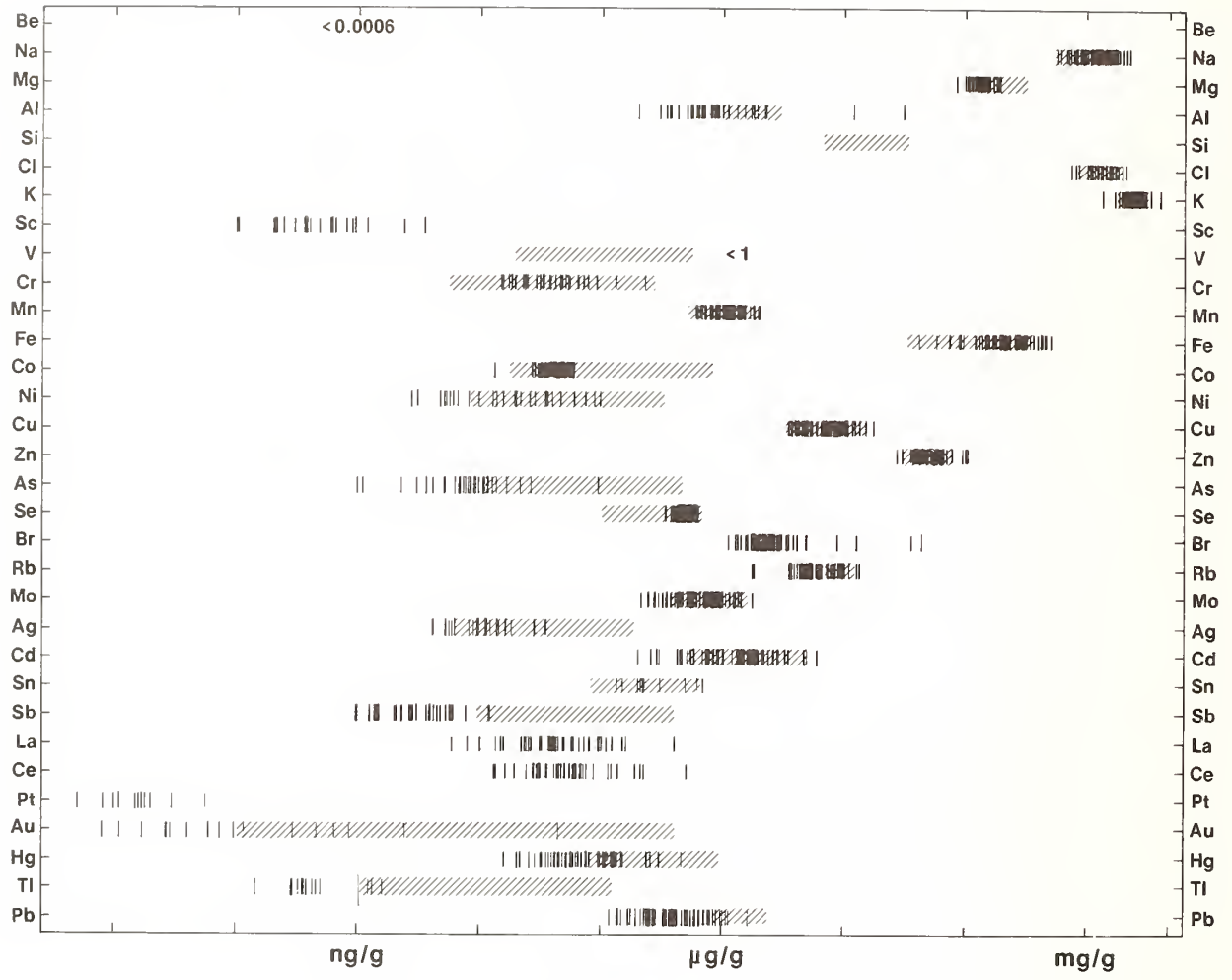

Figure 3. Elemental concentrations in 66 individual liver specimens and the concentration ranges reported in the literature [32]. Data from the pilot specimen bank program denoted by one line for each data point. Literature ranges denoted by the shaded areas.

number of specimens analyzed. However, observations are described below to illustrate the potential utility of a specimen banking program in relation to monitoring environmental trends.

It is interesting to note the narrow range of concentration data for many essential trace elements as compared to the wide scatter of pollutant element concentrations. For example, the concentrations of Se vary by only a factor of 1.8 and other essential elements (Mg, Co, Cu, and Zn) vary by only a factor of 2-3.5, whereas several pollutant elements (Al, As, Cd, Hg, Pb, and Tl) vary by as much as a factor of 100. Since the biological variability of the individual specimens appears to be reasonably small based on the above data for essential trace elements, the broad ranges for the pollutant elements in the data may indicate sources of pollution to which the individuals were exposed.

The range of the selenium data is much lower if individuals from the same geographical area are studied. Evaluation of the data from the first year (36 liver specimens) revealed a statistically significant difference in average Se values for samples from Baltimore (0.44 ± 0.06 µg/g wet weight) compared to Minneapolis (0.51 ± 0.06 µg/g), and Seattle (0.56 ± 0.08 µg/g). Similar evaluations indicated that possible geographic differences may exist for a number of other elements. The trace element data were also evaluated with respect to possible correlations of variables such as age and sex with elemental concentration and correlations of different elements with each other. Because of

the relatively small data set, the results were only partially successful in suggesting some possible correlations. However, as real-time analyses provide additional data in each year of the pilot program, the potential for substantiating these correlations will increase significantly.

Many pollutant trace elements were found at concentration levels which were on the low side or below previously reported data. Specifically, levels of Al, As, Tl, and Pb are significantly lower than the concentrations reported in the literature compiled through 1976. In the case of Pb, a direct comparison of 1973 data from Baltimore residents [33] with the data in this study illustrates this trend within a specified geographical location. In 1973 the mean Pb concentration of 22 livers from residents of Baltimore was 2.5 μg/g with a range of 1.0-6.3 μg/g. In 1981 (this study), the mean Pb concentration of 8 livers from Baltimore residents was 0.58 μg/g with a range of 0.25-1.15 μg/g. However, there is an element of uncertainty in this comparison, since the applied analytical approaches (i.e., sampling protocol, sample preparation, and analytical methodology) cannot be readily compared.

This observation of lower Pb levels could be attributed to improvements in analytical methodology and control of sample contamination and/or a decrease in the level of lead in the environment because of the decrease in the use of leaded gasoline, as some have suggested [34]. The precautions taken to preserve the integrity of the samples in the pilot specimen bank program, especially the minimization of possible sources of contamination during sampling and sample preparation, would obviously result in lower concentrations. However, this question would have been readily answered if liver samples (or other human tissues) collected and stored in 1970 in a reliable, non-contaminating manner had been available in 1983 for analysis. Perhaps similar environmental questions in the 1980's will be answered by a retrospective look at the 1980's through samples from an environmental specimen bank.

D. ORGANIC ANALYTICAL SCHEME

Screening for large numbers of organic compound classes is difficult because of the requirements of selective extraction, digestion, isolation procedures, and/or detection for various compound classes. As a result, organic analysis of the human liver samples in the pilot program have focused on the determination of organochlorine pesticide residues. The multitude and diversity of environmental organic pollutants and the analytical requirements necessary to even attempt to monitor all of these pollutants serve as justification for the need to archive environmental specimens for retrospective analyses.

Analytical methodology for the determination of organochlorine pesticide residues in human liver samples was developed and used for the analyses of 30 samples from the second-year sample collection in the pilot program. The detailed methodology and results of these analyses are reported elsewhere [26]. A typical gas chromatogram from the analysis of a liver sample is shown in Figure 4. The following organochlorine compounds, which are present in most human liver samples, were measured: hexachlorobenzene, the β-isomer of hexachlorocyclohexane (β-HCH), heptachlor epoxide, trans-nonachlor, p,p'-DDE, dieldrin, and p,p'-DDT. The results of the analyses of 30 liver samples are summarized in Table 2. In general, the most abundant pesticide residue was p,p-DDE, the dehydrochlorinated metabolic derivative of p,p-DDT.

A sample of a human liver homogenate (a composite of about 10 livers) from the German Pilot Environmental Specimen Bank was analyzed as part of an interlaboratory comparison of methods. The gas chromatogram from the analysis of this liver homogenate is shown in Figure 4. Of particular interest in comparing the two samples is the large quantity of hexachlorobenzene (~2 μg/g extractable fat) in the German sample as compared to the U.S. sample (see range of values for HCB in Table 2). Even though some reports [35] suggest that within the United States broad geographical differences in human tissue pesticide levels do not exist (since the pesticide levels are predominately derived from diet), this example illustrates the potential differences in baseline values which may be discernable on a larger international scale.

IV. Conclusions

Even though the NBS specimen bank program is a "pilot" effort, the experience gained provides the basis for establishing specimen banking as a viable long-term mechanism for evaluating environmental pollutants and monitoring pollution in our bioshpere. The protocols developed for sample collection, processing, and storage in a contamination-free mode yield benefits which extend beyond the application to human livers in the NBS pilot specimen bank; the lessons learned are equally applicable to any occurrence requiring contamination-free, trace analysis for impact/hazard assessment. Finally, with pilot experience at its current state of maturity, one can envision the broad applicability of the specimen bank concept to real-time and trend monitoring. Beyond the concern of environmental pollution, this concept is equally valuable for monitoring nutritional status, occupational exposure, and key chemicals related to the health status of a populace.

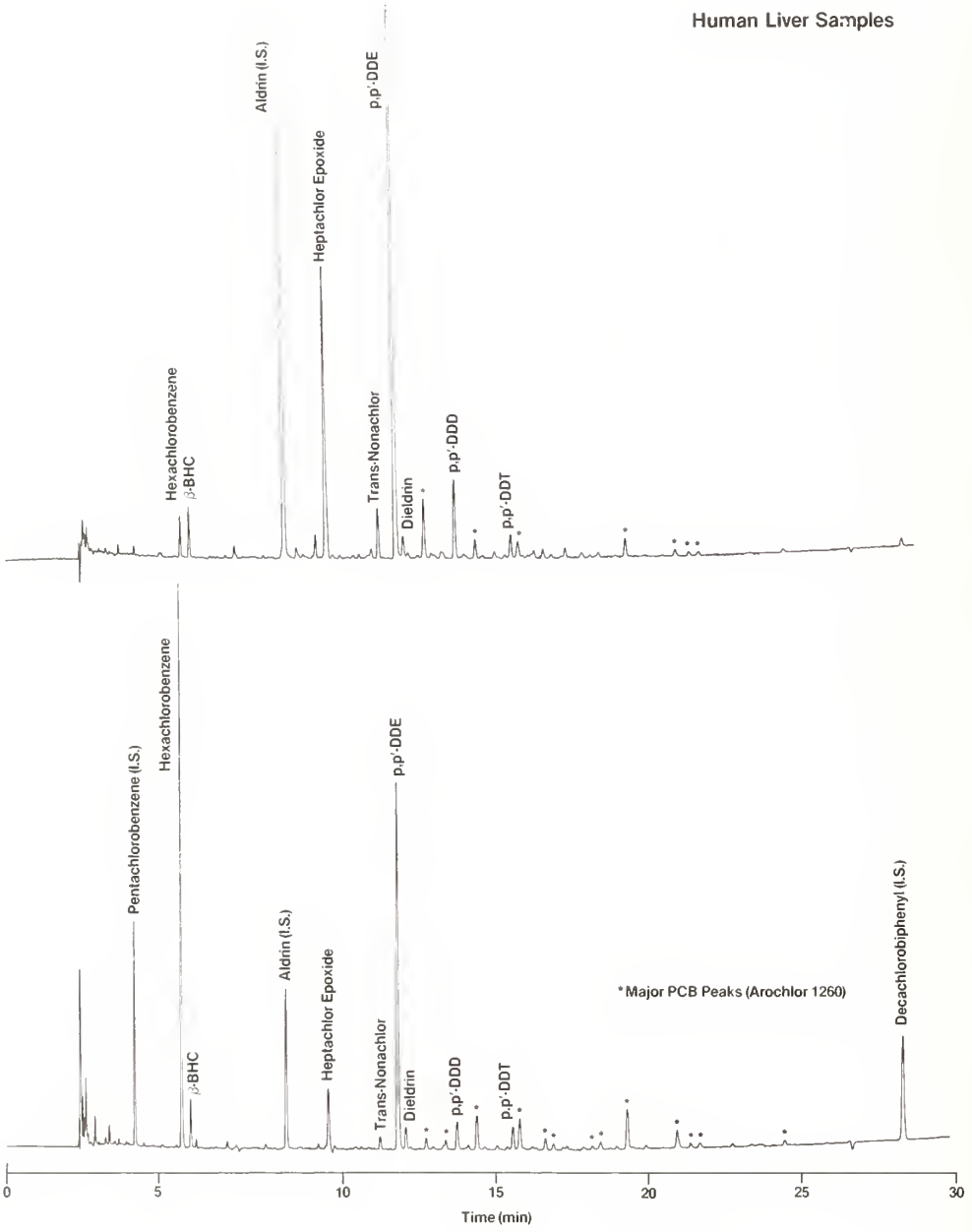

Figure 4. Gas chromatograms from the analysis of human liver samples.

(A) Liver from NBS/EPA Pilot Environmental Specimen Bank Program,

(B) Liver homogenate from the German Pilot Environmental Specimen Bank Program.

TABLE 2. Concentration of organochlorine pesticide residues in human liver specimens (μg/g extractable fat).

	No. of Samples Detected	Range	Mean Value	Median Value
Hexachlorobenzene	30	0.036 - 0.52	0.13	0.11
β-HCH	30	0.062 - 0.63	0.20	0.19
Heptachlor Epoxide	30	0.044 - 1.2	0.42	0.34
Trans-Nonachlor	28	0.005 - 0.60	0.17	0.13
p,p'-DDE	30	0.21 - 9.07	2.19	1.53
Dieldrin	24	0.031 - 2.94	0.39	0.26
p,p'-DDT	24	0.041 - 0.80	0.24	0.19

V. Acknowledgement

The Pilot National Environmental Specimen Bank Program is supported in part by U.S. Environmental Protection Agency, Office of Research and Development, Office of Exploratory Research; Project Officer: George M. Goldstein, Inhalation Toxicology Division, Health Effects Research Laboratory, Research Triangle Park, NC. The authors acknowledge the participation of the following members of the Center for Analytical Chemistry for providing analytical research and data for this program: K. A. Brletic, T. A. Butler, S. N. Chesler, K. A. Fitzpatrick, J. W. Gramlich, R. R. Greenberg, S. H. Harrison, W. F. Kline, J. K. Langland, E. J. Maienthal, T. J. Murphy, L. J. Powell, T. C. Rains, T. A. Rush, and S. F. Stone.

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APPENDIX I

PROGRAM

Eighth U.S.-German Seminar of State and Planning on Environmental Specimen Banking

September 19-20, 1983

National Bureau of Standards
Gaithersburg, Maryland

Monday, September 19	Working Sessions
8:30 a.m.	Registration, Administration Building, Lecture Room B
9:00 a.m.	Welcome/Introductory Remarks
	Marvin Rogul, U.S. Environmental Protection Agency Ulrich Boehringer, Umweltbundesamt, Federal Republic of Germany
	Current Activities of the Pilot Specimen Bank Programs in the U.S. and Germany
	Session Chairman: Stephen A. Wise, Organic Analytical Research Divison, NBS
9:30 a.m.	"Specimen and Site Selection for Environmental Specimen Banking:
	Gerhard Wagner, University of Saarland, Saarbrücken
9:50 a.m.	"The Role of Human Tissue in Environmental Specimen Banking"
	"Fritz H. Kemper, University of Münster, Münster
10:10 a.m.	"New Specimens for the U.S. Pilot Environmental Specimen Bank"
	Rolf Zeisler, Inorganic Analytical Research Division, NBS
10:30 a.m.	"Importance of Cow's Milk in a Specimen Banking Project"
	Walther Heeschen, Bundesanstalt für Milkforschung, Kiel
10:50 a.m.	Coffee Break
11:10 a.m.	"Sampling for Organic Trace Analysis"
	Karlheinz Ballschmiter, University of Ulm, Ulm
11:30 a.m.	"Determination of Chlorinated Pesticide Residues in Human Liver Specimens for the U.S. Pilot Environmental Specimen Bank Program"
	Walter F. Kline, Stephen N. Chesler, and Stephen A. Wise, Organic Analytical Research Division, NBS
11:50 a.m.	"Experiences with Organohalogen Determination with Respect to Environmental Specimen Banking"
	István Gebefügi, Gesellschaft für Strahlen- und Umweltforschung, Munchen
12:30 p.m.	Lunch, Dining Room A

1:30 p.m. "Analysis and Stability of PAH and Azaarenes During Long-term Storage"

Jürgen Jacob, Biochemisches Institut für Umweltcarcinogene, Ahrensburg

1:50 p.m. "Recent Results on the Stability of Cholesterol in Stored Human Samples"

Hans-Werner Dürbeck, Kernforschungsanlage Jülich, Jülich

2:10 p.m. "Homogenization of Solid Samples--A General Problem of Environmental Specimen Banking"

Johann D. Schladot, Kernforschungsanlage Jülich, Jülich

2:30 p.m. "Recent Results on Trace Elements in Human Livers"

Rolf Zeisler, Kathy A. Fitzpatrick, and Susan F. Stone, Inorganic Analytical Research Division, NBS

2:50 p.m. "Recent Improvements for the Determination of Trace Metals in Materials of the German Pilot Environmental Specimen Bank"

Markus Stoeppler, Kernforschungsanlage Jülich, Jülich

3:10 p.m. Coffee Break

3:30 p.m. "Application of Thermal Ionization Isotope Dilution Mass Spectrometry for Determination of Trace Elements in Human Liver"

John W. Gramlich, Inorganic Analytical Research Division, NBS

3:50 p.m. "Instrumental Analysis of Marine Bivalve Samples"

Susan F. Stone, Rolf Zeisler, and Eduardo Cortes Toro, Inorganic Analytical Research Division, NBS

4:10 p.m. "Determination of Trace Elements in Biological Tissue by Atomic Absorption Spectrometry"

T. C. Rains, T. A. Rush, and T. A. Bulter, Analytical Research Division, NBS

4:30 p.m. "Ultratrace Determination of Critical Elements Using Radiochemical Neutron Activation Analysis"

Robert R. Greenberg, Inorganic Analytical Research Division, NBS

Tuesday, September 20 **Workshop Discussion**

Session Chairman: Stephen A. Wise

9:00 - 12:00 noon **Discussion Topics and Discussion Leaders**

(1) Experience with Homogenization and Storage; Problems and Unanswered Questions in Specimen Banking; Are Samples Stable?

Rolf Zeisler

(2) Reference Material Needs for Environmental Specimen Banking

Markus Stoeppler

(3) Statistical Aspects and Representative Sampling

Robert A. Lewis

(4) Collaborations/Interactions Between U.S./Germany and Other Countries

Karlheinz Ballschmiter

(5) Uses of Banked Samples - Potential Uses of Specimen Banking

John E. Elliot

12:00 noon - 1:30 p.m. Lunch

1:30 p.m. - 2:30 p.m. Visit NBS Specimen Bank Facility

International Review of Environmental Specimen Banking

September 21, 1983

National Bureau of Standards (NBS)
Gaithersburg, Maryland

Wednesday, September 21

8:30 a.m. Registration, Administration Building, Lecture Room B

9:00 a.m. Welcome/Remarks

Donald R. Johnson, National Measurement Laboratory, NBS

9:10 a.m. Introductory Remarks

Carl R. Gerber, Office of Exploratory Research, U.S. Environmental Protection Agency

"The Environmental Specimen Banking Project of the Federal Republic of Germany"

Ulrich Boehringer, Umweltbundesamt, Federal Republic of Germany

Plenary Lectures

Session Chairman: Karlheinz Ballschmiter, University of Ulm, Federal Republic of Germany

9:50 a.m. "The Role of Specimen Banking in Environmental Management"

John E. Gannon, International Joint Commission, Great Lakes Regional Office, Windsor, Ontario, Canada

10:20 a.m. "Technical Highlights of Specimen Banking Program in the Federal Republic of Germany"

Fritz H. Kemper, University of Münster, Münster, Federal Republic of Germany

Markus Stoeppler, Kernforshungsanlage, Jülich, Federal Republic of Germany

11:10 a.m. Coffee Break

11:30 a.m. "Specimen Banking and Technology"

Edward M. Chait, E. I. du Pont de Nemours and Company, Inc., Wilmington, DE

12:00 noon "Specimen Banking, Quality Assurance, and Reference Materials"

Harry S. Hertz, Center for Analytical Chemistry, NBS

1:00 p.m. Lunch, Senior Lunch Club

Plenary Lectures

Session Chairman: Curt W. Reimann, National Measurement Laboratory, NBS

1:45 p.m.	"The Role of Specimen Banking in the Evaluation and Management of Toxic Chemicals" Robert A. Lewis, University of Saarland, Saarbrücken, Federal Republic of Germany
2:15 p.m.	"Status of the Pilot Specimen Banking Program in the U.S." Stephen A. Wise, Organic Analytical Research Division, NBS
2:45 p.m.	"Specimen Banking in Canada" John E. Elliott, National Wildlife Research Centre, Ottawa, Canada
3:15 p.m. - 3:45 p.m.	Concluding Remarks/Discussion
3:45 p.m. - 5:00 p.m.	Visit NBS Specimen Bank Facility

APPENDIX II

LIST OF PARTICIPANTS

Eighth U.S.-German Seminar of State and Planning on
Environmental Specimen Banking

and

International Review of Environmental Specimen Banking

National Bureau of Standards
Gaithersburg, Maryland

September 19-21, 1983

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In September 1983, the "8th U. S. - German Seminar of State and Planning on Environmental Specimen Banking" and the "International Review of Environmental Specimen Banking" were held at the National Bureau of Standards. At these meetings the current status of Environmental Specimen Banking Program in the U. S., Federal Republic of Germany (FRG), and other countries was presented and discussed. This publication contains a brief summary of these meetings and separate contributions describing the specimen banking activities in Canada, FRG, Japan, Sweden and the U. S.

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