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Project Summary

Pilot Program for the National Environmental Specimen Bank—Phase I

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This work was performed under a joint NBS/EPA research program to develop state-of-the-art protocols for the sampling, storage, and analysis of biological and environmental-type matrices. This report summarizes the procedures used in the initial phase of a pilot program for determining the feasibility of the National Environmental Specimen Bank. A special clean-laboratory/storage facility has been designed and completed for use in this program. Detailed protocols for sampling, storage, and analysis of human liver samples have been designed for this study. The implementation of these protocols for human liver samples is described in this report.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

In response to the growing concern for the potential dangers to human health and the environment by the increasing influx of man-made substances in our ecosystem, the U.S. Environmental Protection Agency (EPA) and the National Bureau of Standards (NBS) are currently studying the feasibility of establishing a National Environmental Specimen Bank (NESB). The program for such a bank would incorporate a well-defined system of collection, analysis, and long-term storage of selected environmental samples to provide (1) real-time monitoring data for pollutant trend analysis and (2) properly collected and stored samples for retrospective analysis.

The National Bureau of Standards is currently involved in a Pilot Environmental Specimen Bank Program which will provide actual working experience in all stages of the banking effort: specimen collection, processing, storage, analysis, and data management. The purposes of this pilot study are: (1) to develop analytical protocols for sampling and storage of four types of environmental samples, (2) to improve analytical methodologies for both trace elements and trace organics, (3) to evaluate the feasibility of long-term specimen storage at various conditions (-25°C, -80°C, -120°C, and freeze-dried at room temperature), and (4) to provide a "bank" of samples for retrospective analysis in future years as analytical methodologies improve. The experience gained in this pilot study will be used to evaluate the feasibility of the NESB Program. The rationale for this specimen banking program, a review of the NBS activities relating to this program, and an outline of the proposed pilot specimen bank study at NBS have been described previously. In these previous reports, the preliminary plans for sample collection and storage were reviewed. During this

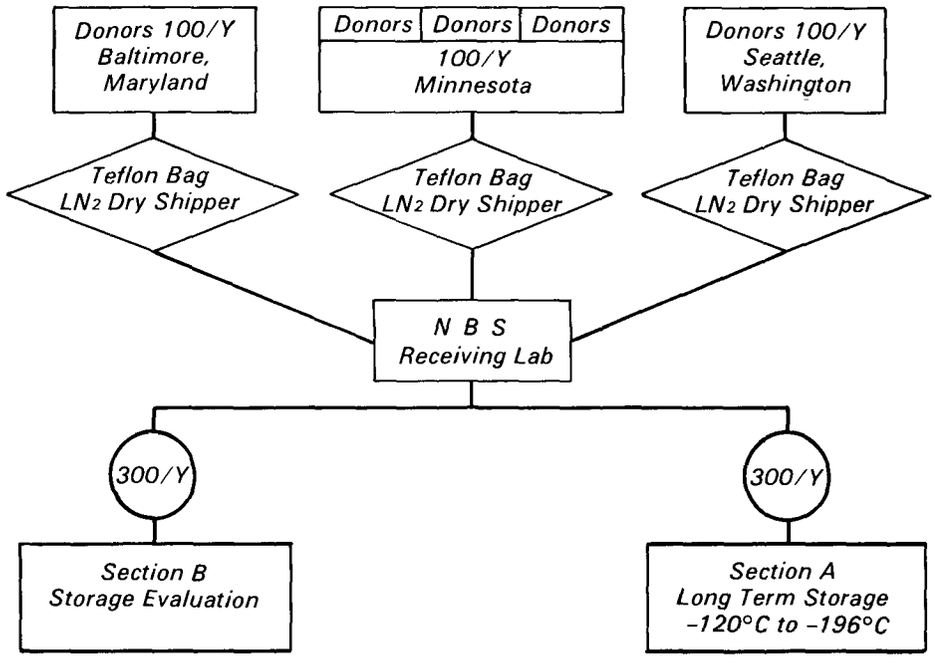

Figure 1. *Sample collection scheme for human livers.*

initial year of the proposed pilot study, the collection, processing, storage evaluation, and trace element analysis of the first environmental specimens, human livers, were implemented. These activities are described in detail in this report. In addition, the design of the clean laboratory/storage facility is discussed.

Collection of Liver Samples

Contracts for procurement of liver samples were negotiated and awarded to three medical schools located in Baltimore, Maryland; Minneapolis, Minnesota; and Seattle, Washington. Each contractor is providing 100 liver samples this year for a total of 300 samples (see Figure 1).

Due to the extremely low levels of trace elements and organic pollutants in human liver tissue, the analytical chemist must exercise extreme caution to avoid possible contamination of the sample. (The lack of contamination control has made existing banks of samples useless for general trace constituent analyses.) A detailed sampling protocol, designed from the viewpoint of analytical chemists, is utilized to minimize contamination. The written sampling protocol outlines in detail the case selection criteria and the steps to be followed by the contractor in obtaining a "valid" sample. The implementation of this protocol is a result of extensive interaction of NBS personnel with the contractors to insure the quality of the samples.

The individual collecting the liver samples uses (1) non-talced gloves, (2) a special titanium-bladed knife for bisecting the liver (to avoid contamination from other elements, i.e., Cr, Ni, etc.), (3) Teflon sheets upon which the sample is placed, and (4) high-purity water to rinse the sample. The samples are then sealed in Teflon bags, frozen at liquid nitrogen temperature, and shipped to NBS. A data form, sent with each liver sample, contains information about the donor and specimen, e.g., date of birth, sex, residence, ethnic group, height, weight, smoker, occupation (if known), diagnosis of autopsy, date and time of death and autopsy, and weight of sample. Approximately 100 samples have been received and stored in the pilot bank facility at NBS.

The schemes for the collection and allocation of subsamples for storage evaluation are shown in Figures 1 and 2. The liver specimens are received as duplicate sections of the left lobe identified as sections "A" and "B." All "A" sections are placed in liquid nitrogen freezers for long-term storage, and the "B" sections are used for storage evaluation, i.e., real-time analysis and long-term storage evaluation at different conditions. Approximately 10 percent of the samples will be homogenized using a cryogenic brittle fracture technique. This homogenization provides about 20 aliquots of 5-8 g which are stored in Teflon jars. To solve the question of

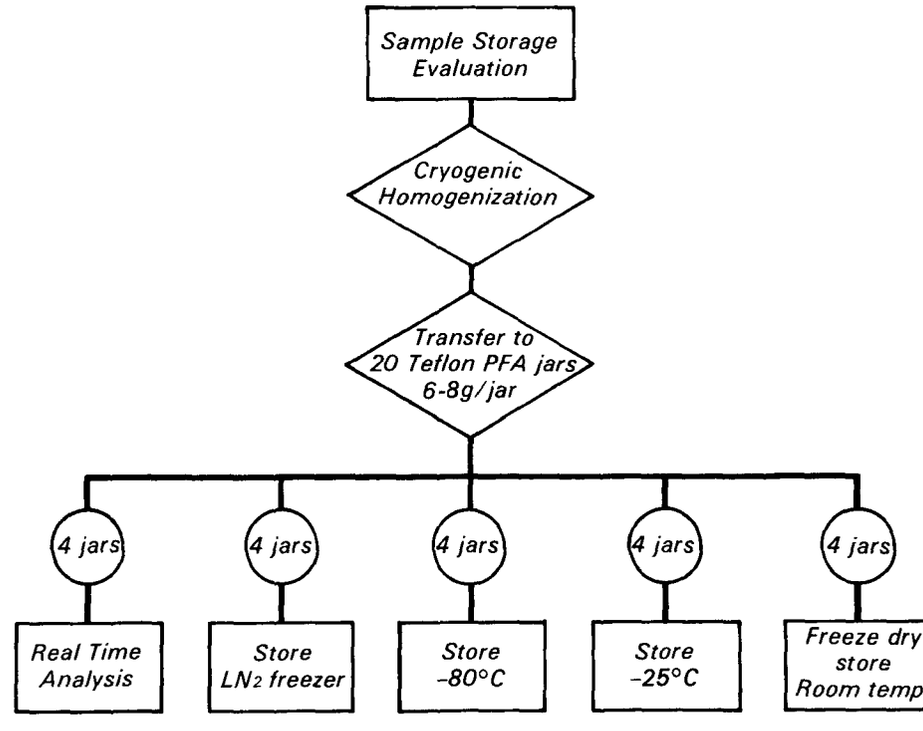

Figure 2. *Allocation of subsamples for storage evaluation.*

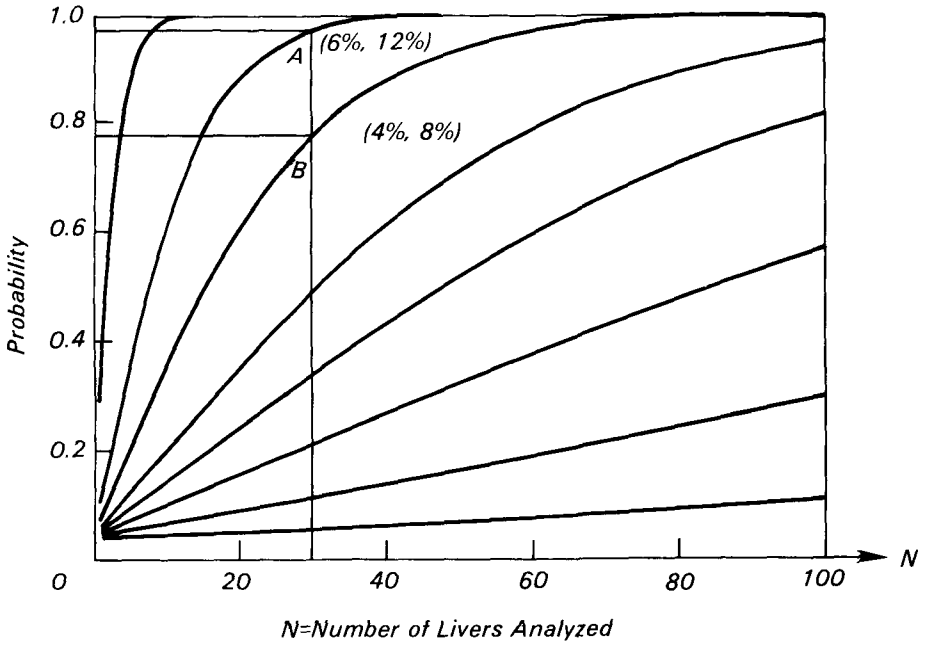

Figure 3. Probability of detecting change in the concentration due to storage condition.

appropriate temperature for sample storage, these sample aliquots will be stored under four different conditions: freeze-dried at room temperature, frozen at -25°C, at -80°C, and in liquid nitrogen vapor at -120°C to -190°C. The concentrations of trace constituents found after storage under those conditions will be compared to the data found in the "real-time" analysis of each specimen (Figure 2). The "A" sections are reference samples which may be used to re-evaluate results on a particular "B" section. At the end of the pilot program, the "A" sections stored at liquid nitrogen temperatures will represent a valuable bank of validated samples available to the scientific community. In addition, a large quantity of data, from the analyses of the "B" sections, will be available on many of these samples.

The success of the NESB will be determined in a large part by the ability to preserve the integrity of the trace substances (i.e., organic, inorganic, and organometallic species) in samples during long-term storage. Changes in the forms and concentrations of the numerous environmentally important substances in specimens stored for extended periods may occur in several ways. Processes such as surface adsorption and sample degradation may reduce the concentrations of various components. In addition, continued biochemical and enzymatic activity may produce species which may not have been present in the original sample. On the other hand, contamination of the specimen fluids could lead to apparent increases in trace substances' concentrations. Superimposed on these processes are the factors which will affect the process rates, such as container material, contact time and area, storage temperature, pH, and initial species concentration. All of these factors are important considerations in evaluating the suitability of long-term storage.

The probability for the discovery of changes in the concentrations of trace constituents during storage has been assessed by statistical treatment of the expected experimental parameters, e.g., number of specimens analyzed, homogeneity of subsamples, and analytical error (K. R. Eberhardt and L. DeRobertis, Center for Applied Mathematics, Statistical Engineering Division, NBS). Figure 3 consists of a series of curves generated from the experimental parameters of 5 percent inhomogeneity and 5 percent analytical error. These curves determine the probability of detecting a particular percent change in concentration (e.g., 6 and 4 percent for curves 1.0 and 0.7, respectively) for a given number of liver samples analyzed. For example, we would have a 98 percent probability of detecting a 6 percent change (Point A) and 78 percent probability of detecting a 4 percent change in concentration (Point B) if 30 livers are analyzed. If the sample inhomogeneity or analytical error increased to 10 instead of 5 percent, the percent change detectable at Points A and B would be 12 and 8 percent, respectively. Based on these curves, the number of samples to be analyzed was set at 30. Analyses of a smaller number of samples would significantly reduce the probabilities of detecting small changes, whereas increasing the number of samples ana-

Table 1. *Number of Livers to be Analyzed for Storage Evaluation Study*

Year		*80*	*81*	*82*	*83*	*84*
Real Time Analysis		*36*	*30*	*30*	*30*	
1 Year Storage	LN_2		*12*			*30*
	-85°C	*Samples*	*12*		*Samples*	*30*
	-25°C	*from '80*	*12*		*from '83*	*30*
	RT		*12*			*30*
2 Years Storage	LN_2					*30*
	-85°C				*Samples*	*30*
	-25°C				*from '82*	*30*
	RT					*30*
3 Years Storage	*LN:*					*30*
	-85°C				*Samples*	*30*
	-25°C				*from '81*	*30*
	RT					*30*
4 Years Storage	LN_2					*24*
	-85°C				*Samples*	*24*
	-25°C				*from '80*	*24*
	RT					*24*
Livers Analyzed/Year		*36*	*78*	*30*	*30*	*456*

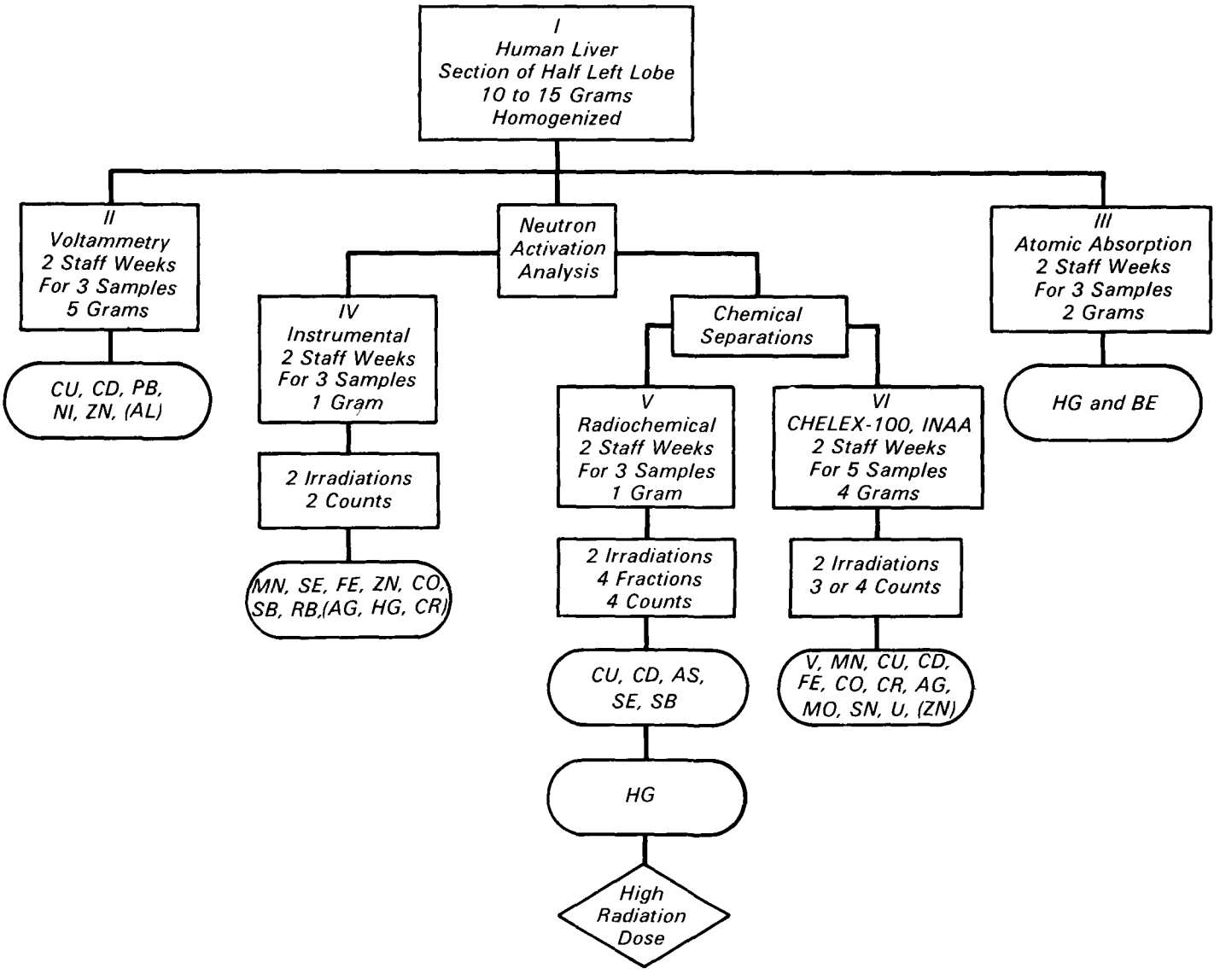

Figure 4. *Analytical scheme for the determination of trace elements in human livers.*

lyzed would not increase greatly the probability of detecting changes.

The 30 specimens used for the storage evaluation represent approximately 10 percent of the specimens collected per year. A major limitation to this number is the present analytical capacity. In order not to exceed the currently available analytical capability, most of the analytical work is deferred to the year 1984, when the program should have a larger analytical capacity. For the evaluation of the storage of human livers, Table 1 gives the analytical needs in terms of numbers of livers to be analyzed each year.

Trace Element and Trace Organic Analysis

The trace element analysis activities of the specimen bank project have concentrated on three main tasks this year: (1) development of a comprehensive analytical scheme, (2) evaluation of the sample collection protocols, and (3) initiation of the analysis of the first year's collected samples.

The current analytical protocol is directed toward the analysis of most of the first-priority elements proposed by the International Workshop on Monitoring Environmental Materials and Specimen Banking. Additional elements occurring as biological major elements and trace elements in the samples are included in the protocol because their levels might supply information about the "normal" state of the specimen. The elements under consideration, their priority, and the analytical techniques to be used appear in Table 2. The analytical scheme (Figure 4) incorporates the NBS Center for Analytical Chemistry approach of multi-technique analysis for quality control. The first three techniques listed, i.e., atomic absorption spectroscopy (AAS), anodic stripping voltammetry (ASV) or linear sweep voltammetry

(LSV), and instrumental neutron activation analysis (INAA), comprise the current routine analytical scheme. Radiochemical neutron activation analysis (RNAA) will be performed on a limited number of samples to provide multi-technique quality control. According to the proposed storage evaluation scheme, 36 livers are being analyzed during the first year of sample collection.

Analytical methods for the analysis of two classes of organic compounds, i.e., organochlorine pesticides and polycyclic aromatic hydrocarbons (PAH) are currently being developed. Preliminary results have been obtained using the following analytical procedures: (1) extraction with acetonitrile, (2) liquid-liquid partition into hexane, (3) concentration by evaporation, (4) high-performance liquid chromatography (HPLC) to isolate the compounds of interest, and (5) gas chromatography (GC) analysis on capillary columns with selective electron capture detection. An HPLC method, using a chemically bonded amine column has been developed to isolate the organochlorine pesticides and PAH from interfering organic constituents. GC methods have been developed for the determination of the 16 pesticides on the priority pollutant list. Research is continuing on the methodology for quantitation of these compounds in liver samples in order to initiate real-time analyses for organics in 1981. Future plans include (1) the evaluation of homogeneity for organics, (2) the development of procedures for comparison of organochlorine content determined by GC and determined by neutron activation analysis, and (3) expansion of the pilot program to include development of analytical protocols for the mussel/oyster samples.

Safety Requirements

The handling of human liver samples carries the potential risk of exposure to infectious diseases, particularly hepatitis B virus or "serum hepatitis." As a result, certain precautions are necessary to insure protection of the analysts involved in sample processing and analysis.

To eliminate potentially infectious liver specimens from the specimen bank samples, a blood sample is removed from the donor at the time of autopsy to be used for hepatitis B screening. In addition, liver specimens from the right lobe are removed for preparation of histological slides. These slides are examined by the pathologists for evidence of infectious diseases. Liver samples are placed in temporary storage at liquid nitrogen temperatures at NBS until the results of the screening are received from the hospitals supplying the livers.

A Safety Advisory Committee has been established to advise the Center for Analytical Chemistry concerning suitable safety precautions for the handling and processing of these liver samples at NBS. Recommendations were made by the committee regarding procedures to safeguard personnel. The Safety Advisory Committee will be requested to review annually the procedures of the pilot specimen bank program.

Table 2. *Analysis of Trace Elements in Human Liver*

Element	*Concentration (μg/g)*[a]	*Analytical Method*[b]	*Category*[c]
Be	*ND*	*AAS*	*1*
F	*0.06 - 1.4*		*1*
Al	*1.6 - 2.6*	*ASV/LSV*	*2*
V	*< 0.007- 0.09*	*R*	*1*
Cr	*0.005- 0.27*	*INAA*	*1*
Mn	*0.5 - 1.9*	*INAA*	*1*
Fe	*70 -210*	*INAA*	*2*
Co	*0.017- 0.16*	*INAA*	*1*
Ni	*0.009- 0.32*	*ASV/LSV*	*1*
Cu	*3.2 - 14.7*	*ASV/LSV, RNAA*	*3*
Zn	*31 - 80*	*ASV/LSV, INAA*	*1*
As	*0.006- 0.46*	*RNAA*	*1*
Se	*0.097- 0.68*	*INAA, RNAA*	*1*
Rb	*7 - 12*	*INAA*	*2*
Sr	*0.01*		*3*
Mo	*0.4 - 1.6*	*RNAA, INAA*	*1*
Pd	*ND*		*1*
Ag	*0.006- 0.07*	*INAA*	*2*
Cd	*0.5 - 4.9*	*ASV/LSV, RNAA*	*1*
Sn	*0.08 - 0.65*	*R*	*1*
Sb	*0.01*	*INAA, RNAA*	*2*
Ba	*0.01*		*3*
Pt	*ND*	*R*	*3*
Hg	*0.005- 0.25*	*AAS*	*1*
Tl	*0.001- 0.009*	*R*	*1*
Pb	*0.8 - 2.3*	*ASV/LSV*	*1*

[a] *Fresh weight, range of concentrations from reference 4.*

[b] *Techniques used at NBS for Pilot Specimen Bank Program:*
AAS = atomic absorption spectroscopy,
ASV/LSV = anodic skipping voltammetry/linear sweep voltammetry,
INAA = instrumental neutron activation analysis,
RNAA = radiochemical neutron activation analysis.

[c] *1) First priority element (3);*
2) Trace elements available with applied methods to monitor "normal" body concentrations for non-pollutants.
3) Trace elements of environmental importance not included in first priority list.
R = Research initiated ND = No data available

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***George M. Goldstein** is the EPA Project Officer (see below).*

The complete report, entitled "Pilot Program for the National Environmental Specimen Bank—Phase I," (Order No. PB 81-173 320; Cost: $8.00, subject to change) will be available only from:

National Technical Information Service
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