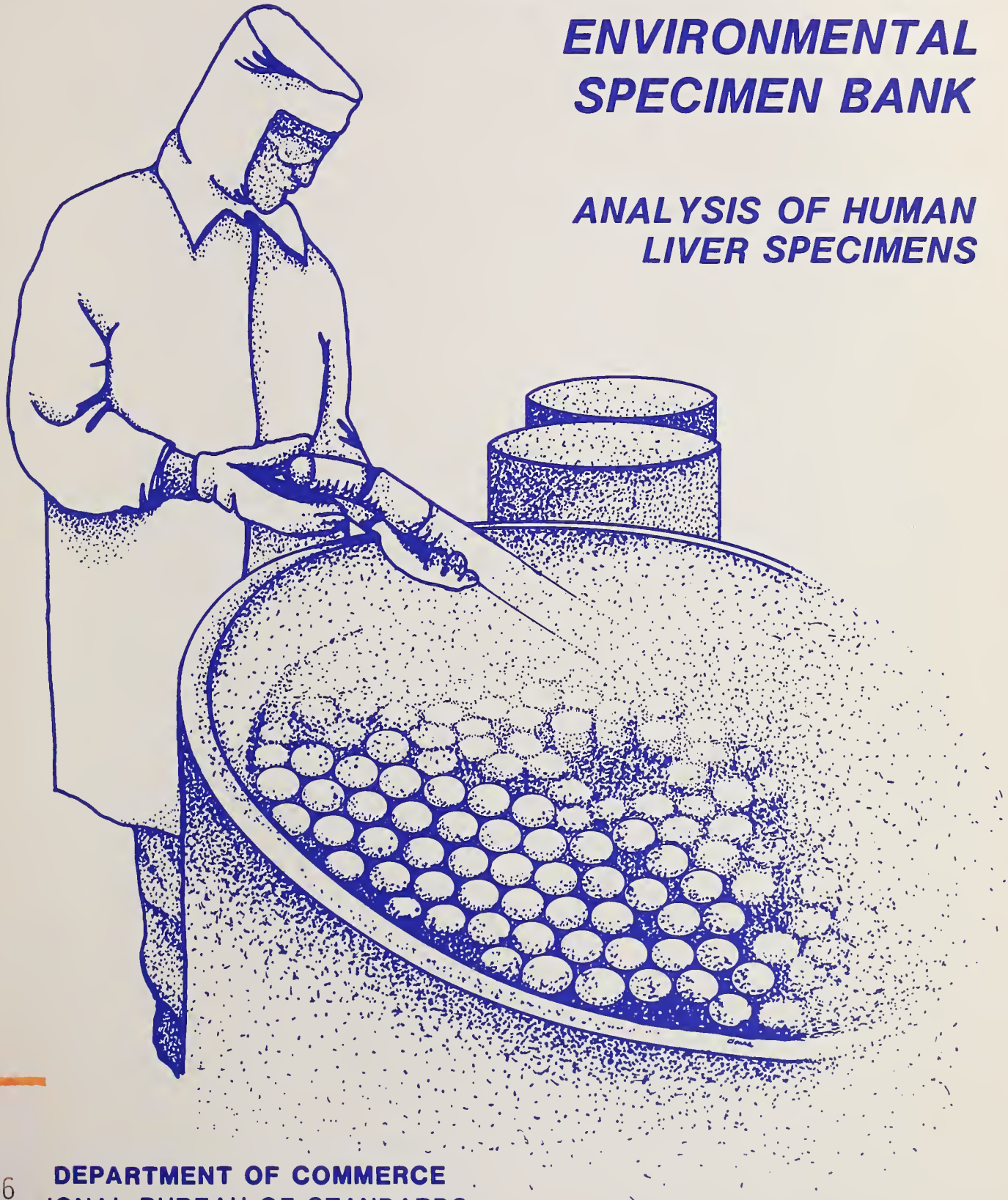
NBS SPECIAL PUBLICATION 656

THE PILOT NATIONAL ENVIRONMENTAL SPECIMEN BANK

ANALYSIS OF HUMAN LIVER SPECIMENS



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The Pilot National Environmental Specimen Bank —
Analysis of Human Liver Specimens

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Foreword

In 1979, the Environmental Protection Agency and the National Bureau of Standards completed construction on a Pilot National Environmental Specimen Bank at NBS. This pilot program was initiated to (1) develop and evaluate protocols for contamination-free sampling of environmental specimens; (2) develop analytical methods for the trace analysis of inorganic and organic pollutants; (3) develop and evaluate storage conditions which would permit the long-term storage of samples without change in pollutant concentrations; and (4) evaluate a specimen bank as a means of storing samples for pollutant trend monitoring over time and permitting retrospective analyses as concerns for new pollutants arise and as new analytical techniques are developed.

In 1975, an international agreement to share knowledge and samples between the United States and the Federal Republic of Germany was instituted and in 1981, a pilot environmental specimen bank was initiated in Germany. This international collaboration has led to a fruitful exchange of ideas and analytical intercomparisons which have demonstrated the comparability of data on an international scale.

The report which follows summarizes several years of careful research and lays the groundwork for evaluation of specimen banking as a viable long-term mechanism for evaluating environmental pollutants and monitoring pollution in our biosphere. In addition to the obvious benefits which arise from meeting the goals outlined above, there are several additional and noteworthy benefits which have accrued from the pilot program. It is this sum total of knowledge gained that should be evaluated in judging the concept of specimen banking. The sampling, storage, and analysis protocols developed when coupled with the statistical treatment of the data, have given added insight into quality assurance mechanisms for environmental analyses. It is our opinion that improved data quality through careful research is of itself a major benefit to the complex field of pollutant analyses. The protocols developed for sample collection and storage in a contamination-free mode yield benefits that go well beyond the sampling of human livers for the pilot specimen bank; the lessons learned are equally applicable to any occurrence which would require contamination-free analysis for impact/hazard assessment. Furthermore, experience gained through this pilot program is a valuable lesson in the development of a reference laboratory capable of performing high priority, problem-solving analyses when a calamity has occurred and capable of serving as an advisory laboratory for field analyses performed on a continuing basis.

Finally, with the pilot experience at its current state of maturity, one can envision the broad applicability of the specimen banking concept to real-time and trend monitoring. Beyond the concerns of environmental pollution the concept is equally valid to monitoring nutritional status, occupational exposure and key chemicals related to health state of a populace. It is with all these aims and benefits in mind that the concept of a full-scale specimen bank needs to be evaluated by the American scientific community.

Harry S. Hertz
Acting Director
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ABSTRACT

This work was performed under a joint EPA/NBS research program to develop state-of-the-art protocols for the sampling, storage, and analysis of biological and environmental-type matrices as part of a Pilot National Environmental Specimen Bank program. The purpose of this report is to summarize the experience and results obtained for the first sample type, human liver. The sample collection protocol was evaluated with respect to costs and suitability of donor selection criteria. An analytical protocol was implemented for the determination of trace elements in the liver samples using the techniques of atomic absorption spectrometry, isotope dilution mass spectrometry, neutron activation analysis, and voltammetry. Individual sections of this report describe in detail the procedures used and the results obtained for each of these analytical techniques. The analytical results for the determination of 31 trace elements in 36 liver specimens are presented and discussed. Analytical methodology for the measurement of organochlorine compounds is also described.

This report is submitted in partial fulfillment of EPA Interagency Agreement EPA-81-D-X0105 by the National Bureau of Standards.

Key words: atomic absorption spectrometry; cryogenic homogenization; gas chromatography; human liver; inorganic analysis; isotope dilution mass spectrometry; neutron activation analysis; organic analysis; organochlorine pesticides; specimen banking; trace elements; voltammetry.

DISCLAIMER

Certain commercial equipment, instruments, or materials are identified in this Report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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SECTION 1

THE NATIONAL ENVIRONMENTAL SPECIMEN BANK — BACKGROUND AND HISTORY

by

S. A. Wise

The concept of environmental specimen banking for archiving and retrospective analysis of biological and environmental samples has been recognized recently as an important component of systematic environmental monitoring. Participants at three international workshops, held in 1977, 1978, and 1982 issued recommendations for the establishment of environmental monitoring and specimen banking programs (1-3). The historical development during the 1970's of the concept of a National Environmental Specimen Bank (NESB) in the United States has been reviewed previously by Goldstein (4,5). The history of the Environmental Specimen Bank in the U.S. 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. The purpose of such a national system would be [1] to detect changes in the environment on a real-time basis using bioaccumulators as indicators, (i.e., monitoring) to assess the effectiveness of environmental control measures, [2] to distinguish such changes from natural inputs, and [3] to provide a "bank" of well preserved and documented environmental samples for retrospective analyses in future years as analytical techniques improve or as new pollutants are identified.

Since 1975 the National Bureau of Standards (NBS), in conjunction with EPA, has been involved in research relating to the establishment of a National Environmental Specimen Bank. The initial plans and preliminary results of the evaluation of methodology for the NESB have been described previously (6-11). At an EPA/NBS Workshop on "Recommendations and Conclusions on the National Environmental Specimen Bank" held in 1976 (6), a pilot NESB program was proposed. 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, analysis, and data management. The major goals of this pilot study were: [1] to develop analytical protocols for sampling, processing, and storage of four types of environmental accumulators; [2] to evaluate and improve analytical methods for the determination of both trace element and organic pollutants in biological matrices; [3] to establish baseline data on selected environmental specimens; [4] to evaluate the feasibility of long-term storage at various conditions; and [5] to provide a "bank" of samples for retrospective analyses

TABLE 1. HISTORY 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	Collection and Analysis of Mussel Samples Initiated
	International Workshop on "Environmental Specimen Banking and Monitoring as Related to Banking" held in Saarbrucken, Germany

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

in future years. The experience gained during the pilot study will be necessary to evaluate the feasibility of establishing a National (or International) Environmental Specimen Bank.

Four types of environmental specimens, which represent environmental accumulators, were selected for inclusion in the NBS pilot program as a result of the EPA/NBS Workshop on "Recommendations and Conclusions on the National Environmental Specimen Bank". These sample types and specific samples representative of the sample type are: [1] human soft tissue — liver, [2] aquatic accumulator — marine mussels, [3] food accumulator — grain or milk, [4] air pollutant accumulator — moss, lichen, or air particulate filters. Human liver was selected as the first sample type for inclusion in the pilot

specimen banking program. As each new sample type is incorporated into the program, collection, storage, and analysis of the previous sample type(s) will continue. The number of sample types and samples was 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. In November, 1979 a special "clean" laboratory/ storage facility (12) was completed at NBS to initiate the Pilot National Environmental Specimen Bank program. Since 1980, NBS has been involved in the sampling, sample processing, storage, and analysis of the first two sample types, human liver and mussels. In this report the experience gained by NBS during this pilot program relating to sample collection, processing, and storage of human liver samples is described.

At the "International Workshop on Monitoring Environmental Materials and Specimen Banking" held in 1978 in Berlin, a major conclusion and recommendation was that "Pilot specimen banks should be established as soon as possible in order to study the problems of organizing long term storage in banking programs" (Ref. 2, p. 1). The joint U.S. EPA/NBS Pilot National Environmental Specimen Bank is an example of the implementation of such recommendations.

Since 1975, EPA/NBS and the Environmental Agency of the Federal Republic of Germany (FRG) have been pursuing collaborative research in the area of environmental specimen banking as part of a bilateral agreement on the environment. In 1981 the Pilot Environmental Specimen Bank Facility for the FRG was completed. The activities of the U.S. EPA/NBS pilot program are being coordinated closely with the efforts of the German Pilot Environmental Specimen Bank Program (13-15). Participants of both programs meet annually to exchange information and experience for the benefit of both programs. Samples have also been exchanged between the two programs for interlaboratory comparison purposes.

Through the EPA/NBS pilot program, the U.S. is looked upon as a leader in environmental specimen banking research. In recent years interest in specimen banking has increased and several other nations (e.g., Japan and Canada) have initiated specimen banking programs.

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SECTION 2

EVALUATION OF THE SAMPLING AND STORAGE PROTOCOL FOR HUMAN LIVER SAMPLES

by

K. A. Fitzpatrick, S. H. Harrison, R. Zeisler, and S. A. Wise

A major goal of the Pilot National Environmental Specimen Bank (NESB) Program is the development of protocols for the sampling, processing, and storage of four types of environmental samples. During the past three years (1980-1982) the pilot NESB program has been involved in the collection, processing, storage, and analysis of the first sample type, human liver. In this section the experience gained during this program relating to the collection and storage of the human liver samples will be described. This experience will provide the basis for evaluating the feasibility of establishing a National Environmental Specimen Bank.

SAMPLE COLLECTION

Sampling Protocol

Due to the extremely low levels of trace element and organic pollutants found in most environmental samples, caution must be exercised during sample collection and sample processing to avoid contamination. A detailed protocol, designed to provide samples suitable for both trace element and organic analysis, has been developed and implemented for the collection of human liver samples. A summary of the liver sampling protocol for the pilot program is given in Appendix A [for the complete detailed protocol see Harrison et al., (1,2)]. This liver sampling protocol was developed in conjunction with those individuals performing the autopsies, and the implementation of the protocol required periods of education and close cooperation to achieve a suitable protocol within the bounds of practicality. Different disciplinary views and the need for careful communication were evident from perceived meanings of such terms as "clean", i.e., interpreted as "sterile" by autopsy personnel vs. "non-contaminated chemically" by analytical chemists.

The sampling protocol was designed to avoid possible contamination of the sample by either inorganic or organic constituents. 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

*Three types of Teflon® material were used: perfluoralkoxy (PFA), tetrafluoroethylene (TFE), and fluorinated ethylene propylene (FEP).

constituents and for low diffusion rates of water (3-5). The protocol specifies the use of such non-contaminating items as dust-free non-talced vinyl gloves, pre-cleaned dust-free Teflon® FEP sheets and bags, high-purity water, and a titanium/Teflon® TFE knife. These items are provided by NBS to each collection site to achieve uniformity in sampling materials. A special titanium-bladed knife with a Teflon® TFE handle was designed and constructed for use during cutting of the specimen. This special knife is 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 a single element which is currently of no environmental or biological interest, i.e., titanium. The use of a titanium knife for removal of human tissues was a recommendation of previous workshops on specimen banking (6,7). The liver samples are sealed in Teflon® FEP bags, frozen in liquid nitrogen (LN_2), and shipped to the pilot NESB facility at NBS in a special biological shipper at LN_2 vapor temperature.

To eliminate potentially infectious liver samples from the specimen bank, a blood sample to be used for hepatitis B screening is removed from the donor at the time of autopsy. In addition, a liver section from the right lobe is removed for preparation of histological slides. These slides are examined by a pathologist for evidence of abnormality and/or infectious diseases and then stored in the specimen bank facility. The liver samples are placed in "temporary" storage in a LN_2 vapor freezer at the bank until the results of the hepatitis screening are available. Any samples found to be infectious are returned to the pathologist for incineration. These precautions are followed to protect the analysts involved in sample processing and analysis and to provide biologically normal livers. Even after this screening, the human liver samples are regarded and handled as potentially infectious. Processing of the liver samples (i.e., sample transfers during homogenization and subsampling) is performed in a biological safety hood.

A data form, provided with each liver sample, contains information about the donor and specimen, e.g., date of birth, sex, residence, ethnic group, height, weight, smoking history, occupation (if known), date and time of death and autopsy, diagnosis of autopsy, and liver specimen weight (see Appendix A and Refs. 1 and 2).

The sampling protocol for human livers developed for the pilot specimen bank program was recently adopted as a model for collection of human tissue at a workshop entitled "Protocols — Mineral/Elemental Analysis of Human Tissues" held in St. Louis, MO (8). The goal of this workshop was to prepare specific protocols for sample selection, collection, preservation, and analysis of human tissues. These protocols were to be published as guidelines for those involved in research on human body burdens. 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" (9).

Sampling Protocol Evaluation

During the first two years of the pilot specimen bank, 350 liver samples from three geographical locations (Baltimore, MD; Minneapolis, MN; and Seattle, WA) were collected using this protocol. During the remaining years

of the pilot study, liver samples will be received at a rate of approximately 100 per year and predominately from one location (Seattle). At the completion of the first year of human liver collection, the sample collection protocol was evaluated with respect to such items as: initial set-up costs for each site, sample procurement costs, transport time, time required for receipt of hepatitis results and histological slides, and suitability of the donor selection criteria.

Sampling Costs

The costs associated with the sample collection are summarized in Table 1. After the initial set-up cost of about $3000 per collection site, the liver samples were obtained at an approximate cost of $250 each during the first year of collection (300 samples total). The major portion of the sampling cost was the sample procurement fee which included such items as: part-time salary for a technician to work with autopsy personnel to obtain samples, fees for hepatitis testing, and miscellaneous supplies (e.g., liquid nitrogen, solvents for cleaning the knife, etc.). The second largest expense was the shipping cost of the biological shipper to and from the collection site. During the second year, the cost of materials and sample procurement increased by about 10 percent; however shipping costs were lower due to the more efficient use of the large shippers (5 samples) rather than the small shippers (1 sample).

TABLE 1. SAMPLE COLLECTION COSTS ($U.S.)

Initial Set-up Costs Per Site

Item	Cost/Site
Biological shipper	
Large (5 samples) or	1125
Small (1 sample)	(290)
Shipping container (large)	375
Knife-titanium blade/Teflon® TFE handle	150
Heat sealing unit	1380
	$3030

Sampling costs (non-reusable items)

Item	Average Cost/Liver Sample
Non-talced vinyl gloves (4 pair)	2.00
Teflon® FEP sheets (3)	6.00
Teflon® FEP bags (4)	8.00
High purity water	3.00
Shipping (to NBS and return, express air service)	46.00
Sample procurement contracts (year 1) (i.e., technician salary, liquid nitrogen, hepatitis testing, and miscellaneous expenses)	180.00
Miscellaneous items (e.g., labels, cardboard containers, etc.)	2.00
	$247.00

Donor Selection Criteria--

The donor selection criteria were designed to provide samples which are representative of a "normal" liver immediately prior to death (see Appendix A for the selection criteria). Livers were excluded which were physically injured, diseased, infected, or chemically abnormal (e.g., drug overdose). Medical examiners' offices rather than hospitals are more likely to have sufficient cases which satisfy these criteria. In all three sampling sites most of the samples were obtained through the medical examiners' office. In Baltimore and Seattle the samples were obtained almost exclusively from medical examiners' offices (100 percent and 96 percent, respectively). At the Minneapolis site, samples were obtained from four different locations including medical examiners' offices and hospital autopsy rooms. The majority of the samples in Minneapolis (60 percent), however, were provided by a research clinic autopsy room.

Regarding donor selection criteria, the sampling experience at one site (Seattle), i.e., total cases available for sampling, types of cases, number of cases rejected, and reasons for rejections, is summarized in Table 2. At this site the major factor for case rejection ($\sim$20 percent of the cases autopsied) was the time requirement that the autopsy be performed within 24 hours after death. An additional 10-20 percent of the cases available were lost because the part-time technician was not available for sampling on afternoons and Saturdays. In Table 3 the percentages of samples obtained from various sources (i.e., cause of death) at each location are summarized. At the Baltimore and Seattle sites where the samples were obtained predominately from medical examiners' cases, the primary sources were accidents, suicides, and homicides. However, at the Minneapolis site the samples provided by the research clinic were primarily from deaths due to natural causes. Legal constraints in the State of Washington prevented the use of any tissue from a probable homicide case (i.e., the body is considered to be evidence). However, at the Baltimore site, where such restrictions do not exist, homicide cases provided a major source of samples (34 percent). Of the 350 samples collected through 1981, 2 percent were rejected because of positive hepatitis results and 4 percent were rejected based on the results of the histological examination. Additional samples (4 percent) were rejected due to lost hepatitis results or no hepatitis test possible. The samples which were rejected because of possible hepatitis contamination were returned to the pathologist for incineration.

TABLE 2. EVALUATION OF CASE SELECTION CRITERIA (SEATTLE)

Total cases in first year = 1290

Samples needed = 100

Samples obtained during first year = 88 (∿7% of total cases available)

Technical reasons for sample rejection

	Case Type	Total Cases	Cause for Rejection	Estimated Percent Rejected
1.	View only (no autopsy)	28%		28%
2.	Homicides	7%	Used for evidence	7%
3.	Natural death	17%	Puncture wounds (medic assistance)	9%
4.	Traffic accidents	18%	Torn, hemorrhage, contaminated	7%
5.	Suicides	9%	Not autopsied	2%
6.	Accidents	21%	Drugs, fire, alcoholics	6%
			Laceration by pathologists/technicians	1%
			Autopsy performed more than 24 hour after death	30-40%
			Technician unavailable during autopsy	

TABLE 3. PERCENTAGE OF HUMAN LIVER SAMPLES FROM VARIOUS SOURCES DURING 1980-1981

	Baltimore	Seattle	Minneapolis	Total
Suicides	13	20	3	13
Homicides	34	0	0	10
Traffic accidents	36	36	12	29
Other accidents (e.g., falls, burns drownings, etc.)	5	15	9	11
Natural causes (e.g., diseases, heart problems, etc.)	3	26	73	33
Miscellaneous	9	3	3	4

The age and sex distribution of the donors for the 350 samples collected during 1980-81 are illustrated in Figure 1. As shown in Figure 1, the age and sex distributions of the donors for the Baltimore and Seattle sites are predominately young and male because these samples were obtained from medical examiner's cases; whereas for the Minneapolis samples which were predominately from a research clinic (60 percent), the majority of the donors were older (>60 years old). For all sites the majority of the donors were male (~76 percent). Thus, the selection criteria and the major source of samples (i.e., medical examiners' offices) tend to provide "abnormal" age/sex distributions of donors when compared to the Census Bureau data on the age/sex distributions of the U.S. population (see Figure 2). Regarding racial information, 83 percent of the total 350 donors were Caucasian, 14 percent were Black, and 3 percent were other (e.g., Hispanic, Oriental, and American Indian). The majority of the non-Caucasian donors were included in the 100 donors from the Baltimore site (54 percent Caucasian and 45 percent black). Both the Seattle and Minneapolis sites provided mainly Caucasian donors (93 and 97 percent, respectively). The collection of a set of samples with age/sex/race distributions representative of the normal population was not the primary goal of the pilot program. However, the sample collection process could be more selective to provide a representative sample set for a NESB. Of the first 350 liver specimens collected, approximately 10 percent were analyzed. Donor information for the 36 samples analyzed is summarized in Table 4. As additional samples are analyzed during the pilot program to provide baseline data (~30/year), a more normal age/sex/race donor profile will be selected.

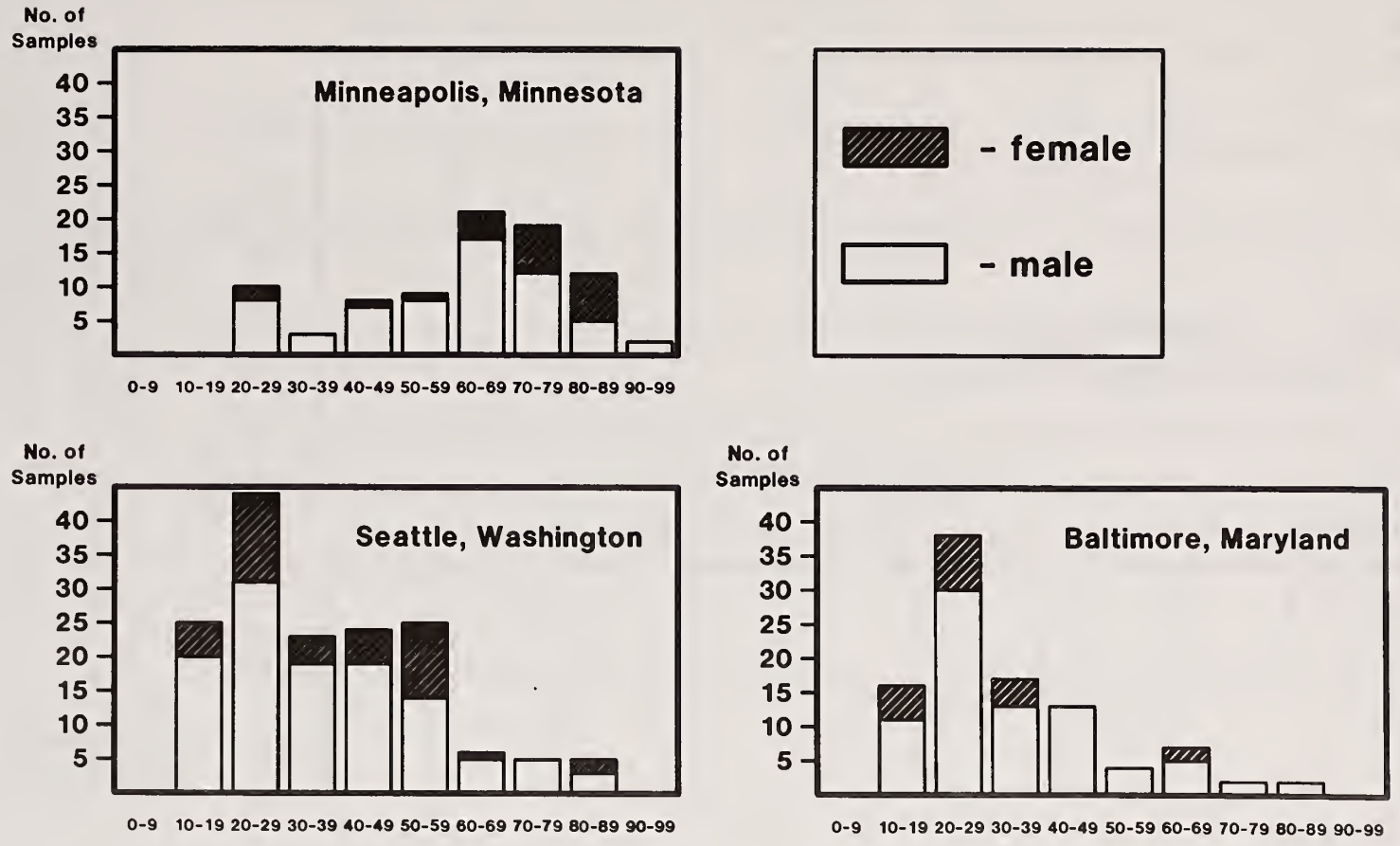

Figure 1. Age and sex distribution of donors for the liver samples collected during 1980-81 in the Pilot National Environmental Specimen Bank Program.

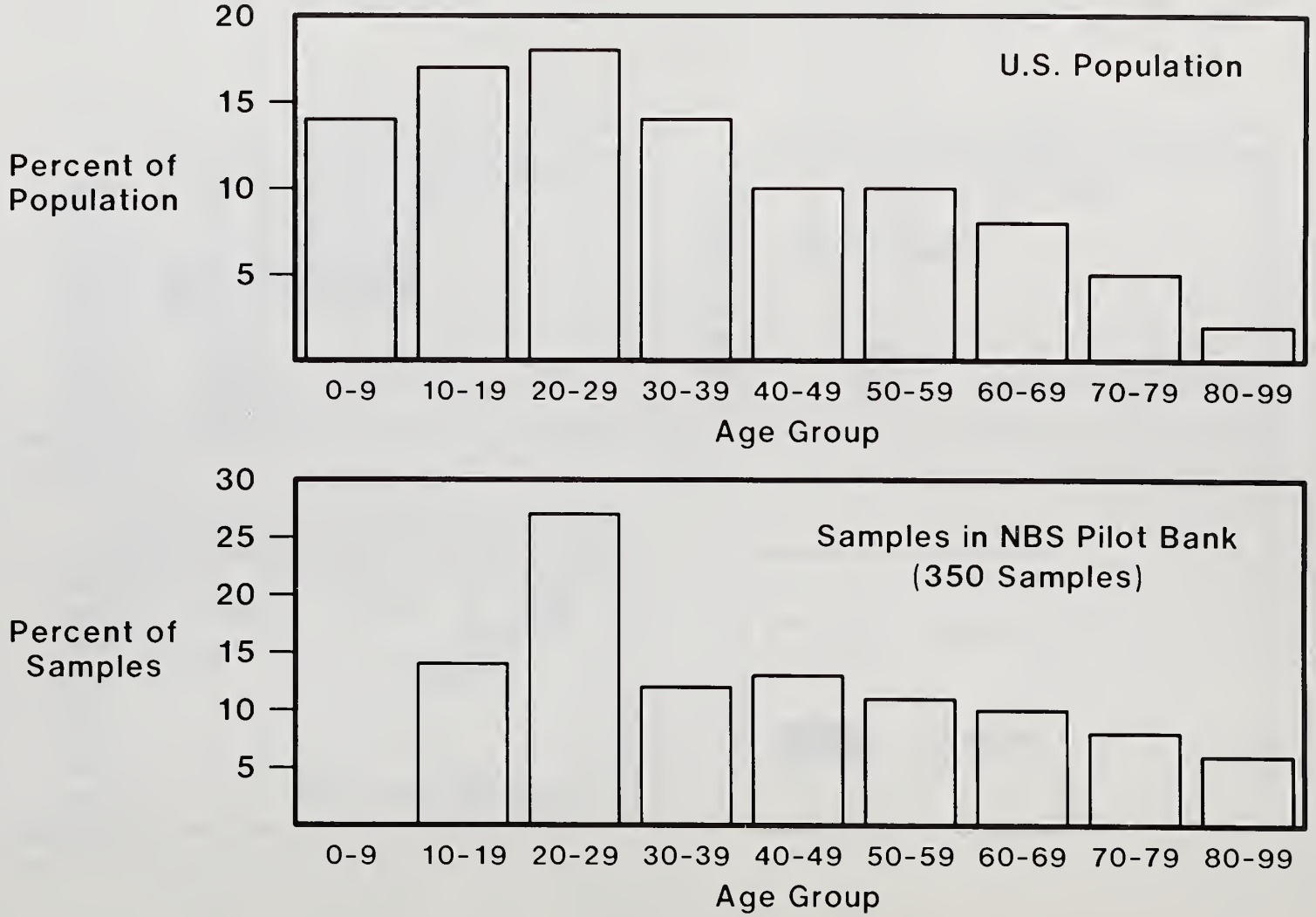

Figure 2. Age distribution of the U.S. population (1980) compared to the donors of the first 378 liver samples in the Pilot National Environmental Specimen Bank Program.

TABLE 4. DONOR AND SAMPLE INFORMATION FOR HUMAN SPECIMENS ANALYZED DURING THE FIRST YEAR OF SAMPLE COLLECTION (1980-81)

Sample Identification No.[a]	Sex[b]	Age	Ethnic Group[c]	Hours Between Death and Autopsy	Smoker[d]	Concentration Factor (dry weight/wet weight)
L1B0002	M	61	4	14.5	2	0.279
L1B0005	M	27	1	8	2	0.302
L1S0018	M	43	4	12.5	2	0.287
L1M0023	F	22	4	8.5	2	0.240
L1S0027	M	52	4	11.5	2	0.226
L1S0037	M	82	4	15.5	0	0.235
L2B0041	M	45	4	13.3	2	0.256
L2B0042	M	17	4	19	2	0.248
L1B0045	F	21	4	7.7	2	0.278
L2M0048	M	17	4	12.0	2	0.230
L2S0049	M	24	4	5.8	2	0.240
L1S0053	M	60	4	11.5	2	0.278
L2M0062	F	84	4	14.3	2	0.221
L1B0074	M	16	1	14.5	2	0.281
L1S0080	M	32	4	15	2	0.230
L1M0083	M	29	4	10	2	0.300
L1S0086	M	19	4	12	2	0.299
L1S0090	M	67	3	10	2	0.253
L1S0091	M	38	4	14.8	1	0.305
L1S0092	M	36	4	21.3	1	0.262
L1B0100	M	66	4	21.5	2	0.302
L1M0105	M	74	4	4.5	0	0.269
L1S0107	M	33	2	21.5	1	0.287
L1S0110	M	75	4	22.8	2	0.280
L1M0118	M	33	1	9	2	0.293
L1M0119	M	49	4	17	2	0.240
L1B0121	M	26	1	12	2	0.257
L1S0123	M	27	4	13.5	2	0.280
L1M0126	M	70	4	11.8	2	0.297
L1M0127	M	86	4	11.8	2	0.217
L1S0128	M	54	4	19	2	0.265
L1S0129	M	69	4	14.5	2	0.279
L1M0130	M	23	4	5	2	0.259
L1M0131	M	56	4	5	2	0.270
L1M0140	M	27	4	20.5	2	0.285
L1M0141	M	69	4	17.5	2	0.206

[a] The third letter/number in the identification no. indicates the geographical location, i.e., B = Baltimore, MD; M = Minneapolis, MN; and S = Seattle, WA.
[b] M = male; F = female.
[c] 1 = Black; 2 = Hispanic; 3 = Oriental; 4 = Caucasian; and 5 = unknown.
[d] 1 = yes; 0 = no; and 2 = unknown.
[e] Concentration factor for conversion of results from dry weight to wet weight basis.

A major conclusion from the experience of the first year of collection was the need for a technician whose primary responsibility was to work closely with autopsy personnel to select, remove, and prepare the liver samples. Since these technicians were responsible for implementing the sampling protocol, NBS personnel worked closely with them to stress the importance of following the protocol precisely. When a sample stored in the specimen bank is analyzed, the researcher must have confidence that the sample was collected exactly as prescribed in the protocol.

STORAGE SCHEME

The storage scheme for the liver samples will be described in detail to illustrate the approach used in the pilot NESB program. This scheme is designed to evaluate the question of appropriate temperature for storage of biological samples and to provide a "bank" of well-characterized reference samples. The liver samples are received at the specimen bank facility as duplicate sections of the left lobe, identified as Sections "A" and "B" (see Figure 3). 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 a special cryogenic homogenization technique (see Section 3) to provide about 20 aliquots per sample of 6-8 g each. The sample remains frozen during homogenization and the sample aliquots are transferred to the Teflon® PFA jars inside a cold nitrogen atmosphere dry box to minimize water condensation on the frozen samples. To investigate the question of the appropriate temperature for long-term storage, these aliquots are stored in Teflon® jars under four different conditions: frozen at -25 °C, at -80 °C, and in liquid nitrogen vapor (-120 °C to -190 °C) and room temperature after freeze-drying. These aliquots will be reanalyzed at one-year intervals during the pilot study and the results compared to the data found in the "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 re-evaluate 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.

The storage area of the NBS pilot facility (see Figure 4), contains three LN_2 vapor freezers (500-L capacity each), four compressor type biological storage freezers (382-L capacity each), and a shelf cabinet for storage at room temperature. The Teflon® packaged liver specimens are received at NBS in cylindrical cardboard tubes (57 mm O.D. x 117 mm) which provide a standard geometry to facilitate easier sample storage. These samples are stored in the LN_2 vapor freezers in larger cylindrical tubes (6.35 cm O.D. x 64.8 cm) with five specimens (sections) in each tube. Each LN_2 vapor freezer will hold a maximum of 170 tubes or 850 sections. In the compressor freezers whole liver sections are stored in trays containing 16 samples each, three trays per shelf, six shelves per freezer for a total of 288 samples per

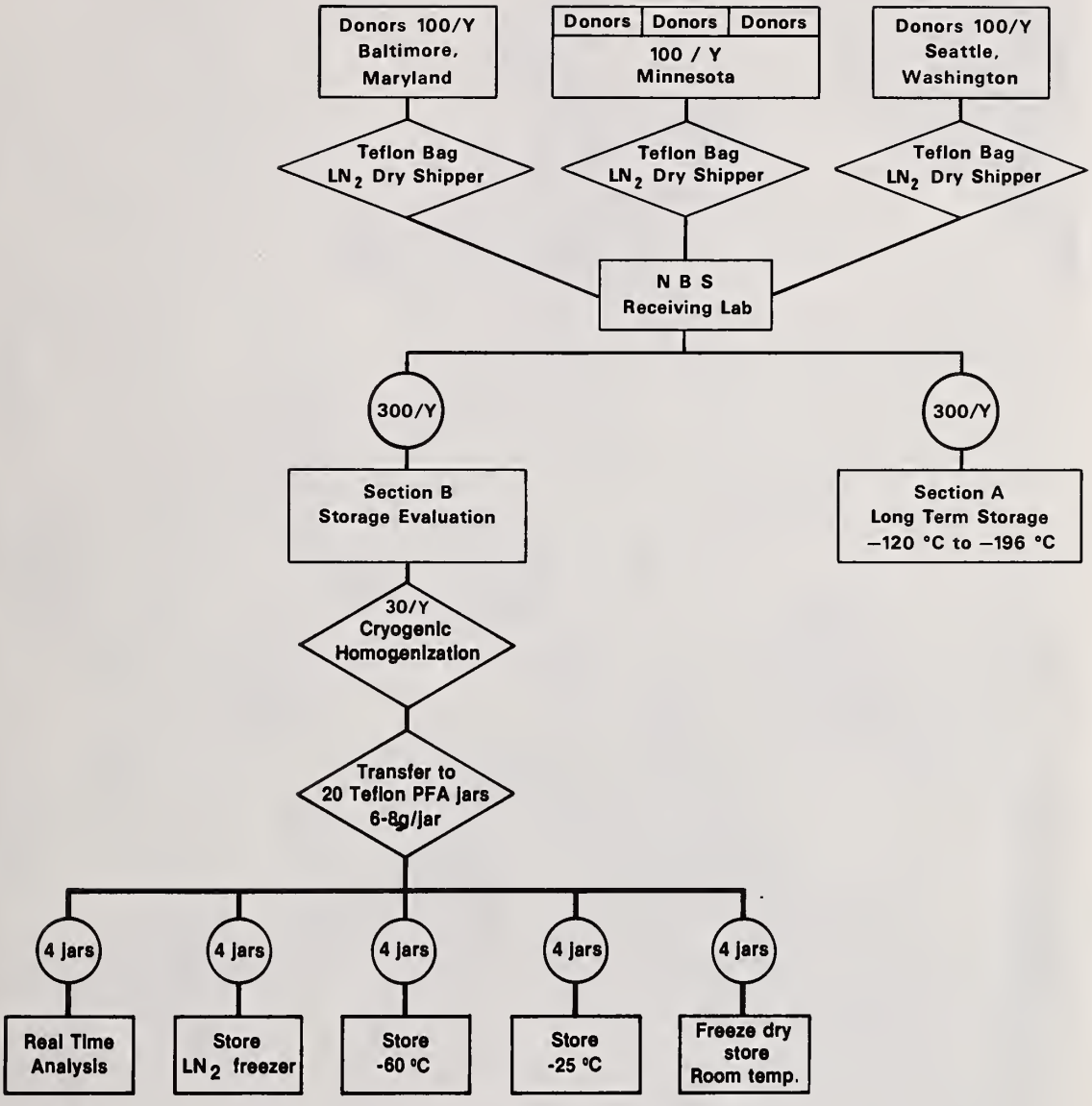

Figure 3. Sampling and storage scheme for human liver samples during the first year.

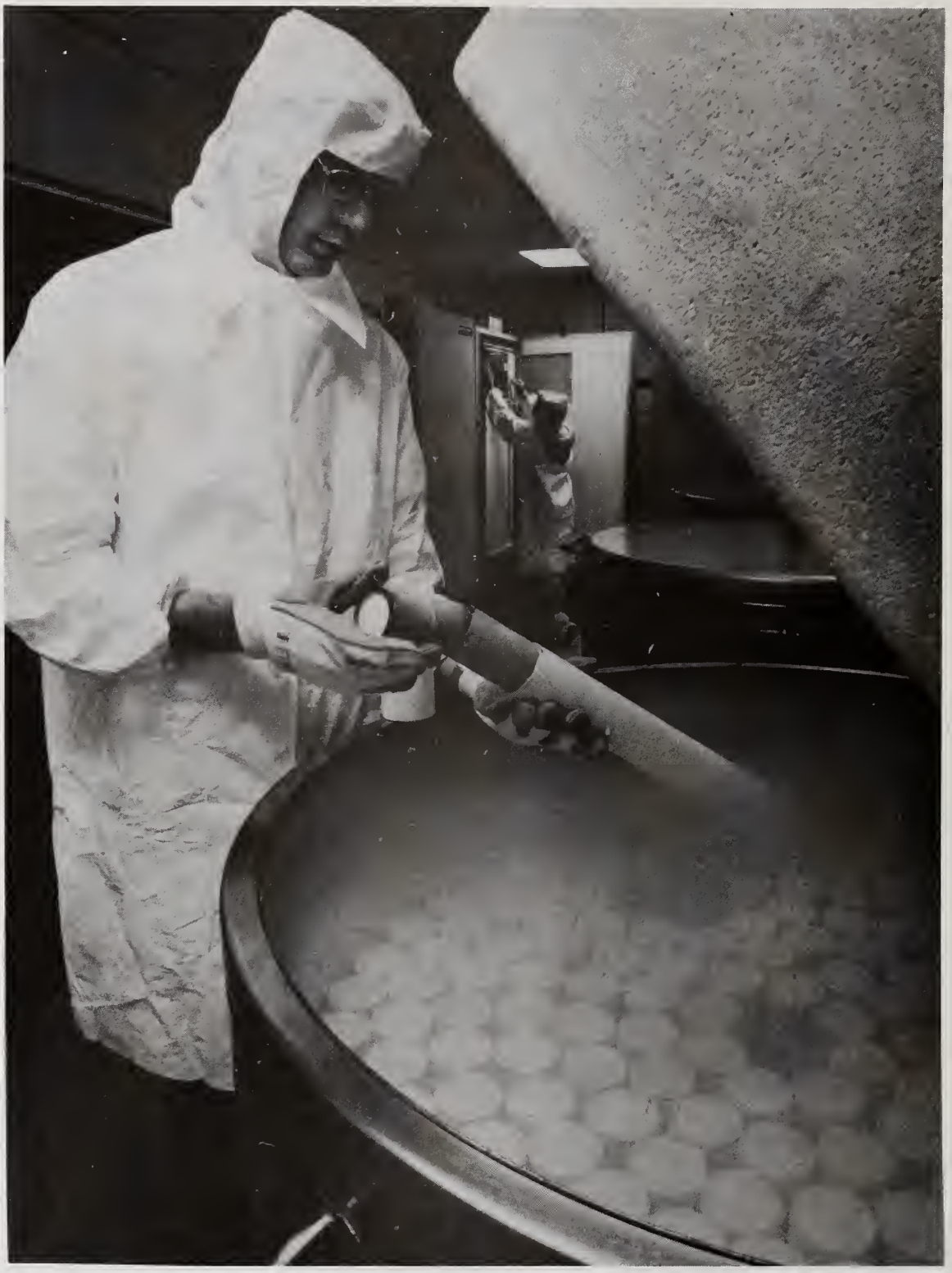

Figure 4. Storage of a liver specimen in the storage facility of the Pilot National Environmental Specimen Bank at the National Bureau of Standards.

freezer. Sample storage location is identified by freezer, shelf, tray, and position (or tube identification for a LN_2 vapor freezer). The homogenized liver subsamples are stored in 15-mL Teflon® PFA jars. The outside surface of the jar lids contains a removable Nomex® Aramid (a synthetic paper) identification label. These jars are stored in square cardboard boxes (140 mm x 140 mm x 50 mm) which are labeled with a three-digit number embossed on the side. These boxes (eight jars/box) are stored in racks in both the LN_2 vapor and compressor freezers. When a sample is placed in the bank, a storage file (presently on hard-copy) is created which includes the following information: sample identification number, storage identification number (describing storage location in the freezers), sample weights, and storage dates (in and out) [see Harrison et al., (1,2)]. Currently, three compressor-type freezers are used for -80 °C storage and one freezer is used for -25 °C storage. Freeze-dried samples are also stored in boxes in an enclosed, dust-free cabinet at room temperature. A cost summary of the major items used in the NBS pilot storage facility and for the sample processing is provided in Table 5.

TABLE 5. SAMPLE PROCESSING/STORAGE COSTS

Sample Processing Costs ($U.S.)	
Cold dry box	2750
Disk mill instrument	5300
Teflon® TFE mill head	1200-1500
Teflon® PFA jars (@3.65 ea.)	365/100 jars
Teflon® TFE scoops (10)	200
Storage Facility Costs	
Liquid nitrogen vapor freezer (4) @ 6320	25,280
Compressor type freezers (4) @ 3853 with LN_2 backup, remote alarm	15,412
Storage shelves, racks, containers	792
Automatic dialing alarm system	2100
Biological safety hood	4100
Histological slide cabinet	495
Dust free cabinet	213
Cardboard tubes	114/100 tubes
Liquid nitrogen per freezer @ $0.10/L	360/year
Oxygen monitor	990
Fuel cells	110/year
LN_2 storage containers (210L) 6 @ 1300	7800

As a safety precaution to protect the stored specimens, the operating temperatures and power supply of the compressor freezers, LN_2 vapor freezers, and storage facility are monitored by an automatic dialing alarm system. This alarm system will dial selected telephone numbers to report a fault when the temperature exceeds a pre-selected limit or the power fails. The compressor freezers are also equipped with a LN_2 back-up system to cool them in the event of an extended power failure.

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SECTION 2

APPENDIX A

Human Liver Sampling Protocol

A. Donor Case Selection Criteria

1. Liver expected to be "normal" and uninjured (lacerated or punctured).

2. Donor has no known history of alcoholism, hepatitis, drug abuse, gross sepsis, long-term disease treatment, tuberculosis, cirrhosis, liver carcinoma, chronic circulatory failure, or congestion.

3. Death was not due to chemical or drug overdose/exposure.

4. Autopsy performed less than 24 hours after death.

5. Donor has not been embalmed or frozen.

6. Liver has a total weight greater than one kilogram.

B. Donor/Sample Information Requested:

1. Autopsy identification number;

2. Date and time of death and autopsy;

3. Place of autopsy and attending pathologist;

4. Date of birth, sex, and ethnic group (e.g., Caucasian, Black, Oriental, etc.);

5. Height and weight of donor;

6. Occupation, diseases, smoking history (if known);

7. Diagnosis of autopsy;

8. Comments regarding any deviations from the prescribed protocol or anything special about the donor/sample.

C. Sample Collection

1. Personnel involved in liver removal/handling should wear the dust-free non-talced vinyl gloves provided by NBS; no smoking is allowed in autopsy room during sample collection.

2. The liver is removed as soon as possible after opening the body and is placed on a clean Teflon® FEP sheet (If liver membrane is ruptured, sample is not acceptable for this pilot program).

3. Liver is rinsed slightly with high purity water to remove blood and surface contamination, allowed to drain, and transferred to a clean Teflon® FEP sheet on the autopsy room scales for weighing.

4. Liver is placed on a Teflon® FEP sheet on a flat surface for dissection. Using the titanium knife, the left and right lobes are separated to obtain a left lobe sample of $\leq$400 g.

5. Using the titanium knife, the left lobe sample is divided into anterior and posterior sections (designated as A and B) of approximately equal size.

6. Each section is placed in a dust-free Teflon® FEP bag, the air is squeezed out, and the bag is then sealed with a heat sealing unit. The sample is weighed and a label containing the autopsy identification number and the sample weight is placed on the bag; the sample is then placed in a second bag and sealed again.

7. The double-bagged samples are placed in the cylindrical cardboard containers provided and immersed in liquid nitrogen for 10 minutes.

8. The liver specimens should be stored in the cryogenic biological shipper for no longer than 10 days before shipping to the pilot facility using 24 hour express package service.

9. A copy of the completed donor information form is sent with the sample.

10. During the autopsy a sample of blood (generally from within the heart) is collected for hepatitis B antigen screening and a liver section is removed from the right lobe for preparation of histological slides.

11. The hepatitis results are reported to NBS as soon as available and the histological slides are sent to NBS with results of the histological examination. Another copy of the donor information form is sent to NBS containing any additional information from the autopsy report.

SECTION 3

CRYOGENIC HOMOGENIZATION OF BIOLOGICAL TISSUES

by

J. K. Langland, S. H. Harrison, B. Kratochvil, and R. Zeisler

INTRODUCTION

A difficult task in the Pilot NESB program is the chemical characterization of a bulk sample, the human liver. Despite the macroscopic homogeneous appearance of human liver, large inhomogeneities have been reported within a single liver when one gram test portions were used (1). The concentrations of essential trace elements may vary by a factor of four, while other trace elements may vary by as much as a factor of 1000 (2). Most analytical techniques use relatively small test portions for the measurements and therefore, the analyst is confronted with this inhomogeneity problem when a precise and accurate characterization of the bulk sample is desired. To obtain trace data on the bulk sample and to make results from different test portions of the same liver equivalent for the purpose of storage and storage evaluation in the pilot program (see reference 3 for a discussion of the storage evaluation scheme), homogenization of the bulk sample is required.

The reduction of the bulk sample to a laboratory sample suitable for the analytical techniques used is a critical step in an analytical measurement. Heterogeneity and contamination of the analytical sample may produce poor results which do not reflect the status of the bulk sample. If no precautions are taken, the uncertainties due to the sample inhomogeneity and the sampling process may become the limiting factor, thus reducing the usefulness of highly precise and accurate analytical techniques. Thus, a major research effort of the Pilot NESB Program has been directed towards the development and evaluation of a system for contamination-free homogenization of soft tissues.

METHODS FOR HOMOGENIZATION OF TISSUE

A variety of devices are in use for particle size reduction and homogenization of tissue, e.g., Waring-type blenders, ball mills, glass tube and pestle grinders, and hydraulic or cutting shear homogenizers. The size reduction step requires mechanical interaction (e.g., cutting, chopping, and grinding) of an implement with the biological matrix. These implements are commonly made from metals (e.g., stainless steel) or from plastic laminates or glass. These implements provide sharp edges and/or high resistance which are necessary to reduce the size of tissue subdivisions. These interactions

can cause severe contamination of the biological material; therefore blenders and similar apparatus have been excluded from use in the Pilot NESB Program. In addition, some types of blenders generate excessive heat which can lead to losses of trace constituents or changes in organic compound structures.

Several years ago Iyengar and Kasperek (4) evaluated the technique of cryogenic homogenization (Brittle Fracture Technique) for relatively small biological samples (5 to 15 g each). Cryogenic homogenization utilizes the principles of milling and grinding of hard materials after the tissues are frozen at temperatures below -140 °C with liquid nitrogen. At this temperature the hardness of all tissues is increased to a point where impact or pressure causes fracture and reduction to smaller pieces. The mill is constructed of Teflon® which has several essential advantages for the homogenization procedure. [1] Teflon® is relatively pure, and therefore, the danger of contamination of the sample with trace elements (other than carbon and fluorine) and organic compounds by abrasion and leaching is minimal (5). [2] Parts made from Teflon® are chemically inert and withstand thorough cleaning procedures with acids and organic solvents. These cleaning procedures also reduce the rish of contamination greatly. [3] Teflon® provides the specific heat capacity (1.05 kJ/kg·K) and insulation (heat conductivity = 0.07 W/m·K) to maintain mill and sample at cryogenic temperatures throughout the homogenization process, thereby preserving the physical and chemical state of the sample. [4] Teflon® has the density required for the grinding balls and disks and, at near liquid nitrogen temperature, the hardness and strength (without becoming brittle) to efficiently grind frozen biological tissues.

DESIGN AND CONSTRUCTION OF CRYOGENIC HOMOGENIZATION APPARATUS

The capacity of the "Mikro-Dismembrator" described by Iyengar (4) was too small for the Pilot NESB Program. Initially, sample sizes of up to 50 g were considered for the human liver banking program. During the final implementation of the storage and analysis plan (3), the sample size was increased to 150 — 200 g; for future specimens (e.g., mussels), a sample size of up to 1000 g is anticipated. These requirements led to a series of prototype developments summarized in Table 1. For the initial experiments the ball mills were patterned after that of Iyengar (4) (Figures 1a and 1b); however, mechanical limitations and the performance evaluation (see below) made the search for another type of mill necessary. The Teflon® disk mills developed during this study fulfill all the necessary requirements; therefore, the description of the construction details will be limited to the disk mills.

TABLE 1. CRYOGENIC HOMOGENIZATION UNITS USED IN THE PILOT NESB PROGRAM

	Teflon® Milling Chamber			Drive Unit	
	Volume Total	Milling Body	Capacity for Soft Tissue	Type	Motor Power/Revolutions
Ball Mill A	920 cm^3	ball, 65 cm^3	60 g	NBS shaker	0.25 kW, variable
Ball Mill B	1000 cm^3	ball, 200 cm^3	150 g		
Disk Mill #1	1400 cm^3	disk and 1 ring, 700 cm^3	100 g	Shatter box by Spex Industries[a]	0.25 kW, 825 rpm
Disk Mill #2	2500 cm^3	disk and 1 ring, 900 cm^3	250 g	Disk Mill TS 250 by Siebtechnik[b]	0.75 kW, 825 rpm
		disk and 2 rings, 1300 cm^3	400 g		
Disk Mill #3	6500 cm^3	disk and 3 rings, 2800 cm^3	~1000 g		

[a]Spex Industries, Inc., Metuchen, NJ.

[b]Siebtechnik GmbH, Mülheim (Ruhr), Federal Republic of Germany.

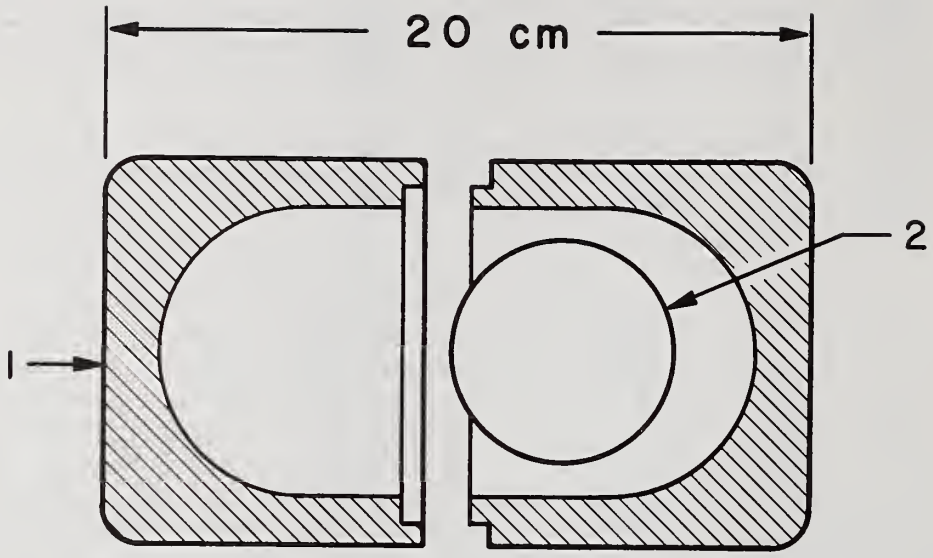

Figure 1a. Ball mill B (see Table 1): (1) milling body, (2) grinding ball.

Figure 1b. Teflon® ball mill, Teflon® scoop, and Teflon® jar used in the homogenization and storage of human liver specimens.

A "Shatterbox" disk mill made by Spex Industries, Inc. (Metuchen, NJ) was available. A Teflon® mill head was constructed (disk mill #1) to fit into the holding clamps of the "Shatterbox" (Figure 2). This disk mill head was patterned exactly after the commercial steel heads with only a slight increase in volume in spite of the much larger outer dimensions due to the increased wall thickness.

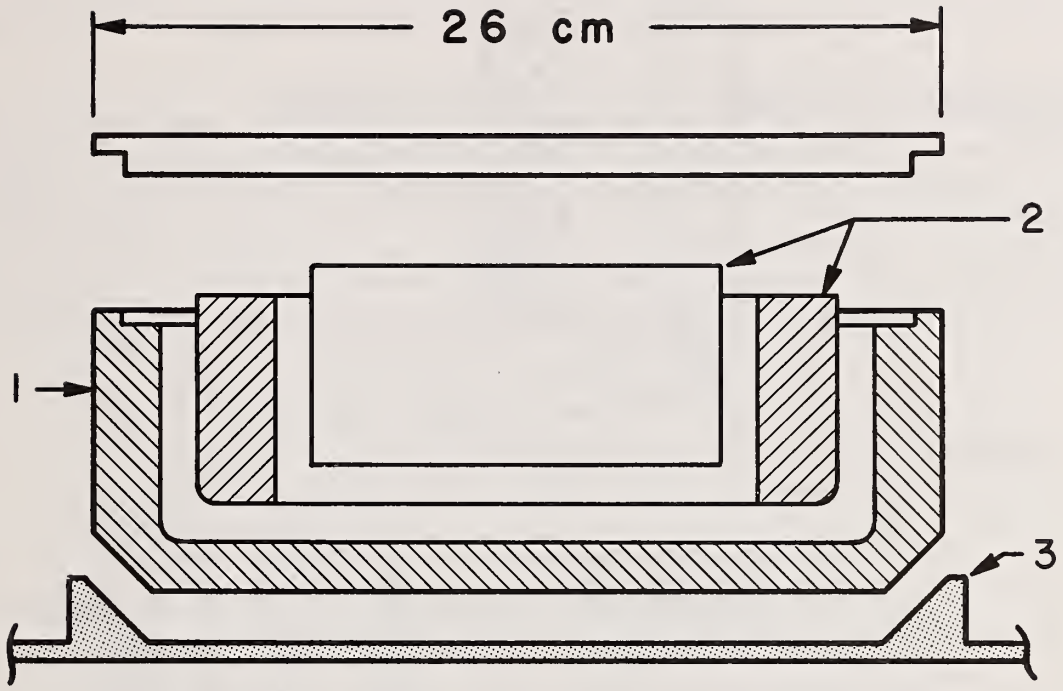

Figure 2a. Disk mill #2 (see Table 1): (1) milling body, (2) grinding disk and ring, and (3) aluminum holder.

Figure 2b. Teflon® disk mill and aluminum adapter plate.

After a short time of preliminary use (which gave excellent performance, but provided insufficient capacity), a stress problem created a fracture along the bottom inside corner of the mill head (Figure 3 upper diagram). This crack was probably caused by rapid changes in temperature. The sharp edge of the bottom of the chamber provided a starting point for the fracture. Later designs (disk mills #2 and #3) have a smooth curve between the bottom and the wall (Figure 3 lower diagram). As an additional precaution, the heads are cooled and warmed slowly to avoid rapid temperature changes.

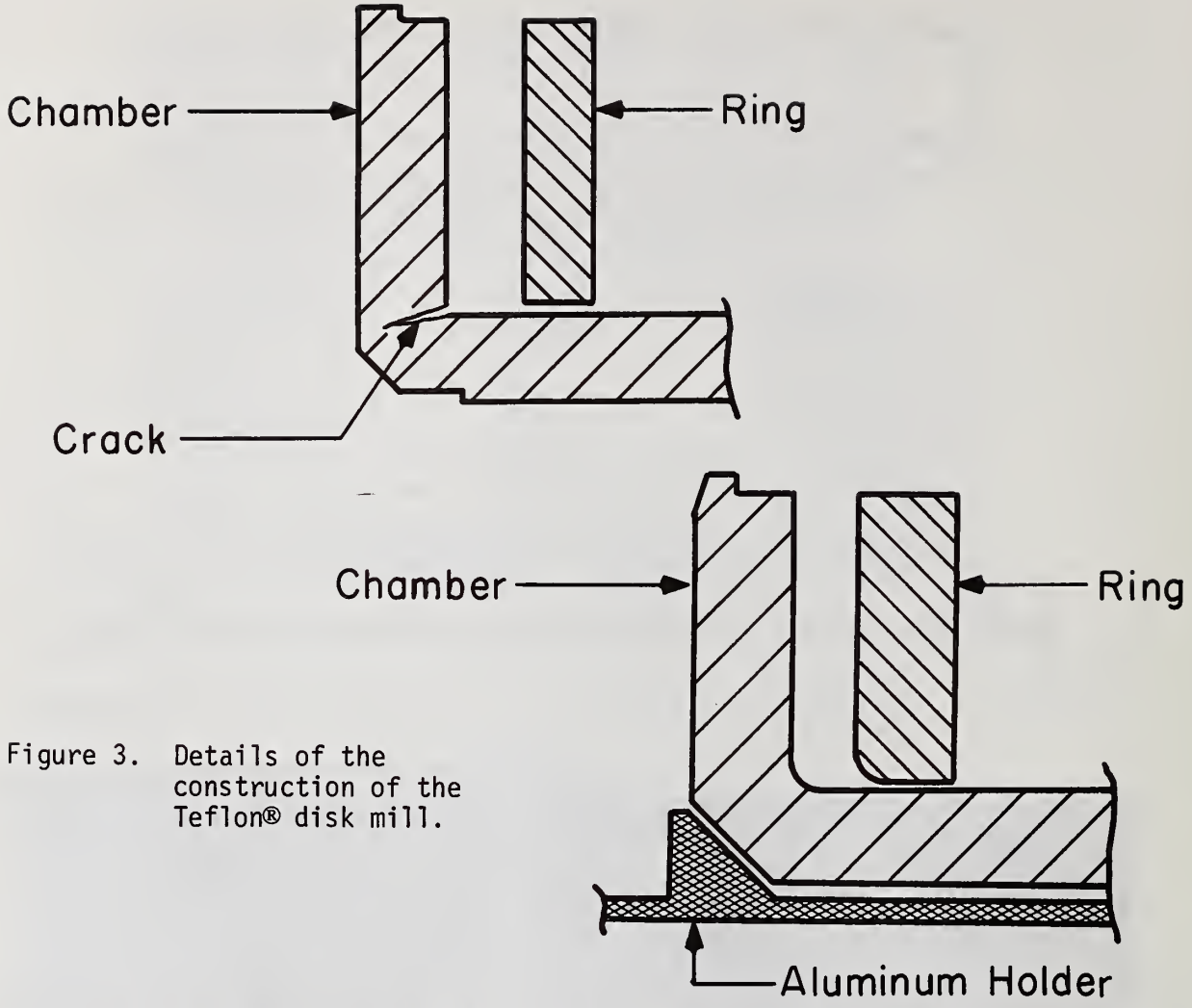

Figure 3. Details of the construction of the Teflon® disk mill.

The larger heads (#2 and #3) were adapted to fit a modified Siebtechnik G.M.B.H. (Mülheim (Ruhr), Federal Republic of Germany) "Swinging Mill". The large heads undergo significant changes in size with changes in temperature. The linear expansion coefficient of Teflon® was determined to be about 1.2×10^{-4} °C^{-1}. With a change of about 40 °C during operation, the diameter of the head increases by 1.2 mm. Therefore, the bottom of the mill is beveled and fits into a beveled aluminum holder on the Siebtechnik machine (Figure 3). With this arrangement a firm support of the mill head is ensured throughout its operation.

All mill heads, disks, and rings were machined from solid Teflon® PTFE stock. Rods with the desired diameters were commercially available. However, working with Teflon® pieces the size of the mill heads is not easy. Teflon® (TFE) is an unstable material, i.e., it will cold flow, "move", warp, etc. During machining, close tolerances are difficult to maintain. The additional problem of possible different contraction of the various parts during cooling down also had to be considered. For example, seals are flat surfaces on the top of the heads pressed together by the clamping holder of the Siebtechnik machine.

AUXILIARY EQUIPMENT FOR CRYOGENIC HOMOGENIZATION

The liver samples used in the Pilot NESB program are frozen at liquid nitrogen temperature. They are in a cylindrical shape about 10 cm long and 5 cm diameter. These tissue blocks do not fit into the disk mill and must be broken into smaller pieces. This operation is accomplished by direct impact in a Teflon® mortar with a Teflon® plunger (Figure 4). The liver is placed into the cooled chamber (LN_2 vapor temperature) and the plunger is inserted. An 18 kg weight is dropped from a height of about one meter onto the plunger. After several hits, the liver is fractured into small pieces with a maximum size of about 2 cm^3.

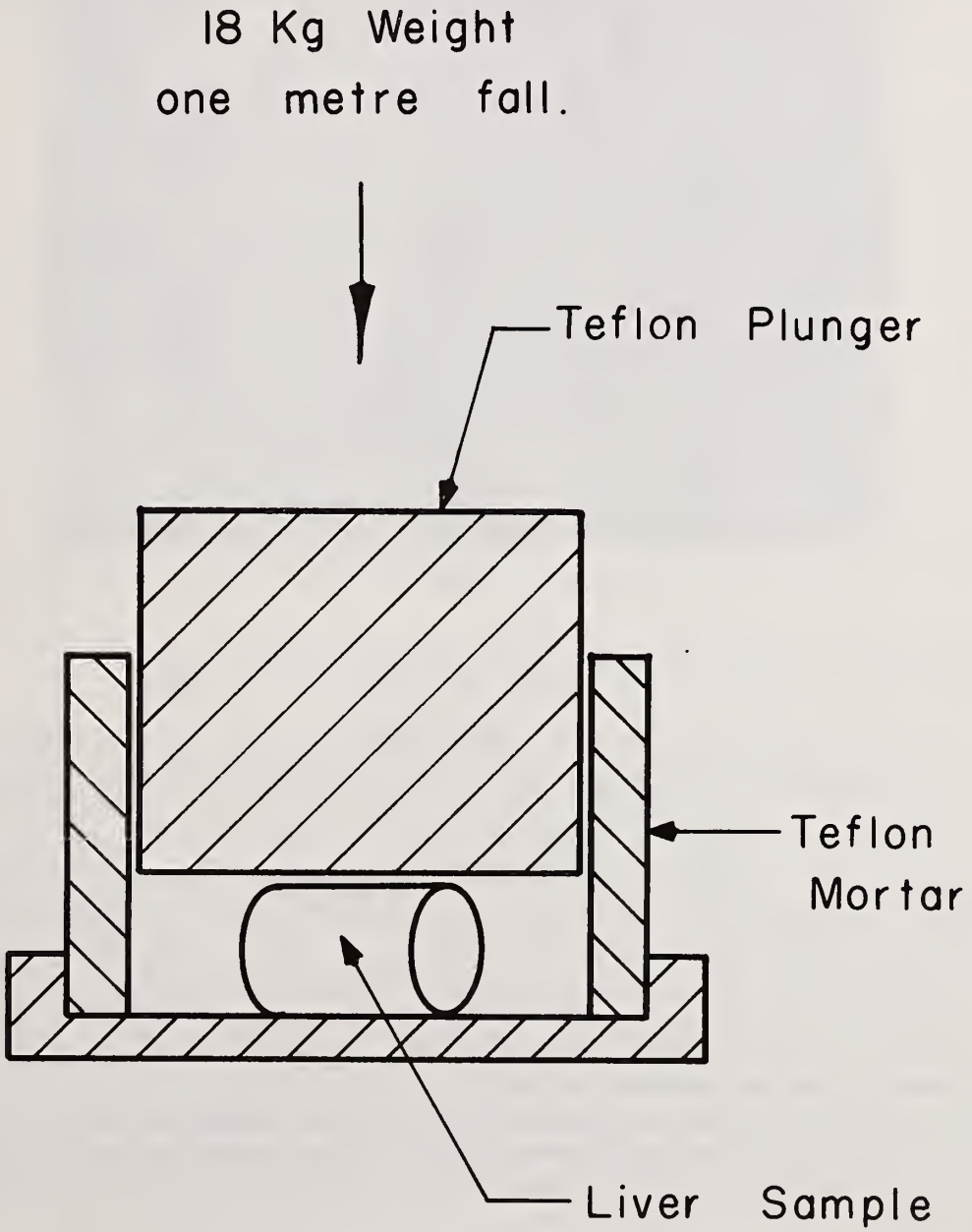

Figure 4. Teflon® mortar and plunger used to prefracture liver specimens.

The samples are maintained at cryogenic temperatures (below -60 °C) to insure the chemical stability of the samples. These temperatures are maintained from the time of removal of the specimen from storage through sample preparation. After the prefracture, the sample pieces are inserted into the cooled disk mill and after two minutes the homogenization is complete resulting in a fine, frozen powder (particle size <40 mesh). The powder is subsampled into Teflon® containers in a cold box (Figure 5) at temperatures below -60 °C. A variety of small sample handling tools (e.g., scoops, scrapers, brushes, etc) have been constructed of Teflon® to prevent contamination of the samples.

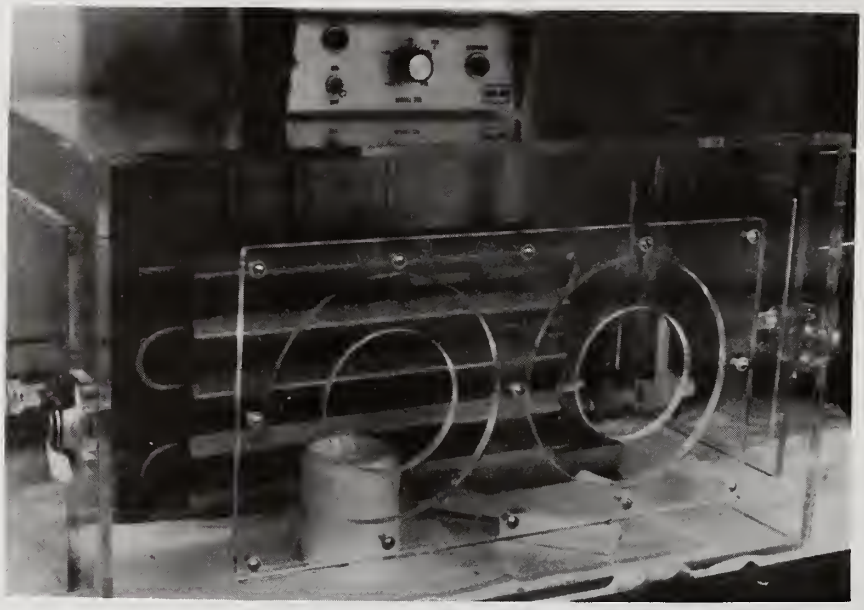

Figure 5. Cold dry box.

PERFORMANCE EVALUATION OF CRYOGENIC HOMOGENIZATION TECHNIQUE

In the frozen state the liver homogenate has a particulate physical nature. The sampling properties of particulate materials have been studied extensively (6,7), and Ingamells (8) has developed a very practical model to assess sampling. Ingamells' proposed sampling constant, K_s, can be used for direct comparison of the performance of the different cryogenic homogenization systems. To determine K_s experimentally, a particulate homogenate is subsampled at different sample sizes and analyzed. If the analytical error is sufficiently small, the sampling constant K_s can be determined using the equation $K_s = wR^2$, where R is the observed relative standard deviation 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 1 percent with 68 percent confidence. A sampling diagram derived from this relation can be used to predict analytical errors and their dependence on sample size.

To evaluate the performance of the homogenization procedure, a radiotracer experiment was performed using ^{24}Na. In this experiment a 5-g subsample was irradiated and then homogenized with the bulk material. Subsamples of different weights were then removed from the homogenate and the amount of ^{24}Na determined. From these experiments the sampling diagrams shown in Figure 6 were generated. The comparison of ball mill B with disk mill #1 (100-g capacity) is shown in Figure 6. For the ball mill, a sampling constant of K_s = 32 g was obtained indicating that the material was not sufficiently ground and mixed to allow accurate sampling using a typical 1-g analytical subsample. The inadequate performance of the ball mill led to the design of a disk mill which yielded a K_s = 0.95 g. Using the disk mill, the precision of the analytical data from a 1-g sample will be determined by the precision of the analytical techniques rather than by sampling uncertainty.

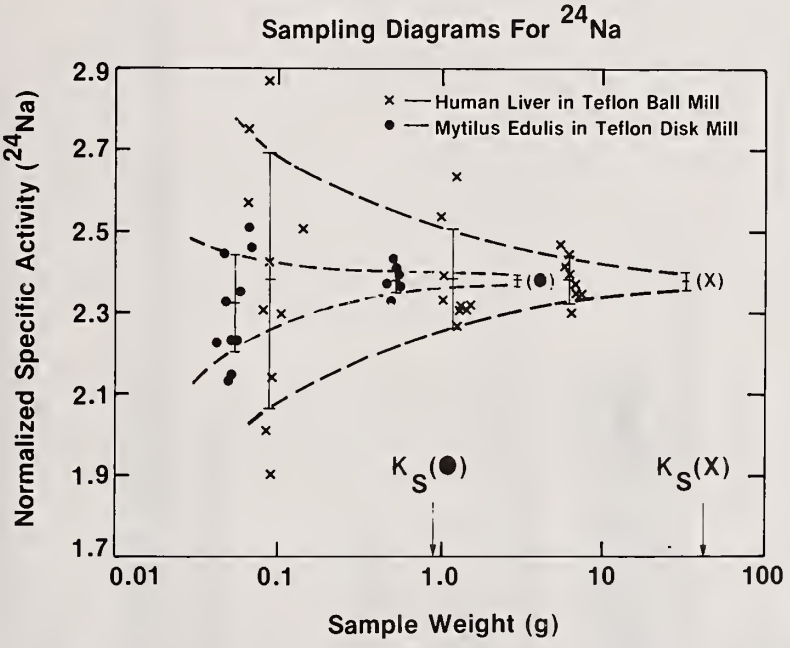

Figure 6. Sampling diagram of ^{24}Na in human liver homogenate.

To evaluate the performance of the mills with respect to particle size reduction, efforts were undertaken to measure the actual particle size distribution of the homogenate obtained from the two different milling procedures. In general, sieving is an easy way to determine particle sizes; however, cryogenic sieving of frozen samples is much more difficult. After some procedure development, the sieving experiments were performed in a large LN_2 freezer which provided the necessary cold and dry atmosphere to minimize melting and particle agglomeration due to moisture condensation. A standard set of metal sieves with the following mesh sizes, 40 (top), 60, 80, 100, 140, and 200 (bottom) was used to determine the particle size distribution of the homogenized tissues.

The performance of the mills was evaluated using three different types of tissue and the results of these experiments are summarized in Figure 7. A direct comparision of the results obtained with the ball mill and the disk mill reveals that the disk mill is much more effective in producing a small particle homogenate from beef liver. This matrix was selected to simulate the behavior of human liver tissue. After homogenization with the disk mill, virtually all of the material passes through the 40 mesh sieve, i.e., the particles are less than 0.46 mm in diameter. The results shown in Figure 7 are reproducible within ±10 percent (1s) for constant weight, temperature, homogenization time, and sieving time. Therefore, only the disk mills were considered for further evaluation and development.

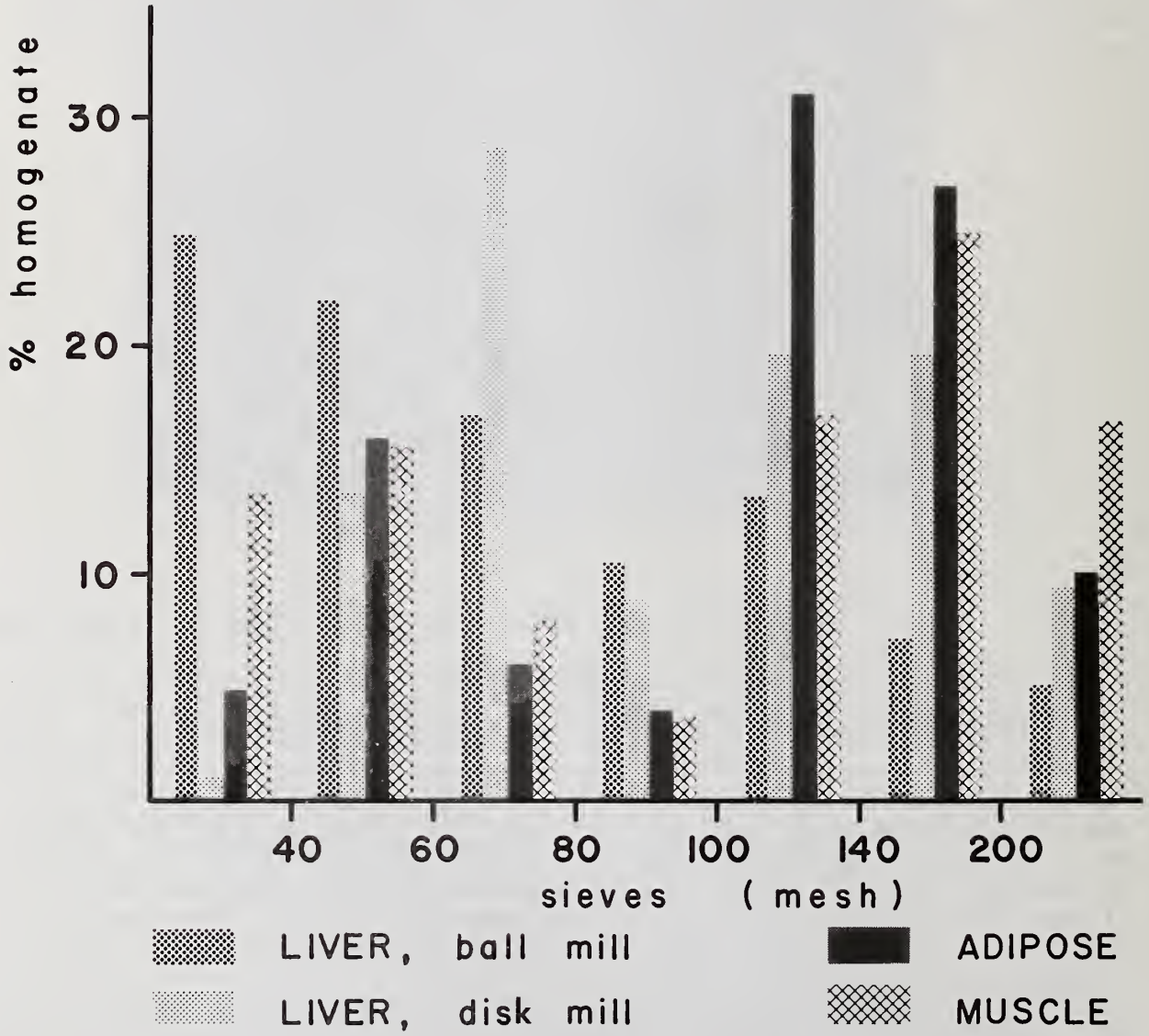

Figure 7. Particle size distribution of frozen tissue after homogenization in Teflon® mills. Approximately 150 g of tissue were homogenized with a grinding time of 4 min; liver homogenized in ball mill B and liver, adipose, and muscle tissue homogenized in disk mill #2.

Two additional tissue types were selected to test the performance of the disk mills: pork fat (adiposis abdominalis) to simulate human adipose tissue (which is used frequently for monitoring of organic pollutants) and beef muscle to simulate a fibrous tissue. The adipose tissue was the most difficult tissue for this homogenization technique. In all experiments with the pork fat, a fraction of up to 16 percent remained on top of the 40 mesh sieve; many times this fraction still contained several 1 cm^3 chunks which were not broken up during the homogenization process. These larger pieces may have hindered the action of the mill. It was necessary to reduce the weight of the sample to about 120 g for appropriate functioning of the mill since the volume of the fat tissue homogenate is considerably larger than a corresponding mass of liver. In addition, the mill had to be maintained as close as possible to LN_2 temperature to maintain the fat in a brittle state. Therefore, the aluminum support plates were also precooled in LN_2. Although the mill still did not perform as well with the fat tissue as with the liver tissue, the properties of the homogenate appeared to be suitable for analysis. The fibrous muscle tissue was also not completely homogenized during the selected 4 min cycle. About 14 percent of the material remained on the 40 mesh sieve. This fraction consisted mostly of fibers about 1 cm long and 1 mm thick. Although a considerable number of smaller fibers were found in the 40 to 60 mesh fraction, these fibers could be broken up by further milling. After an additional 4 min homogenization, only 2 percent of the material was left in the course fraction above 40 mesh. For the particles passing through the 60 mesh sieve, the particle size distribution for the fiberous tissue and the adipose tissue was smaller compared to the liver sample.

Using the information about particle size distribution and some assumptions as to the chemical nature of the liver, the weight of sample required to hold the sampling standard deviation to a preselected level can be theoretically calculated starting with the computation of N, the number of particles.

The value of N may be calculated from the relation

$$N = \left[\frac{d_1 d_2}{\overline{d}^2}\right]^2 \left[\frac{100(P_1 - P_2)}{R\,\overline{P}}\right]^2 p(1 - p)$$

where d_1 and d_2 are the densities of the two kinds of particles, $\overline{d}$ is the density of the sample, P_1 and P_2 are the percentage compositions of the component of interest in the two kinds of particles, $\overline{P}$ is the overall average composition in percent of the component of interest in the sample, R is the percent relative standard deviation (sampling error) of the sampling operation, and p and 1-p are the fractions of the two kinds of particles in the bulk material (7,9). For a two component particle mix made up of cells and fluids and for 40 mesh particles, the element Na requires a one-gram sample to reduce the sampling error to one percent. This is very consistent with the sampling constant of 0.95 g found as described above.

In addition to demonstrating the reduction in particle size, data supporting the homogeneity of the material was obtained by elemental analysis of the homogenate. Two differently prepared human liver homogenates were each subsampled into 20 jars with each jar containing about 7.5 g (fresh weight).

The subsamples were lyophilized and randomly selected test portions of 250 mg lyophilized material (corresponding to about 1 g fresh weight) were analyzed for 26 elements using neutron activation analysis. The analytical procedures are described in Section 7. The results which include the observed standard deviation R from the set of 10 samples and the uncertainty σ due to counting statistics of the individual measurements are summarized in Table 2. If the analytical uncertainty (in this case σ) is small or insignificant relative to R, then R is an estimate of the inhomogeneity in the material. None of the elements determined showed any inhomogeneous distributions exceeding the analytical uncertainty when either the ball mill or the disk mill was used. The sample standard deviation R depends mainly on the dominant analytical uncertainty σ which is due to the counting statistics. Therefore, no significant difference can be observed between ball mill and disk mill. However, the disk mill provides a finer particulate homogenate as shown in the sieving experiments and, because of mechanical requirements, can homogenize larger samples. For the majority of elements, R is smaller than 5 percent and less than 2 percent for several essential trace elements, for which large differences among 1-g test portions were observed previously (1). This suggests that subsampling errors due to inhomogeneity can be confined to less than 2 percent.

In another experiment, mussel (*Mytilus edulis*) samples were homogenized in a disk mill and then sieved. The sieved fractions were then analyzed by instrumental neutron activation analysis (INAA) for determination of selected trace elements. Representative percentages of the amount of material in each sieve fraction were obtained. Some interesting results from this experiment are shown in Table 3. The sodium and chlorine concentrations increase with decreasing particle size. These results may indicate that a fraction containing NaCl, probably due to ice crystals from the salt water contained inside the mussel shell at the time of shucking, grinds to a finer particle size than the organic material.

The results obtained during the performance evaluations of the various mills provide important knowledge on the relation of the analytical test portion to the bulk material. This information significantly increases the quality and the usefulness of the analysis. The favorable comparison of the theoretical calculations of sampling parameters with experimental results validates the assumption that frozen tissue homogenate has a particulate physical nature.

Though handling of the parts of the mill and of the materials at low temperatures is inconvenient, the Teflon® disk mill is recommended as an effective, contamination-free device for particle size reduction and homogenization of biological tissue. Homogenization at cryogenic temperatures reduces the loss of volatile components and changes in composition during the size reduction step. Samples as large as 1000 g can be homogenized using this technique. The procedure uses a commercially available drive unit which is fast and simple to operate. The quality and quantity of the samples produced should make this technique useful for sample preparation of biological tissues for analysis.

TABLE 2. HOMOGENEITY OF TWO CRYOGENICALLY HOMOGENIZED LIVERS, MEAN CONCENTRATIONS BASED ON WET WEIGHT.[a]

Element	Method	Ball Mill B, Liver ID: L1B0014B N	$\bar{X}$	R (%)	σ (%)	Disk Mill #2, Liver ID: L1M0344B N	$\bar{X}$	R (%)	σ (%)
Major and Minor Components			(mg/g)				(mg/g)		
C	PGAA	8	143	5.0	3.0	—			
N	PGAA	8	29.5	4.1	2.0	—			
H	PGAA	8	20.2	2.6	0.3	—			
P	PGAA	8	3.01	7.0	16	—			
K	PGAA	8	2.95	3.1	1.0	—			
K	INAA	10	2.92	3.6	2.3	11	2.8	6.0	9
S	PGAA	8	2.13	3.0	2.5	—			
Cl	PGAA	8	1.08	3.0	1.5	—			
Cl	INAA	10	1.00	3.3	1.0	11	1.38	2.2	1.0
Na	INAA	10	0.84	1.4	0.2	11	1.10	1.2	1.2
Trace Components			(μg/g)				(μg/g)		
Fe	INAA	10	196	0.85	0.7	10	430	1.4	0.4
Mg	INAA	10	150	20	10	11	150	11	9
Zn	INAA	10	41.9	0.87	0.2	10	59.5	1.2	0.3
Rb	INAA	10	9.52	2.2	1.3	10	(1)[b]	1.2	0.7
Cu	INAA	10	5.88	16	10	—			
Br	INAA	10	2.75	1.5	0.8	10	2.49	1.2	1.6
Cd	PGAA	8	1.31	3.4	1.5	—			
Cd	INAA	10	1.34	7.0	6.0	10	0.98	4.9	6.5
Mn	INAA	10	1.23	5.0	3.5	11	1.31	3.2	2.3
Ultratrace Components			(ng/g)				(ng/g)		
Se	INAA	10	518	1.5	0.7	10	512	1.9	1.0
B	PGAA	8	486	3.4	6.0	—			
Mo	INAA	10	180	7.0	7.0	10	528	7.2	10
Hg	INAA	10	140	6.0	2.5	10	107	11	5.0
Co	INAA	8	47.1	4.1	1.0	10	46.5	1.8	0.9
Cr	INAA	8	25	30	25	10	26	17	15
La	INAA	10	21	12	13	10	20	25	25
Cs	INAA	10	(1)[b]	7.0	5.0	10	14.9	5.4	3.8
Sb	INAA	10	16.7	5.0	4.0	10	6.1	4.6	6.6
Sc	INAA	10	0.17	12	13	10	0.40	14	10

[a] The analysis was carried out on freeze-dried samples. Dry weight factors of the individual samples (f=0.2626 to 0.2703) were used for conversion to wet weight.

[b] Relative values of specific activity only (no standards used).

NOTE: N = number of subsamples analyzed. $\bar{X}$ = mean concentration. R = relative sample standard deviation. σ = Relative uncertainty of individual determination due to counting statistics. PGAA = neutron capture prompt gamma activation analysis. INAA = instrumental neutron activation analysis.

TABLE 3. CONCENTRATION OF SODIUM AND CHLORINE IN MUSSEL SIEVE FRACTIONS (mg/g DRY WEIGHT).

Sieve Fraction Mesh Range	Unsieved	>40	40-60	60-80	80-100	100-400	140-200	<200
Weight Percent		2	25	18	14	126	12	3
Na	61.0	54.0	53.8	57.2	61.9	65.5	70.7	71.8
Cl	111	79.8	96.7	102	110	116	121	128

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SECTION 4

DEVELOPMENT AND VERIFICATION OF A PROTOCOL FOR THE ANALYSIS OF INORGANIC CONSTITUENTS IN HUMAN LIVERS

by

R. Zeisler

INTRODUCTION

General criteria for the selection of analytical techniques for specimen banking activities have been discussed previously (1). These selection criteria are: sensitivity, accuracy, reliability, availability, cost, precision, speed, applicability to specific elements and species, minimal sample pretreatment, and multielement capabilities. Although a large number of trace element analytical techniques which fulfill all or most of the above criteria can be mentioned, each with their own advantages and limitations, only a few can be selected for a routine monitoring program. From the well-established analytical techniques available at NBS, the following were considered and evaluated for the human liver analyses: atomic absorption spectrometry (AAS), inductively coupled plasma emission spectrometry (ICP), isotope dilution mass spectrometry (IDMS), neutron activation analysis (NAA), voltammetry (VOL), and x-ray fluorescence analysis (XRF).

COVERAGE OF PRIORITY ELEMENTS

Although all stable elements (and some radioactive elements) may occur in biological tissues, only a small number are known for their biological functions: the major constituents of the organic matrix, the mineral elements, and the essential trace elements. Equally limited is the number of elements which have known toxic effects on biological systems at trace levels. The first priority in the development of the specimen bank analytical protocol has been the determination of as many elements of interest from the above groups as possible. The elements of interest are those of environmental concern [pollutant elements as defined by the 1978 workshop on specimen banking (1) and EPA (2)] and minerals and trace elements of biological interest which may be important for the assessment of nutrition and health related aspects of the individuals (Table 1). With the measurement of as many elements as possible from these groups, a set of data will be created which has the same analytical basis (i.e., sampling protocol, processing, and measurement procedures), thereby possibly providing new information about interelemental relationships, the relationships of elements to health and nutrition, and the impact of pollutant elements on the biological system. Some additional elements are

TABLE 1. ANALYTICAL PROTOCOL FOR HUMAN LIVER SPECIMENS

	Priority[a]	Method	Detection Limit (μg/g)[b]
Be	1	AAS	0.0006
Na	3	NAA	1.0
Mg	3	NAA	2.0
Al	2	AAS/NAA	0.1/0.5
Cl	3	NAA	20
K	3	NAA	100
Sc	4	NAA	0.0001
V	1	NAA	0.02
Cr	1	NAA	0.02
Mn	1	AAS/NAA	0.2/0.05
Fe	3	NAA	1.0
Co	1	NAA	0.0005
Ni	1	AAS	0.01
Cu	2	AAS/IDMS NAA/VOL	0.2/0.1 1.0/0.2
Zn	1	NAA/VOL	0.1/1.0
As	1	AAS/NAA	0.001/0.02
Se	1	AAS/NAA	0.05/0.01
Br	3	NAA	0.1
Rb	3	NAA	0.1
Mo	1	NAA	0.2
Ag	2	NAA	0.02
Cd	1	AAS/NAA/VOL	0.01/0.1/0.05
Sn	1	NAA[c]	0.005
Cs	4	NAA	0.01
La	4	NAA	0.05
Ce	4	NAA	0.05
Pt	1	NAA[c]	0.000002
Au	4	NAA	0.0001
Hg	1	NAA	0.005
Tl	1	IDMS	0.0001
Pb	1	IDMS/VOL	0.01/0.01

[a] 1 = First priority elements from Ref. 1.
2 = Additional pollutant elements from Ref. 2.
3 = Biological mineral and trace elements.
4 = Elements possibly suitable for monitoring contaminations.

[b] For human liver samples with the applied analytical protocol using ∿1 g (wet weight).

[c] Newly developed radiochemical NAA procedures, not yet routinely applied.

determined since they are detectable by a multielement technique thereby providing additional information at relatively little additional cost.

The techniques considered for this program are limited in their sensitivities. These limitations are made greater by the small sample size available for analysis in the Pilot NESB Program. The attractive multielement techniques ICP and XRF could not be readily applied to the NESB liver samples bcause of the limited amount of sample available for analysis and, therefore, the lack of sensitivity for many of the elements of interest. Using the remaining four techniques (i.e., AAS, IDMS, NAA, and VOL), nearly complete coverage of the elements of interest (31 elements) has been achieved. The implemented analytical techniques are summarized in Table 1. Table 1 also includes the experimental detection limits which were achieved during the analysis of 36 liver specimens collected during the first year of the Pilot NESB Program.

During the first year not all of the first priority elements (Ref. 1 and Table 1) were determined in the complete set of 36 human liver samples. Fluorine was excluded from the protocol because of the use of Teflon® materials for homogenization and storage. Four elements (Be, Cr, V, and Ni) were at or below the detection limits of the applied analytical procedures. A modification in the AAS procedure allowed the determination of nickel in some of the samples. The NAA procedure was not sensitive enough for the low concentrations of chromium in most of the samples. However, improvements in the NAA instrumentation will provide improved sensitivity, thereby allowing for determination of chromium at these low levels in the second-year liver analyses. The analytical protocol for the second year will also be modified to include the determination of chromium by AAS in addition to NAA. Vanadium is also expected to be determined in the future by NAA using improved counting techniques. To determine the low levels of beryllium successfully, research has to be directed towards a more sensitive procedure, unless much larger samples are made available for the AAS procedure to allow a preconcentration step before the analytical measurement.

The experimental detection limits reported in this work (see Table 1) are somewhat higher than literature data which are generally reported for optimized conditions. Since these techniques are obviously more sensitive if the experimental parameters are optimized, there is significant potential for improved procedures which would result in greater sensitivity and more complete elemental coverage in the future. In addition, recently developed analytical methodologies (see Appendices I, II, and V) will be incorporated. In spite of the above mentioned limitations of the applied analytical protocol, the data set for the first year liver samples (31 elements in 36 liver specimens) represents the most complete data set available using four analytical techniques.

QUALITY ASSURANCE

The second criterion in the development of the analytical protocol was the implementation of two procedures to assure the quality of chemical analysis: (1) the simultaneous analysis of standards (Standard Reference Materials) with the same or a similar matrix as the analyte and (2) the determination of constituents by two or more independent analytical methods.

All of the methods which were considered for use in this protocol have been used for the certification of biological and environmental Standard Reference Materials (SRM's). During the planning of the analytical phase of the Pilot NESB Program, the capabilities of various analytical methods were evaluated on the basis of results from the ongoing certification analyses of SRM 1577a and b, bovine liver, (renewals of the widely used SRM). The similarity of the SRM matrix made the analytical procedures immediately available for the human liver analyses. During the course of the first-year human liver analyses, several of the analytical procedures were validated by analyzing the SRM 1577 parallel to the NESB specimens (see Sections 6 and 7 and Appendix I).

The parallel analyses of SRM's provided an essential method of validation for the acceptability of the results during actual routine implementation. However, occasional blunders, contamination, and/or analytical errors still may occur and provide incorrect data particularly when only one sample is available for analysis. Good analytical practice calls for replicate and independent determination of the constituents in a sample. Due to the limited amount of analytical test portions of each liver sample in the pilot program, a three-fold approach was incorporated into the analytical protocol to attain a second means of continuous quality assurance. First, NAA was selected to analyze the homogenate of each specimen in duplicate because of the small sample size required for this technique. Second, as many elements as economically possible were determined using different techniques on additional sample aliquots (Table 1). The use of two different techniques serves in place of replicate analysis by one technique to eliminate the occasional analytical blunder and also to protect against systematic errors of a single technique. Third, sufficient sample homogenate is banked (as well as the complete second section of each liver sample) to repeat an analysis if a result appears to be questionable.

CONCLUSION

The outlined philosophy for the selection of analytical methodology for the analysis of human liver samples in the Pilot NESB Program has resulted in a working analytical protocol for the determination of a large number of elements in each of the selected specimens. Using this analytical protocol, in conjunction with the previously described sampling protocol, should provide high quality data for most elements of environmental and biological interest. This protocol is a flexible analytical plan which is open for improvements in analytical methodology as they may occur as well as for the addition of new procedures which provide for the inclusion of additional priority elements or for better quality control.

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SECTION 5

DETERMINATION OF SELECTED TRACE ELEMENTS IN HUMAN LIVER SAMPLES BY ATOMIC ABSORPTION SPECTROMETRY

by

T. C. Rains, T. A. Rush, and T. A. Butler

INTRODUCTION

Atomic absorption spectrometry (AAS) is a unique analytical technique for the determination of metallic elements. The absorption of radiant energy by ground state atoms in the gaseous state is the basis of AAS. When radiation of proper wavelength passes through a vapor containing ground state atoms, some of this radiation will be absorbed by excitation of particular atoms (1). The magnitude of the absorption signal is directly related to the number of these ground state atoms found in the optical path. Ground state atoms necessary for the measurements are produced from the sample material by vaporization of the material introduced into an appropriate excitation source followed by dissociation of the molecular species into neutral atoms. Normally these steps are carried out by a nebulizer associated with a flame or electrochemical device (1-3). The basic components of an AAS instrument are described in detail in the literature (2).

Since AAS is a relative technique, a quantitative determination can only be carried out by comparison to a reference solution. If the behavior of the sample is different than the behavior of the reference solution, it is implied that an interferent is present. Depending upon the cause, the interferent can be classified as chemical, physical, background, or spectral. While background and spectral interferences are based on measurement of non-specific signals, chemical and physical interferences have an influence (positive or negative) on the total number of atoms formed per unit volume of the absorption cell. Of the interferences encountered in biological tissue, such as human liver, the chemical form is most prevalent. Also, chemical interferences are most likely to prevail in electrothermal atomization than in flame excitation devices. For those elements being determined in human liver and described in this report, (i.e., Cu, Mn, Al, Be, Ni, Cd, As, and Se), AAS with electrothermal atomization was used because the concentration of the analytes were below the detection limits by flame excitation devices.

The first furnace or electrothermal atomization device proposed for AAS was described by L'vov (4). In his early work, a solution was placed on the end of an electrode and evaporated to dryness. The sample was then vaporized

by a dc arc into a carbon furnace. This device produced impressive detection limits but was limited because of power requirements and poor precision. Massmann (5) constructed a somewhat simple graphite furnace which is basically being used by all manufacturers today.

In this report procedures are described for the determination of selected trace elements in human liver by AAS using electrothermal atomization or hydride generation.

EXPERIMENTAL

Reagents

High purity acids were prepared using subboiling distillation (6). All reagents used in sample preparation or matrix modification were prepared and stored in clean Teflon® FEP bottles unless otherwise stated.

Instrumentation

The instrumental systems used in this study consist of a Perkin-Elmer Model 603 and Perkin-Elmer Model 5000 atomic absorption spectrometers with HGA-2100 and HGA-500 graphite furnaces, respectively. A 20-µL aliquot of sample was introduced into the furnace using an autosampler. The instrumental parameters are given in Table 1.

TABLE 1. INSTRUMENTAL PARAMETERS FOR ATOMIC ABSORPTION SPECTROMETRY

Element	Wavelength nm	SBW[b] nm	Scale Expansion	Drying T-s[c]	Charring T-s	Atomization T-s
Al[a]	309.3	0.7	3	125-40	800-40	2700-6
Be	234.7	0.7	5	125-40	1000-40	2800-6
Cd[a]	228.8	0.7	1	125-40	800-40	2300-6
Cu	324.7	0.7	1	125-40	700-40	2700-6
Mn[a]	279.5	0.7	1	125-40	800-40	2700-6
Ni[a]	223.0	0.2	5	125-40	800-400	2700-6
As	193.7	0.7	3	Hydride generation/heated quartz cell		
Se	196.0	0.7	3	Hydride generation/heated quartz cell		

[a] L'vov platform used within the graphite cell. The internal gas was argon.

[b] SBW = Source bandwidth.

[c] T-s = Temperature (°C) and seconds.

L'vov Platform

Interferences encountered with electrothermal atomization devices are more pronounced than in most flame systems, and the analyst has to rely upon the standard addition technique or close matching of standards with unknowns to correct for the interferences. Recent improvements in electrothermal atomization-AAS have greatly reduced analytical interferences. Graphite used in the absorption cell is a porous material which is easily penetrated by liquids and gases. Atomic vapor can freely pass through a 1-mm thick wall of hot graphite. Coating the graphite tubes with a thin layer of pyrolytic graphite has been found to greatly reduce the effects of the porosity of the graphite and increase the sensitivity for some elements. By inserting a L'vov platform in the graphite absorption cell, it is possible to atomize the sample at nearly constant temperature conditions (8). This reduces analytical interferences by volatilizing the sample into a gas which is hotter than the surface from which the sample is volatilized.

While these platforms can be obtained commercially, they can be prepared in the analyst's laboratory with a minimum of cost. For the system used in this work, the platforms are constructed by cutting the two ends of a graphite tube into six (three from each end) 7-mm x 5-mm grooved, curved sections. These cuts are made using a small stainless steel saw. After the sections are cut, the sides of each section are filed so that the platform will fit the inside contour of the graphite tube (8).

The graphite tube is positioned in the furnace head. The right window is temporarily removed and the platform inserted. The platform is then centered directly beneath the sample port using a metal rod. Adjustments of the automatic injector tip are made to insure that it does not come into contact with the platform surface.

Interferences

Sublimation of the analyte during the processing step is virtually unknown in flame AAS; however, in AAS with electrothermal atomization it is of major importance. Since the atomization of a sample in a graphite tube furnace can only take place from the graphite surface, it is essential that the sample remains in good contact with the hot graphite surface for as long as possible to become completely atomized. In contrast to flame AAS, the formation of sulfate, phosphate or oxides in the graphite tube is often advantageous.

Standardization

Working standards were prepared by serial dilution of aqueous stock solutions, as described by Dean and Rains (2). These were acidified with HNO_3 to one percent to match the acid concentration in the sample solution. Standard addition procedures were used to check for the presence of matrix suppression or enhancement and to correct for these interferences when they were present (7).

Sample Preparation

The freeze-dried samples were dried for 24 hours at ambient temperature in a vacuum oven equipped with a liquid nitrogen trap. From a dried sample, a 0.5 to 1 g test portion was transferred to a Teflon® beaker. The sample was digested overnight after the addition of 10 mL of high purity HNO_3. Then 5 mL of $HClO_4$ and 1 mL of H_2SO_4 were added and the sample evaporated to near dryness. Then 1 mL of HNO_3 was added and the sides of the beaker washed down. The solution was warmed to redissolve the solids. Then, the solution was transfereed to a 10-mL volumetric flask and diluted to the calibrated volume. The sample solution was transferred into a clean plastic (common polyethylene) bottle for storage. The elements Al, Be, Cd, Cu, Mn, and Ni were determined using the instrumental conditions as described in Table 1. The validity of each analytical result was checked by the single addition method (7).

The samples for As and Se determination were prepared as described below. A 0.5-1 g test portion of the previously dried sample was transferred to a digestion flask and 10 mL of HNO_3 and 3 mL of H_2SO_4 were added. A water condenser was attached and the solution heated gently for one hour. The water from the condenser was drained and 5 mL of $HClO_4$ added, the sample was then heated to sulfuric acid fumes. The flask was allowed to cool and 10 mL of HCl was added slowly, the solution was reheated for 10-15 minutes and then transferred to a 50-mL volumetric flask. The sample was then diluted to the calibrated volume with water. Arsenic and selenium were then determined by AAS using hydride generation.

The influence of some acids upon the analyte in the graphite furnace can be attributed to the formation of easily volatile compounds which sublime as the molecule. Losses owing to deflagration of the salt at increased temperature play a part, especially when using perchloric acid and occasionally with nitric acid. Since perchloric acid is necessary to completely oxidize the human liver sample, a method had to be devised to remove the perchlorate before atomization. Different matrix modifiers were found not to be effective when perchlorate was present. To alleviate the interferences of perchlorate the digestion procedure was modified to include the addition of sulfuric acid and heating the sample to sulfuric acid fumes. While the sulfate ion was found in previous studies to suppress many analytes by AAS with electrothermal atomization, with the L'vov platform the sulfate suppression was largely removed.

Even with the L'vov platform, calcium caused a severe suppression of the absorption signal for beryllium. In order to compensate for the interference, calcium was added to all standards to match the calcium concentration in the liver sample.

Cadmium compounds are known to sublime at relatively low temperatures; therefore, it is difficult to volatilize the matrix during the charring step without loss of cadmium. To alleviate this difficulty, a one percent solution of $(NH_4)_2HPO_4$ was added to sample and standards. With the addition of phosphate the interference of the matrix on the cadmium absorption signal was largely removed.

Hydride Generation

The determination of arsenic and selenium is considerably influenced by the fact that their resonance lines are in the vacuum UV spectrum range. Arsenic can only be determined satisfactorily in the best AAS instrumentation because its strongest resonance line is at 193.7 nm. Very few chemical interferences were observed for arsenic in an air-acetylene flame; however, the disadvantage of the flame is that 60 percent of the light energy emitted by the radiation source (hollow cathode lamp or electrodeless discharge lamp) was absorbed (9). The argon (entrained air) — hydrogen flame produced the same sensitivity with only 10 percent absorption of the light energy. A typical detection limit for arsenic and selenium with the argon (entrained air) — hydrogen flame is 0.02 μg. A further increase in sensitivity for these elements can be obtained with the hydride system. With $NaBH_4$ as the reducing agent and an electrodeless discharge lamp, a detection limit of 0.0005 μg was attained. These elements are relatively interference free provided the arsenic is prereduced from As^{V} to As^{III} with potassium iodide and Se^{VI} to Se^{IV} with HCl.

RESULTS AND DISCUSSION

The results of the determinations of Be, Al, Mn, Ni, Cu, As, Se, and Cd for 36 human liver samples are summarized in Table 2.

TABLE 2. TRACE ELEMENT CONCENTRATIONS IN HUMAN LIVER SAMPLES AS DETERMINED BY ATOMIC ABSORPTION SPECTROMETRY.

Sample Identification	Element Concentration (μg/g wet weight) Be	Al	Mn	Ni	Cu	As	Se	Cd
L1B0002	$\leq 5\times10^{-4}$	0.79	1.8	≤0.01	4.96	0.011	0.44	3.71
L1B0005	$\leq 5\times10^{-4}$	0.61	1.6		2.1	0.013	0.34	0.98
L1S0018	$\leq 5\times10^{-4}$	0.90	2.0		2.8	0.017	0.49	2.97
L1M0023	$\leq 5\times10^{-4}$	0.63	0.47		5.36	0.0023	0.68	2.1
L1S0027	$\leq 5\times10^{-4}$	1.5	1.8	≤0.01	4.44	≤0.0009	0.54	0.94
L1S0037	$\leq 5\times10^{-4}$	0.24	2.46		2.98	0.0078	0.41	2.0
L2B0041	$\leq 5\times10^{-4}$	0.2	1.1		3.24	0.0042	0.50	0.84
L2B0042	$\leq 5\times10^{-4}$	0.51	2.0		5.02	0.0053	0.46	0.66
L1B0045	$\leq 5\times10^{-4}$	0.34	1.6		2.0	0.0075	0.49	1.4
L2M0048	$\leq 5\times10^{-4}$	0.70	0.83	≤0.01	6.84	0.0052	0.65	0.79
L2S0049	$\leq 5\times10^{-4}$	0.81	1.5		2.2	0.0091	0.48	0.45
L1S0053	$\leq 5\times10^{-4}$	0.30	0.95	≤0.01	5.80	0.027	0.65	2.4
L2M0062	$\leq 5\times10^{-4}$	2.2	1.8	≤0.02	5.92	0.0031	0.47	3.54
L1B0074	$\leq 5\times10^{-4}$	0.68	1.6	≤0.01	5.87	0.0092	0.46	1.7
L1S0080	$\leq 5\times10^{-4}$	0.2	1.3	0.01	4.97	0.0095	0.50	0.54
L1M0083	$\leq 5\times10^{-4}$	0.99	0.64	≤0.01	5.25	0.011	0.50	0.82
L1S0086	$\leq 5\times10^{-4}$	0.45	1.4	≤0.01	6.38	0.023	0.59	0.59
L1S0090	$\leq 5\times10^{-4}$	0.93	1.1	≤0.01	8.23	0.0085	0.53	2.2
L1S0091	$\leq 5\times10^{-4}$	0.56	1.2	0.02	5.58	0.0082	0.45	1.4
L1S0092	$\leq 5\times10^{-4}$	0.64	1.2	≤0.01	10.8	0.0037	0.64	1.9
L1B0100	$\leq 5\times10^{-4}$	1.6	1.6	≤0.02	11.1	0.0069	0.54	2.4
L1M0105	$\leq 5\times10^{-4}$	0.55	1.2	0.04	8.81	≤0.0011	0.55	1.1
L1S0107	$\leq 5\times10^{-4}$	1.1	1.5	0.037	10.1	0.011	0.63	2.6
L1S0110	$\leq 5\times10^{-4}$	0.53	1.0	0.036	4.36	0.0064	0.61	2.1
L1M0118	$\leq 5\times10^{-4}$	0.56	2.0	0.09	4.57	0.0070	0.59	2.5
L1M0119	$\leq 5\times10^{-4}$	31.6	1.8	0.10	4.93	2.0	0.48	1.8
L1B0121	$\leq 5\times10^{-4}$	0.67	1.1	0.013	7.49	≤0.0010	0.49	0.39
L1S0123	$\leq 5\times10^{-4}$	0.42	1.2	0.47	8.41	0.012	0.53	1.8
L1M0126	$\leq 5\times10^{-4}$	11	2.0	0.062	7.39	0.097	0.56	4.84
L1M0127	$\leq 5\times10^{-4}$	2.1	1.1	0.076	4.26	0.0094	0.44	0.63
L1S0128	$\leq 5\times10^{-4}$	0.34	0.61	0.02	8.98	0.022	0.56	1.2
L1S0129	$\leq 5\times10^{-4}$	1.2	1.3	0.02	12.5	0.013	0.62	2.5
L1M0130	$\leq 5\times10^{-5}$	0.2	1.2	0.029	6.6	0.012	0.52	0.45
L1M0131	$\leq 5\times10^{-4}$	0.92	0.62	0.02	4.97	0.0087	0.46	0.76
L1M0140	$\leq 5\times10^{-4}$	0.60	1.4	0.02	6.89	0.010	0.60	1.1
L1M0141	$\leq 5\times10^{-4}$	0.79	0.95	0.014	4.45	0.014	0.41	0.25

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SECTION 6

THE DETERMINATION OF LEAD, THALLIUM, COPPER, AND NICKEL IN HUMAN LIVER SAMPLES BY ISOTOPE DILUTION MASS SPECTROMETRY

by

J. W. Gramlich, L. J. Powell, K. A. Brletic, and T. J. Murphy

INTRODUCTION

Isotope dilution mass spectrometry (IDMS) has been used extensively for the certification of elemental concentrations in Standard Reference Materials (1,2) and is regarded as a "definitive method", i.e., a method of proven accuracy. In IDMS, the quantity of an element present in a material is determined from the change produced in its isotopic composition by the addition of a known amount of a stable isotope of the element. After equilibration and separation of the element, the altered isotopic composition is measured by thermal ionization mass spectrometry and the concentration of the natural element is calculated.

To achieve high accuracy, careful attention must be given to the elimination of possible systematic errors due to errors in the calibration of the spike isotope, isobaric interferences, non-equilibration of spike and natural isotopes, and contamination or blank. In a carefully designed analysis these errors are negligible and the major sources of error are the random errors present in the isotope ratio measurements for the spike calibration and the analysis. These errors are reflected in the precision of the isotopic measurements and are on the order of ±0.05 to 0.2 percent for many elements. Therefore, an absolute accuracy of ±0.1 to 0.4 percent is possible even for a very low concentration in a complex matrix.

Because of the high accuracy attainable with IDMS, this technique was used to determine lead, thallium, copper, and nickel in a number of human liver samples while restricting sample size to 0.5 g or less.

Due to the low concentrations expected for thallium and nickel in human liver, it was necessary to develop procedures for these elements. This method development was accomplished using SRM 1577a, Bovine Liver. This SRM material is certified for lead and copper but not for thallium or nickel. Based on the results using SRM 1577a, the procedures described in this report were adopted for the determination of these elements in the human liver samples.

EXPERIMENTAL

The liver samples had been previously freeze-dried; however, since they may have absorbed water on standing, they were redried by overnight vacuum drying at room temperature. Approximately 0.5-g samples of each liver were spiked with ^{206}Pb, ^{203}Tl, ^{65}Cu, and ^{62}Ni. The samples were then wet-ashed by the addition of 10 g HNO_3 and 1 g $HClO_4$ and heating (covered) on a hot plate. The covers were removed and the samples were heated until fumes of perchloric acid were no longer visible. The sides of each beaker were washed down with water and the heating repeated. The residues were taken up in 20 mL of H_2O, covered with Parafilm and two-50 mm platinum wires were inserted through the film. One wire was connected to the anode of a d.c.-power supply and the other wire was connected to the cathode. The solution was electrolyzed overnight at 2.0 V applied while stirring magnetically. (Lead and thallium deposit on the anode as PbO_2 and Tl_2O_3 and copper deposits on the cathode as Cu metal.) The electrolyte was reserved for the determination of nickel.

Determination of Lead and Thallium

The mixed $PbO_2 - Tl_2O_3$ deposits were stripped from the electrodes with dilute $HNO_3 - H_2O_2$. The resulting solution was evaporated to dryness and the residue was taken up in two percent (w/w) HNO_3 so that the lead concentration was about 100 µg/mL. The concentration of thallium is taken at whatever level is present in the solution, since past experience has shown that the results for thallium are relatively insensitive to the amount of material under the analysis conditions employed.

A single filament rhenium source was used for the sample mounting and analysis. Prior to sample loading, the filament was degassed in a vacuum and under a potential field for 30 minutes at a current through the filaments of 3 A.

A 5 µL drop of a collodial silica gel solution was dried on the filament with a current of 1 A for 5 minutes. A 5 µL drop of the sample solution (0.5 µg Pb) was then dried on the silica gel layer under the same conditions. A 5 µL drop of 0.75 N H_3PO_4 was then dried on the filament at a current of 1.3 A for 5 minutes followed by 1.5 A for 5 minutes. The filament was then heated at a current of 2 A until the excess H_3PO_4 was seen to fume off. After fuming had stopped, the filament temperature was increased to approximately 800 °C for 2 seconds. The filament was then immediately loaded into the mass spectrometer.

The analysis procedure was as follows: the filament temperature was increased to produce a Tl^+ ion current of approximately 3×10^{-11} A at the collector. The temperature of the filament was about 700 °C. A steady signal intensity was obtained and 20 minutes of data were collected starting at 15 minutes into the analysis. After measurement of the thallium isotopic ratios, the filament temperature was increased to 1100 °C. After 5 min and 10 min the filament temperature was increased to 1150 °C and 1200 °C, respectively. Lead isotopic measurements were obtained between 30 min and 55 min after the filament temperature was increased to 1100 °C.

The thallium blanks for this procedure averaged 0.02 ng Tl or about 1 percent of the thallium in the samples. The lead blanks averaged 2.3 ng or about 0.2 percent of the lowest lead concentration. Due to limited samples, natural lead isotopic compositions were measured for only two samples and one composite from several samples. The average isotopic composition was used for calculation of concentration.

Determination of Copper

The cathode from the electrolysis was used for the determination of Cu in the sample. The electroplated copper was dissolved with a few mL of 25 percent (w/w) HNO_3 and the resulting solution was evaporated to dryness. The residue was taken up in a drop of 2 percent (w/w) HNO_3 for mass spectrographic analysis. The analyses were performed using a triple filament thermal ionization mass spectrometric procedure. In this procedure, approximately 20 µg of copper, dissolved in 2 percent (w/w) HNO_3 solution was electroplated onto each of two zone-refined rhenium sample filaments. After the plating was completed, the filaments were flushed with a stream of high purity water to remove residual acid and dried under a heat lamp. The filaments were mounted into a triple filament configuration, loaded into the mass spectrometer, and the system was evacuated to less than 5×10^{-7} torr.

The mass spectrometric procedure is designed to yield a stable ion current of $4\text{-}7 \times 10^{-13}$ A for the ratio measurement. This is accomplished by an initial adjustment of the ionizing filament to 2120 °C and maintaining this as a constant temperature throughout the analysis. The sample filament temperature is increased in a series of discrete increments, at precise time intervals, to yield predetermined signal intensities. At 30 minutes into the heating pattern, a series of three sets of the $^{63}Cu/^{65}Cu$ ratio measurements are obtained.

Determination of Nickel

The samples from the determination of Pb, Tl, and Cu were again electrolyzed at 2.0 V applied using Pt wire anodes and cathodes. If Cu was noted on the cathode, the procedure was repeated until copper was no longer detected. The solution was then made ammoniacal with 0.5 mL of concentrated ammonium hydroxide and Ni was electrodeposited on the cathode by electrolyzing overnight at 2.4 V applied. The electrodeposited nickel was stripped from the electrode with a few mL of 25 percent (w/w) HNO_3 and the resulting solution was evaporated to dryness.

The altered nickel isotope ratios were then measured by thermal ionization mass spectrometry using a silica-gel procedure to enhance ionization. A platinum filament was coated with a layer of silica-gel. The sample residue was taken up in 2 percent (w/w) HNO_3 and a drop equivalent to about 5 µg of Ni was added to the silica gel. A drop of 0.75 N phosphoric acid was added and dried. The filament was transferred to a nitrogen atmosphere and dried at 900 °C for 15 s (temperature was measured using a pyrometer). The filament was then loaded into the source of the mass spectrometer and the analysis was started when the pressure reached 1×10^{-6} torr. At t = 0, the

temperature was increased to 1150 °C, at t = 5 min to 1200 °C and at t = 10 min to 1250 °C. Ratio data were taken from 20 to 40 min.

RESULTS AND DISCUSSION

To monitor the effect of the increasing influx of anthropogenic sources of trace elements on human health, it is imperative to collect accurate baseline data regarding the concentration of environmentally important substances in the present population so that future changes can be detected. Lead and thallium are two elements that are known to be toxic at trace levels. To achieve the required levels of sensitivity and accuracy, an IDMS method was developed and applied to human tissue samples. Since the available samples were small (0.5 g dry weight) and the expected concentrations low (1-5 ppb Tl), blanks for the procedure would have to be extremely low. A method that accomplished these objectives was developed using SRM 1577a, Bovine Liver, as a control sample.

The results of the determinations of Pb on 0.5-g samples of SRM 1577a, Bovine Liver, yielded an average value of 0.136 ± 0.012 μg Pb/g compared to 0.134 ± 0.08 μg Pb/g for one gram samples using a more extensive separation technique (Pb only). The thallium concentration was found to be 3.1 ± 0.1 ng Tl/g. Lead blanks for the procedure averaged 2.0 ng Pb and the thallium blanks averaged 0.03 ng Tl.

In addition, three samples of SRM 1577a were analyzed for copper with this method using 0.5-g samples. The samples averaged 151.7 μg/g Cu. SRM 1577a has not yet been certified for copper, but determinations by spark source IDMS and atomic absorption on large samples yield concentrations of 152 to 164 ppm Cu.

The results of the determination of these elements in 13 human liver specimens are summarized in Table 1. The trace element concentrations were determined on a dry weight basis and then converted to wet weight concentrations using the concentration factors (dry weight/wet weight) for each specimen (see Table 4, Section 2 for concentration factors). The thallium concentrations are estimated to be accurate to ±1 percent mainly due to blank; the lead concentrations are estimated to be accurate to ±1 percent mainly due to uncertainty in isotopic composition. The copper blanks amounted to about 0.5 percent of the copper present in the samples. The total uncertainty in the copper measurement is estimated to be ±1 percent.

TABLE 1. TRACE ELEMENT CONCENTRATIONS IN HUMAN LIVER SAMPLES AS DETERMINED BY ISOTOPE DILUTION MASS SPECTROMETRY

Sample Identification	Element Concentration (μg/g wet weight) Ni	Cu	Tl ($\times 10^{-3}$)	Pb
L1B0002	≤0.08	5.09	0.335	1.15
L1B0005	≤0.03	4.45	0.370	0.39
L1S0018	≤0.03	5.30	0.292	0.811
L1M0023	N.D.	4.98	1.67	0.17
L1S0027	≤0.1	8.56	0.39	0.992
L1S0037	≤0.2	5.25	0.148	0.263
L2B0041	N.D.	6.08	1.38	0.665
L2B0042	≤0.05	9.01	0.518	0.788
L1B0045	N.D.	3.52	0.363	0.25
L2M0048	≤0.07	6.05	0.300	0.776
L2S0049	≤0.60	3.79	0.441	0.513
L1S0053	N.D.	5.42	1.28	1.66
L2M0062	≤0.07	5.08	0.477	0.254

The results of the lead and thallium determinations and the relative ratios of these two elements are shown in Table 2. Results in Table 2 are reported on a dry weight basis for comparison to the bovine liver SRM.

TABLE 2. CONCENTRATIONS (DRY WEIGHT) OF LEAD AND THALLIUM AND THEIR RELATIVE RATIOS

Sample Identification	Concentrations (dry weight) µg Pb/g	ng Tl/g	Ratios Pb/Tl
L1B0002	4.16	1.21	3436
L1B0005	1.31	1.24	1056
L1S0018	2.83	1.02	2774
L1M0023	0.71	7.07	100
L1S0027	4.38	1.70	2576
L1S0037	1.11	0.62	1778
L2B0041	2.56	5.31	482
L1B0042	3.21	2.11	1521
L1B0045	0.90	1.31	687
L2M0048	3.41	1.32	2583
L2S0049	2.15	1.85	1162
L1S0053	6.05	4.66	1298
L2M0062	1.15	2.16	532
SRM 1577, Bovine Liver	0.134	3.1	43
Earth's Crust[a]			20
Granite[a]			33
Shale[a]			20
Basalt[a]			40

[a]Source - R. D. Reeve and R. R. Brooks, "Trace Element Analysis of Geological Material," J. Wiley & Sons, N. Y., NY, 1979, pp. 80-81.

These results show that thallium concentrations in human liver are in the same range as in bovine liver (1-7 ng Tl/g compared to 3 ng Tl/g in the bovine liver), whereas lead in human liver is considerably elevated over bovine liver (1-6 µg Pb/g compared to 0.1 µg Pb/g). This increase becomes more evident when one compares the ratio of lead to thallium found in various types of samples. For most of the human liver samples, this ratio exceeds 1000/1 while the ratio for bovine liver is near that of the earth's crust and various rocks, 20-40/1. These results could be due to the fact that cattle are less exposed to major anthropogenic sources of either lead or thallium. In contrast, humans are exposed to anthropogenic sources of lead but not of thallium. Thus, monitoring the trend of Pb/Tl ratios could be an indicator of the degree of lifetime exposure to anthropogenic lead sources.

The determination of nickel in the liver samples was attempted on a research basis. The expected concentrations, <1 μg/g, were lower than any previous nickel determinations by IDMS. In addition, the dimethylglyoxime-ion exchange separation method which was used previously provided blanks which were too high for these determinations. The silica gel mass spectrographic method was developed which employed much smaller amounts of Ni for isotope ratio determinations. To reduce the blank, Ni separations were performed by electrodeposition from ammoniacal solution. Preliminary experiments showed good nickel recoveries from synthetic solutions. The recoveries of Ni from the SRM Bovine Liver samples were promising; however, the analytical results showed the SRM material to be inhomogeneous for nickel.

Although these initial experiments for the determination of nickel were promising, difficulties were encountered during the actual analyses of the human liver samples. These difficulties were: [1] low signal for some samples during the MS analysis indicating poor recovery, and [2] a few high blanks. Because of these problems, only upper limits are reported for those samples that gave good intensity during the isotopic analysis. Results for samples with low intensities are not reported. The results for nickel are summarized in Table 1. Even though these analyses were not completely successful, further development of this method may yield a procedure that can be used to determine Ni accurately in human liver specimens at the low ppm level on 0.5 g samples.

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SECTION 7

INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS FOR THE DETERMINATION OF MINERAL AND TRACE ELEMENTS IN HUMAN LIVER SAMPLES

by

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INTRODUCTION

Instrumental Neutron Activation Analysis (INAA) is a well-established multielement technique which has been applied to the chemical analysis of a wide variety of matrices and has been used frequently to analyze human tissues (1-3). Using INAA, inorganic constituents can be measured in a broad dynamic range from virtually 100 percent to 10^{-12} g/g and, in special cases, even lower. For most practical applications, INAA is matrix independent and free of chemical blanks since most materials are analyzed without chemical sample preparation. When applied to biological materials, INAA is sufficiently sensitive for the determination of most of the important pollutant trace elements and essential trace elements since the major constituents (H, C, N, O) do not form measurable interferences. Even these matrix elements can be determined using the Prompt Gamma Activation Analysis (PGAA) method which is currently under evaluation (see Appendix V).

The goal of the INAA protocol for the analysis of human livers is the determination of as many priority elements as possible with the limited amount of sample available in the Pilot NESB Program. The "chemical separation" of the elements is achieved by selection of appropriate irradiation and counting conditions according to the nuclear parameters of the elements to be determined. The nuclear parameters and the conditions for the determination of the 22 elements included in this protocol are summarized in Table 1.

PROCEDURE

Samples of 36 human liver specimens were obtained for instrumental neutron activation analysis. Each sample consisted of one Teflon® jar containing 1-1.5 g of freeze-dried liver homogenate. Duplicate samples from each jar were prepared for analysis by pressing 0.3 g of the material into pellets using a stainless steel pellet press. Transfer of the material and preparation of the pellets were performed in a normal fume hood to avoid potential biohazard exposure of the persons handling the material. The pellets were packaged for irradiation in precleaned polyethylene irradiation bags under clean room conditions.

TABLE 1. NUCLEAR PARAMETERS AND INAA CONDITIONS FOR THE ANALYSIS OF HUMAN LIVER SAMPLES

Isotope	$T_{1/2}$ [a]	Irradiation Time at a Neutron Flux of 5×10^{13} n $cm^{-2}s^{-1}$
^{24}Na	15.00h	60s
^{27}Mg	9.48min	60s
^{28}Al	2.31min	60s
^{38}Cl	37.3min	60s
^{42}K	12.36h	60s
^{46}Sc	83.9d	4h
^{52}V	3.75min	60s
^{51}Cr	27.8d	4h
^{56}Mn	2.576h	60s
^{59}Fe	44.6d	4h
^{60}Co	5.263y	4h
^{64}Cu	12.8h	60s
^{66}Cu	5.10min	60s
^{65}Zn	243.8d	4h
^{76}As	26.3h	4h
^{75}Se	120.0d	4h
^{82}Br	35.4h	4h
^{86}Rb	18.6d	4h
$^{99}Mo \xrightarrow{\beta^-} {}^{99m}Tc$	66.2h→6.02h	4h
^{110m}Ag	250.4d	4h
$^{115}Cd \xrightarrow{\beta^-} {}^{115m}In$	53.5H→4.5h	4h
^{134}CS	2.046y	4h
^{140}La	40.27h	4h
^{141}Ce	32.38d	4h
^{153}Sm	46.8h	4h
^{198}Au	2.697d	4h
^{203}Hg	46.59d	4h

[a] G. Erdtmann and W. Soyka, Die λ-Linien der Radionuklide, Band 1, Berichte der Kernforschungsanlage Jülich-Nr. 1003, April 1974.
[b] Value of 1078.8 keV given in the above reference is incorrect.

Decay Time Before Counting	Count Time	λ's Used (keV)
3h	5min	1368.6, 2754.1
2.5min	5min	843.8, 1014.4
2.5min	5min	1778.8
2.5min	5min	1642.4, 2167.5
3h	5min	1524.7
40-70d	24h	889.3, 1120.5
2.5min	5min	1434.2
40-70d	24h	320.1
3h	5min	846.6, 1811.2
40-70d	24h	1099.3, 1291.6
40-70d	24h	1173.2,1332.5
3h	5min	511.0
2.5min	5min	1039.0
40-70d	24h	1115.5
6-8d	4h	559.1, 657.1
40-70d	24h	135.9, 264.5, 279.5, 400.7
6-8d	4h	554.3, 776.5, 1044.0
40-70d	24h	1076.8[b]
6-8d	4h	140.5
40-70d	24h	657.7, 884.6
6-8d	4h	336.2
40-70d	24h	604.7, 795.8
6-8d	4h	1596.2
40-70d	24h	145.4
6-8d	4h	103.2
6-8d	4h	411.8
40-70d	24h	279.2

Primary standard solutions were prepared by dissolving ultrapure metals or compounds in acids. The acids and water were prepared using a sub-boiling distillation technique (5). The concentrations of these primary standards were 10^{-2} to 10^{-4} g/g. From these primary standards, multielement standard solutions were prepared which have concentrations suitable for the irradiation and counting methods used. The elements are mixed together in groups which provide chemical stability of the mixtures as well as groups which avoid interferences in the gamma spectra. 0.1-mL aliquots of the standards were pipetted onto Whatman 41 5-cm diameter filter papers. After air-drying, the filter papers were folded to 1 cm^2 area or pressed into pellets to match the geometry of the samples. The standards were also sealed in precleaned polyethylene bags.

The samples were irradiated twice to obtain data on all the elements of interest. The first, short irradiation with two subsequent counts provides data on Na, Mg, Al, Cl, K, Ca, V, Mn, and Cu. The second, long irradiation with two subsequent counts provides data on Sc, Cr, Fe, Co, Zn, As, Se, Br, Rb, Mo, Ag, Cd, Cs, La, Ce, Au, and Hg. For the short irradiation one sample and standard were irradiated together for 1 min at a nominal flux of $5 \times 10^{13} n \cdot cm^{-2} s^{-1}$. The samples and standards were transfered to clean, unirradiated, polyethylene bags and counted.

After measurement of the short lived isotopes, the pellets were repackaged in clean polyethylene bags and irradiated for 4 hours. Ten samples and two of each primary standard used were packaged together in one irradiation capsule. The samples were irradiated for a 2 x 2 hour flipped irradiation (which results in uniform neutron dose to all samples and standards) at $5 \times 10^{13} n \cdot cm^{-2} s^{-1}$. After 6 days decay the samples and standards were repackaged in clean polyethylene bags and counted for 4 hours each at a distance of 10 cm from the detector. After a 40 to 70 day decay the samples and standards were counted a second time, this time 5 cm from the detector. The second count was for 24 hours for the samples and 4 to 18 hours for the standards. In addition to the human liver samples, samples of NBS SRM 1577 Bovine Liver were analyzed periodically to check accuracy.

The following equipment was used: The counting of the short-lived nuclides was carried out on a Ge(Li) detector (1.88 keV FWHM at 1332, 3.33% efficiency) connected to a 4096 channel Nuclear Data (ND) 100 analyzer. The counting intervals were preset at 5 min real time. Livetime and pileup corrections were accomplished by accumulating a 60 Hz pulser peak at a non-interfering energy in the spectrum. The data were transmitted to the NBS Nuclear Data 6620 system and stored on hard disk. The long-lived nuclides were counted on "Gamma-X" detectors (1.69 keV FWHM, 2.49%, and 1.65 keV FWHM, 2.50%) and spectral data were accumulated directly in 8192 channels of the ND 6620 system and stored on disk. The ND activation analysis software was used for the data reduction. The spectral data were then transferred to magnetic tape for permanent storage.

RESULTS AND DISCUSSION

The results of the INAA determinations in the human liver samples are reported in Table 2. Results are reported for replicate pellets from each sample. For a given liver sample, the first row of data are for the "A" pellets, the second row for "B" pellets. The uncertainty for each sample is $\pm 1\sigma$ due to counting statistics alone. The results on the SRM Bovine Liver check samples are summarized in Table 3 according to the analysis date. The data for Al are somewhat erratic, both for replicate comparisons in the first nine samples and in the detection limits in later samples. At the $\leq$1 ppm level which occurs in most human liver samples, the determination of Al by INAA is marginal primarily due to counting statistics and the high background from Na and Cl activity. The possibility of some Al contamination from the sample processing of the first few samples exists and this may explain the poor agreement between replicates for these samples (e.g., see samples L1B0005, L2B0041, and L1B0045). Due to counting equipment problems, most of the "A" pellets of the later samples had very high detection limits (i.e., $\leq$2-4) because they were counted after much longer decay times than normal which makes replicate comparisons impossible. However, for samples where comparison with AAS data is possible, agreement was generally good. The concentrations of V, Cr, and As were equal to or below the detection limits obtained by the irradiation and counting procedures employed. These detection limits vary somewhat from sample to sample due to the decay time and the background from other elements in the sample.

An incomplete set of data is reported for the elements Cs, La, Ce, Sm, and Au. These elements were not on the environmental or biological priority lists (see Section 4, Table 1); however, when it was found that they could be measured in some or all of the samples, primary standards for these elements were added to the set making quantitative data possible.

Samples of SRM 1577 Bovine Liver were analyzed (Table 3) as periodic check standards for quality control. It is important to note that over the one year time interval during which these analyses were performed, several different sets of primary standards, different analysts, and some different irradiation and counting conditions were used. The use of SRM's provides a good check of the accuracy of the technique. A few aberrant values were observed which require some comment. In the 10/27/81 short analysis, the following elements Na, K, Mn, and Cu (which were determined in the same count) were between 5 and 10 percent high. As this trend is not observed in the samples analyzed at the same time (L1S0090-B, L1B0100-B, L1S0110-B, L1M0118-B, L1B0121-B, L1M0126-B, L1M0127-B, L1M0131-B, and L1M0140-B) when compared to the 'A' pellets of these samples, the high values were probably due to a counting error in this one count. The Hg values in the SRM 1577 analyses are all very high compared to the certified value. Mercury was apparently volatilized from the standards during irradiation. This problem, which certainly varies in degree from irradiation to irradiation, may account for the poor replicate agreement for Hg in the human liver samples.

TABLE 2. TRACE ELEMENT CONCENTRATIONS IN HUMAN LIVERS AS DETERMINED BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

Sample Identification	Element Concentration (μg/g wet weight)				
	Na	Mg	Al	Cl	K
L1B0002	855±25	170±17	<0.6	1180±40	2980±90
	854± 6	210±30	<0.6	1330±20	2770±80
L1B0005	609± 7	136±11	0.49±0.08	869± 9	2910±70
	630±10	145±14	0.92±0.08	869± 9	3160±70
L1S0018	587± 9	139±13	0.94±0.08	722± 8	2850±80
	581± 9	182±15	<0.3	794± 8	2840±70
L1M0023	1190±35	139±26	<3	1460±40	2210±60
	1240±13	130±30	<0.5	1450±20	2190±70
L1S0027	1232±13	142±19	<0.3	1275±11	2540±60
	1211±13	91±19	<0.4	1336±11	2740±60
L1S0037	1073±16	141±16	0.45±0.11	1214±12	2240±60
	1122±16	108±18	0.44±0.10	1278±14	2490±70
L2B0041	810± 9	150±20	0.49±0.08	1112±10	2960±70
	810± 9	203±19	1.07±0.09	1244±10	3010±70
L2B0042	932±14	128±19	0.43±0.08	1016±12	2200±60
	938±14	103±14	0.38±0.09	1035±12	2240±60
L1B0045	740±11	122±16	0.71±0.10	1061±11	2680±70
	645±10	82±16	0.39±0.08	983±11	2410±60
L2M0048	1900±60	120±20	<0.9	1900±60	1370±60
	1880±10	150±40	<0.9	1880±30	1390±70
L2S0049	1191±13	182±19	<0.4	1366±12	2450±60
	1259±13	117±18	<0.4	1376±12	2600±70
L1S0053	960± 3	140±20	<1	1060±30	2510±70
	970± 3	130±30	<0.6	1180±20	2440±80
L2M0062	980±30	290±50	N.D.	1100±30	2340±90
	1010±10	100±30	2.3 ±0.2	1090±20	2120±60
L1B0074	1120±30	122±17	<0.6	1580±50	2290±70
	1134± 7	160±40	<0.7	1730±20	2520±80
L1S0080	1570±50	<82	<2	2020±60	1890±80
	1604±16	<110	<0.8	1980±20	2130±70
L1M0083	720±20	140±30	N.D.	1040±30	3040±90
	720± 7	150±30	<0.8	1010±20	2800±70
L1S0086	930±30	110±18	0.8 ±0.2	1230±40	2970±90
	960±10	150±30	<0.5	1220±20	2840±70
L1S0090	1540±50	190±30	<4	1620±50	2230±90
	1498± 5	140±20	0.77±0.14	1540±20	2240±70

Element Concentration (µg/g wet weight)				
Sc	V	Cr	Mn	Fe
$(1.97\pm0.03)\times10^{-4}$	<0.03	<0.03	1.34±0.03	522 ± 6
$(3.8\pm0.6)\times10^{-4}$	<0.04	<0.03	1.15±0.02	522 ± 4
$<0.2\times10^{-3}$	<0.02	<0.03	1.31±0.05	133 ± 4
N.D.	<0.02	0.039±0.009	1.27±0.05	130 ± 4
N.D.	<0.02	<0.02	1.50±0.05	375 ± 6
$<1\times10^{-4}$	<0.02	<0.02	1.56±0.05	418 ± 3
	<0.07		0.59±0.03	120 ± 9
$<1\times10^{-4}$	<0.04	<0.02	0.61±0.04	121 ± 6
$<2\times10^{-4}$	<0.02	<0.03	1.74±0.06	229 ± 2
---	<0.02	<0.03	1.82±0.06	213 ± 2
N.D.	<0.02	<0.02	1.72±0.05	198 ± 2
$(7.8\pm0.5)\times10^{-4}$	<0.02	<0.02	1.91±0.05	199 ± 2
---	<0.02		0.89±0.05	
$(2.0\pm0.3)\times10^{-4}$	<0.03	<0.02	0.87±0.05	218 ± 2
N.D.	<0.02	<0.02	1.48±0.05	41.0± 0.5
0.07±0.02	<0.02	<0.02	1.57±0.05	46.4± 0.5
$<1\times10^{-4}$	<0.02	<0.03	1.14±0.04	97 ± 1
N.D.	<0.02	<0.02	1.03±0.04	87 ± 8
$<3\times10^{-4}$	<0.04	<0.05	1.00±0.04	190 ± 2
$<1\times10^{-4}$	<0.06	<0.01	0.93±0.04	192.5± 1.5
N.D.	<0.03	<0.01	1.56±0.06	85.3± 1.0
$(2\pm1)\times10^{-4}$	<0.03	<0.02	1.48±0.06	94.2± 1.0
$(1.5\pm0.3)\times10^{-4}$	<0.06	<0.05	1.32±0.12	504 ± 5
$(3.4\pm0.4)\times10^{-4}$	<0.04	<0.02	1.07±0.03	521 ± 4
$(4.8\pm0.4)\times10^{-4}$	<0.07	0.056±0.008	1.51±0.05	296 ± 3
$(4.6\pm0.2)\times10^{-4}$	<0.04	0.089±0.008	1.32±0.04	298 ±10
$<3\times10^{-4}$	<0.06	<0.04	1.45±0.04	309 ± 4
$<2\times10^{-4}$	<0.05	<0.02	1.41±0.04	316 ± 3
$<1\times10^{-4}$	<0.07	<0.04	0.96±0.04	209 ± 3
$<1\times10^{-4}$	<0.05	<0.02	0.94±0.04	215 ±10
$<2\times10^{-4}$	<0.1	<0.03	1.47±0.05	274 ± 3
$<3\times10^{-4}$	<0.04	<0.04	1.33±0.04	266 ±15
$(3.6\pm0.3)\times10^{-4}$	<0.03	<0.03	1.69±0.05	265 ± 3
$<1\times10^{-4}$	<0.04	<0.03	1.67±0.05	268 ±10
$(2.0\pm0.4)\times10^{-4}$	<0.1	<0.02	1.28±0.04	226 ± 3
$(3.7\pm0.3)\times10^{-4}$	<0.03	<0.04	1.19±0.03	222 ± 2

TABLE 2 CONTINUED

Sample Identification	- - - - Element Concentration (µg/g wet weight) - - - - Co	Cu	Zn	As
L1B0002	0.0605±0.0008	5.9±0.8	57.7 ±0.2	≤0.04
	0.0589±0.0007	5.8±0.8	57.82±0.14	≤0.06
L1B0005	0.039 ±0.001	3.0±0.5	43.3 ±0.9	≤0.03
	0.026 ±0.001	4.6±0.6	44.5 ±0.9	≤0.02
L1S0018	0.0424±0.0006	4.6±0.5	39.74±0.08	≤0.03
	0.0498±0.0006	4.6±0.5	41.9 ±0.4	≤0.06
L1M0023	0.0312±0.001	6.1±1.6	39.4 ±0.9	≤0.02
	0.0310±0.001	3.7±0.8	37.9 ±1.5	≤0.03
L1S0027	0.0342±0.0007	7.0±0.7	97.2 ±0.9	≤0.05
	0.0319±0.0005	7.8±0.8	94.97±0.16	≤0.05
L1S0037	0.0286±0.0002	5.3±0.7	64.5 ±0.1	≤0.05
	0.0319±0.0005	3.6±0.7	65.5 ±0.6	≤0.05
L2B0041		5.1±0.6		
	0.0314±0.0005	5.3±0.5	49.5 ±0.5	≤0.05
L2B0042	0.0437±0.0005	8.1±0.5	51.8 ±0.1	≤0.05
	0.0476±0.0005	10.1±0.8	54.2 ±0.5	≤0.05
L1B0045	0.0316±0.0006	5.0±0.7	28.6 ±0.3	≤0.06
	0.0288±0.0003	4.2±0.6	27.04±0.06	≤0.03
L2M0048	0.0461±0.0007	6.1±1	63.51±0.17	≤0.05
	0.0449±0.0003	6.3±1.0	62.78±0.11	≤0.05
L2S0049	0.0331±0.0002	5.1±0.7	37.64±0.07	≤0.05
	0.0372±0.0005	≤2.4	39.4 ±0.4	≤0.05
L1S0053	0.0485±0.0007	≤3.6	54.37±0.16	≤0.04
	0.0484±0.0004	4.7±0.8	55.25±0.12	≤0.03
L2M0062	0.0514±0.0006	≤21	70.30±0.17	≤0.03
	0.0496±0.0020	4.4±0.7	69.2 ±0.8	≤0.02
L1B0074	0.0434±0.0005	5.8±1.1	50.71±0.13	≤0.05
	0.0445±0.0006	4.7±0.9	52.86±0.13	≤0.03
L1S0080	0.0372±0.0006	≤5	52.8 ±0.2	≤0.11
	0.0358±0.0015	7.8±1.0	55.2 ±1.5	≤0.08
L1M0083	0.0500±0.0006	≤7	62.9 ±0.2	≤0.06
	0.046 ±0.002	5.5±0.8	61.4 ±1.0	≤0.02
L1S0086	0.0525±0.0006	6.9±0.9	58.40±0.15	≤0.04
	0.0523±0.0015	7.0±0.9	58.9 ±1.5	≤0.03
L1S0090	0.0472±0.0005	≤6.6	56.0 ±0.2	≤0.05
	0.0483±0.0005	7.3±0.5	55.52±0.11	≤0.04

Element Concentration (µg/g wet weight)				
Se	Br	Rb	Mo	Ag
0.426±0.003	2.70±0.03	13.7 ±0.9	0.47 ±0.05	N.D.
0.456±0.004	1.64±0.05	13.2 ±0.5	0.57 ±0.04	0.011 ±0.002
0.34 ±0.02	1.84±0.03	6.0 ±0.2	0.25 ±0.04	≤0.01
0.38 ±0.02	1.42±0.02	5.8 ±0.2	0.23 ±0.04	≤0.01
0.430±0.006	1.89±0.17	10.3 ±0.08	0.34 ±0.04	≤0.01
0.424±0.006	2.54±0.04	11.6 ±0.08	0.36 ±0.04	≤0.008
0.37 ±0.04	1.68±0.02	3.6 ±0.4	0.28 ±0.03	N.D.
0.49 ±0.02	1.76±0.03	5.6 ±0.4	0.26 ±0.03	0.012 ±0.001
0.546±0.005	2.48±0.04	6.34±0.07	0.63 ±0.04	≤0.009
0.566±0.005	1.84±0.18	5.73±0.07	0.67 ±0.04	≤0.01
0.381±0.005	4.20±0.02	3.67±0.05	0.63 ±0.04	≤0.009
0.371±0.002	5.50±0.09	4.61±0.05	0.58 ±0.04	≤0.007
0.434±0.003	3.18±0.09	8.11±0.05	0.60 ±0.04	≤0.008
0.373±0.005	3.29±0.02	5.06±0.05	0.53 ±0.04	≤0.02
0.370±0.002	4.30±0.10	5.50±0.05	0.59 ±0.04	≤0.007
0.402±0.006	2.84±0.05	4.76±0.06	≤0.14	≤0.01
0.404±0.003	2.12±0.02	4.21±0.03	0.30 ±0.04	≤0.006
0.634±0.005	8.33±0.07	4.0 ±0.4	0.52 ±0.05	≤0.008
0.643±0.003	9.17±0.09	3.47±0.15	0.38 ±0.04	≤0.004
0.534±0.005	3.02±0.02	5.05±0.05	0.43 ±0.04	≤0.007
0.555±0.005	4.01±0.12	5.91±0.05	0.44 ±0.04	≤0.009
0.613±0.005	2.11±0.02	9.2 ±0.8	0.36 ±0.04	0.0117±0.0021
0.645±0.003	2.28±0.03	8.3 ±0.3	0.32 ±0.04	0.0126±0.0014
0.409±0.004	1.90±0.02	5.1 ±0.4	0.76 ±0.04	≤0.008
0.45 ±0.02	2.37±0.04	4.3 ±0.3	0.69 ±0.04	≤0.006
0.424±0.005	1.78±0.11	4.2 ±0.4	0.58 ±0.05	≤0.008
0.473±0.005	1.98±0.03	4.5 ±0.2	0.55 ±0.04	0.0063±0.0018
0.466±0.005	41.2 ±2.5	3.9 ±0.4	0.62 ±0.06	≤0.008
0.50 ±0.02	47.8 ±0.7	4.5 ±0.3	0.55 ±0.06	0.0096±0.0011
0.509±0.003	1.79±0.02	9.9 ±0.6	0.29 ±0.04	≤0.008
0.543±0.0020	1.61±0.02	9.5 ±0.6	0.35 ±0.03	≤0.007
0.564±0.004	1.77±0.02	8.6 ±0.6	0.60 ±0.03	≤0.007
0.626±0.015	2.17±0.03	8.3 ±0.4	0.83 ±0.04	≤0.007
0.544±0.004	2.33±0.14	6.3 ±0.5	0.98 ±0.04	0.0058±0.0013
0.496±0.004	2.69±0.03	5.5 ±0.3	1.02 ±0.05	≤0.005

TABLE 2 CONTINUED

Sample Identification	- - - Element Concentration (μg/g wet weight) - - - Cd	Cs	La
L1B0002	3.34±0.08	0.0139±0.0008	$(7.2\pm0.3)\times10^{-2}$
	1.1 ±0.1	N.D.	N.D.
L1B0005	0.81±0.06	N.D.	N.D.
	0.84±0.06	N.D.	N.D.
L1S0018	2.81±0.06	N.D.	N.D.
	2.74±0.07	N.D.	N.D.
L1M0023	0.56±0.06	N.D.	N.D.
	0.62±0.06	0.0049±0.0003	0.009 ±0.002
L1S0027	2.21±0.07	N.D.	0.022 ±0.003
	2.07±0.07	N.D.	N.D.
L1S0037	1.58±0.07	N.D.	N.D.
	1.75±0.07	N.D.	≤0.009
L2B0041	N.D.	N.D.	N.D.
	0.83±0.06	N.D.	0.078 ±0.003
L2B0042	0.70±0.06	N.D.	N.D.
	0.66±0.06	N.D.	0.0079±0.0021
L1B0045	1.42±0.07	N.D.	≤0.008
	1.31±0.06	N.D.	N.D.
L2M0048	≤0.25	$(4.8\pm0.7)\times10^{-3}$	N.D.
	0.36±0.08	$(5.4\pm0.4)\times10^{-3}$	≤0.01
L2S0049	≤0.2	N.D.	N.D.
	0.43±0.06	N.D.	≤0.010
L1S0053	2.04±0.08	0.0143±0.0008	0.021 ±0.003
	2.19±0.08	0.0154±0.0005	0.032 ±0.003
L2M0062	3.76±0.09	$(8.7\pm0.7)\times10^{-3}$	N.D.
	4.19±0.08	$(9.1\pm0.6)\times10^{-3}$	≤0.012
L1B0074	1.91±0.22	$(3.6\pm0.8)\times10^{-3}$	$(7.7\pm1.4)\times10^{-3}$
	1.41±0.08	$(2.8\pm0.7)\times10^{-3}$	≤0.009
L1S0080	0.52±0.12	$(3.4\pm0.7)\times10^{-3}$	0.139 ±0.006
	0.56±0.12	$(5.8\pm1.1)\times10^{-3}$	0.132 ±0.004
L1M0083	0.79±0.07	$(8.6\pm0.7)\times10^{-3}$	N.D.
	0.66±0.06	$(8.8\pm0.7)\times10^{-3}$	0.026 ±0.003
L1S0086	0.44±0.05	0.013 ±0.001	N.D.
	0.42±0.06	0.008 ±0.001	0.066 ±0.003
L1S0090	2.15±0.09	0.0068±0.0005	$(5.8\pm0.3)\times10^{-2}$
	1.85±0.08	0.0066±0.0006	$(5.7\pm0.4)\times10^{-2}$

Element Concentration (μg/g wet weight)				
Ce	Eu	Sm	Au	Hg
0.123±0.004	N.D.	≤0.001	N.D.	0.101±0.003
0.110±0.004	$\leq 1.7\times10^{-3}$	≤0.03	$(2.7\pm0.5)\times10^{-4}$	0.110±0.002
N.D.	N.D.	N.D.	N.D.	0.117±0.003
N.D.	N.D.	N.D.	N.D.	0.098±0.003
N.D.	N.D.	N.D.	N.D.	0.029±0.003
N.D.	N.D.	N.D.	N.D.	0.063±0.003
N.D.	N.D.	N.D.	$\leq 2\times10^{-4}$	0.127±0.002
0.022±0.002	N.D.	N.D.	N.D.	0.081±0.004
N.D.	N.D.	N.D.	N.D.	0.054±0.005
N.D.	N.D.	N.D.	N.D.	0.050±0.002
N.D.	N.D.	N.D.	N.D.	0.130±0.002
N.D.	N.D.	N.D.	N.D.	0.131±0.002
N.D.	N.D.	N.D.	N.D.	N.D.
N.D.	N.D.	N.D.	N.D.	0.049±0.003
N.D.	N.D.	N.D.	N.D.	0.052±0.002
N.D.	N.D.	N.D.	N.D.	0.071±0.002
N.D.	N.D.	N.D.	N.D.	0.066±0.003
N.D.	N.D.	N.D.	N.D.	0.061±0.002
≤0.02	N.D.	≤0.001	$\leq 2\times10^{-4}$	0.032±0.003
≤0.006	N.D.	≤0.001	N.D.	0.106±0.001
N.D.	N.D.	N.D.	N.D.	0.027±0.002
N.D.	N.D.	N.D.	N.D.	0.048±0.002
0.028±0.005	N.D.	≤0.001	$(8.8\pm0.5)^{-4}$	0.120±0.003
0.030±0.002	N.D.	≤0.0009	N.D.	0.133±0.001
≤0.011	N.D.	≤0.001	$\leq 2\times10^{-4}$	0.026±0.002
≤0.009	N.D.	N.D.	N.D.	0.037±0.002
≤0.02	N.D.	≤0.0014	$\leq 2\times10^{-4}$	0.081±0.003
≤0.01	N.D.	N.D.	N.D.	0.082±0.002
0.114±0.005	N.D.	$(3.9\pm0.4)\times10^{-3}$	$\leq 4\times10^{-4}$	0.113±0.003
0.119±0.004	N.D.	N.D.	N.D.	0.109±0.004
0.013±0.003	$\leq 1.6\times10^{-4}$	$(8.6\pm0.7)\times10^{-3}$	$\leq 2\times10^{-4}$	0.108±0.003
≤0.02	N.D.	$(8.8\pm0.7)\times10^{-3}$	N.D.	0.069±0.005
0.086±0.004	$\leq 1.7\times10^{-4}$	$\leq 9\times10^{-4}$	$\leq 1.1\times10^{-4}$	0.097±0.003
0.078±0.004	N.D.	N.D.	N.D.	0.087±0.003
0.062±0.003	N.D.	$\leq 1\times10^{-3}$	$\leq 1.7\times10^{-4}$	0.081±0.002
0.053±0.005	N.D.	N.D.	N.D.	0.118±0.003

TABLE 2 CONTINUED

Sample Identification	Element Concentration (μg/g wet weight)				
	Na	Mg	Al	Cl	K
L1S0091	960±30	140±20	≤1	1190±40	2620±90
	963±7	140±30	≤0.7	1300±20	2500±80
L1S0092	1410±40	110±30	≤3.3	1500±40	2330±70
	1410±10	110±20	0.55±0.15	1441±10	2230±50
L1B0100	800±20	140±20	≤2	1190±40	2870±90
	790±10	180±20	1.89±0.17	1170±10	2750±50
L1M0105	1270±40	150±30	≤4	1260±40	2710±110
	1280±10	140±40	≤0.7	1490±20	2780±90
L1S0107	888±26	130±30	N.D.	1205±9	2800±90
	933±10	210±30	1.2 ±0.2	1246±15	2900±70
L1S0110	878±28	182±20	≤1	1300±40	2890±110
	890±10	185±22	1.04±0.14	1200±10	2900±50
L1M0118	1010±30	152±30	≤10	1100±30	2530±80
	1010±10	120±20	0.69±0.12	1060±10	2430±50
L1M0119	1160±40	107±24	29 ±2	1400±40	3090±120
	1166±7	180±40	33.1 ±0.5	1675±18	2970±80
L1B0121	1360±40	102±20	≤2	1750±50	2290±80
	1370±10	110±20	≤0.5	1710±10	2290±50
L1S0123	1100±30	149±25	≤3	1390±40	2320±70
	1138±12	160±30	≤0.6	1370±20	2560±70
L1M0126	760±20	≤270	N.D.	840±30	3070±90
	750±10	190±20	13.0 ±0.2	797±9	3000±50
L1M0127	1660±11	N.D.	N.D.	1580±17	2165±60
	1680±10	117±20	1.76±0.14	1540±10	2230±50
L1S0128	1290±40	260±80	N.D.	1470±50	2220±70
	1330±10	130±40	≤0.8	1820±20	2190±80
L1S0129	1100±30	150±20	≤1	1500±50	2720±80
	1111±7	220±40	1.38±0.23	1700±20	2630±100
L1M0130	1280±40	160±30	≤2	1560±50	2220±80
	1317±15	120±30	≤0.6	1540±20	2250±70
L1M0131	1200±40	N.D.	≤1	1780±60	2610±100
	1240±10	126±25	0.89±0.16	1760±10	2600±50
L1M0140	1220±40	95±30	≤4	1640±50	2600±100
	1230±10	130±20	0.61±0.14	1590±20	2470±50
L1M0141	1580±50	150±20	≤2	1610±50	2040±80
	1613±16	130±30	≤0.6	1580±20	2120±70

Element Concentration (μg/g wet weight)				
Sc	V	Cr	Mn	Fe
$\leq 1 \times 10^{-4}$	$\leq$0.06	$\leq$0.05	1.03±0.03	209 ± 3
$\leq 2 \times 10^{-4}$	$\leq$0.05	$\leq$0.02	1.02±0.03	210 ± 2
$\leq 2 \times 10^{-4}$	$\leq$0.08	$\leq$0.07	0.71±0.03	437 ± 5
$\leq 3 \times 10^{-4}$	$\leq$0.03	$\leq$0.05	0.72±0.02	432 ± 4
$(9 \pm 1) \times 10^{-4}$	$\leq$0.06	$\leq$0.02	1.49±0.05	235 ± 3
$(9.3 \pm 0.8) \times 10^{-4}$	$\leq$0.03	N.D.	1.45±0.02	235 ± 2
$(2.0 \pm 0.2) \times 10^{-4}$	$\leq$0.08	$\leq$0.04	1.24±0.13	287 ± 3
$(3.0 \pm 0.5) \times 10^{-4}$	$\leq$0.05	$\leq$0.02	1.08±0.03	295 ± 2
$(4.3 \pm 1.4) \times 10^{-4}$	$\leq$0.2	0.083±0.011	1.34±0.04	188 ± 3
$(6.9 \pm 0.4) \times 10^{-4}$	$\leq$0.04	$\leq$0.03	1.44±0.04	188 ±10
$(2.4 \pm 0.3) \times 10^{-4}$	$\leq$0.06	$\leq$0.07	1.20±0.03	427 ± 5
$(2.1 \pm 0.4) \times 10^{-4}$	$\leq$0.03	$\leq$0.03	1.17±0.02	421 ± 3
$< 4 \times 10^{-4}$	$\leq$0.2	$\leq$0.03	2.07±0.04	57.8± 1.0
$(4.1 \pm 0.2) \times 10^{-4}$	$\leq$0.02	$\leq$0.02	2.00±0.03	60.1± 0.9
$(2.0 \pm 0.5) \times 10^{-4}$	$\leq$0.10	$\leq$0.08	1.39±0.03	68.4± 1.4
$< 2 \times 10^{-4}$	$\leq$0.06	$\leq$0.02	1.27±0.04	124.9± 1.2
$(2.4 \pm 0.7) \times 10^{-4}$	N.D.	$\leq$0.03	N.D.	126 ± 6
$< 8 \times 10^{-5}$	$\leq$0.05	0.234±0.010	0.95±0.04	139 ± 2
$(1.23 \pm 0.18) \times 10^{-4}$	$\leq$0.03	$\leq$0.04	0.94±0.02	141 ± 1.4
$\leq 9 \times 10^{-5}$	$\leq$0.08	$\leq$0.03	1.06±0.03	304 ± 3
$\leq 1 \times 10^{-4}$	$\leq$0.04	$\leq$0.03	1.10±0.04	318 ±12
$(1.2 \pm 0.1) \times 10^{-3}$	$\leq$2	$\leq$0.05	1.83±0.03	88 ± 2
$(1.4 \pm 0.1) \times 10^{-3}$	$\leq$0.03	N.D.	1.66±0.02	N.D.
$(4.0 \pm 0.7) \times 10^{-4}$	N.D.	$\leq$0.04	0.81±0.22	148 ± 2
$(3.3 \pm 0.2) \times 10^{-4}$	$\leq$0.03	$\leq$0.02	0.84±0.03	147.7± 1.2
$\leq 2 \times 10^{-4}$	$\leq$2	$\leq$0.02	0.75±0.03	273 ± 3
$\leq 2 \times 10^{-4}$	$\leq$0.05	$\leq$0.02	0.62±0.03	272 ± 2
$\leq 2 \times 10^{-4}$	$\leq$0.06	$\leq$0.03	1.15±0.03	125 ± 2
$\leq 3 \times 10^{-4}$	$\leq$0.05	$\leq$0.03	1.12±0.03	122.1± 1.3
$\leq 3 \times 10^{-4}$	$\leq$0.07	$\leq$0.03	0.96±0.04	341 ± 4
$\leq 1 \times 10^{-4}$	$\leq$0.04	$\leq$0.03	1.06±0.04	362 ±15
$\leq 2 \times 10^{-4}$	$\leq$0.05	$\leq$0.02	0.70±0.03	270 ± 3
$(1.4 \pm 0.3) \times 10^{-4}$	$\leq$0.04	$\leq$0.03	0.66±0.02	275 ± 2
$< 2 \times 10^{-4}$	$\leq$0.09	$\leq$0.02	1.27±0.03	260 ± 3
$(1.4 \pm 0.3) \times 10^{-4}$	$\leq$0.03	$\leq$0.04	1.19±0.03	260 ± 2
$\leq 1 \times 10^{-4}$	$\leq$0.06	$\leq$0.02	0.84±0.03	428 ± 4
$\leq 2 \times 10^{-4}$	$\leq$0.04	$\leq$0.03	0.78±0.05	440 ±20

TABLE 2 CONTINUED

Sample Identification	Element Concentration (μg/g wet weight)			
	Co	Cu	Zn	As
L1S0091	0.0376±0.0006	≤3	30.66±0.11	≤0.03
	0.0353±0.0005	4.6±0.8	30.35±0.09	≤0.04
L1S0092	0.0435±0.0006	13 ±2	59.06±0.16	≤0.04
	0.0412±0.0010	10.8±0.5	55.3 ±0.2	≤0.05
L1B0100	0.0564±0.0006	12 ±2	54.7 ±0.2	≤0.05
	0.0584±0.0006	9.7±0.4	55.79±0.13	≤0.04
L1M0105	0.0503±0.0005	≤6.4	63.7 ±0.2	≤0.04
	0.0499±0.0006	9.8±1.1	65.02±0.14	≤0.05
L1S0107	0.075 ±0.009	≤11	64.2 ±0.2	≤0.06
	0.055 ±0.001	7.7±0.7	62.4 ±0.6	≤0.03
L1S0110	0.0548±0.0007	5.6±1	41.81±0.14	≤0.03
	0.0526±0.0006	5.0±0.4	41.25±0.10	≤0.04
L1M0118	0.0592±0.0007	≤9.4	44.70±0.13	≤0.04
	0.0601±0.0007	4.5±0.4	45.62±0.11	≤0.04
L1M0119	0.0508±0.0007	≤6	72.81±0.19	≤0.05
	0.0484±0.0005	5.9±0.8	71.88±0.15	≤0.04
	0.052 ±0.002	N.D.	72.4 ±1.0	≤0.03
L1B0121	0.0339±0.0005	6.9±1	42.64±0.12	≤0.05
	0.0335±0.0004	8.2±0.5	42.97±0.009	≤0.04
L1S0123	0.0458±0.0005	9.0±2	56.14±0.14	≤0.03
	0.046 ±0.002	10.2±0.8	57.0 ±2.0	≤0.03
L1M0126	0.135 ±0.001	≤64	65.49±0.18	≤0.06
	0.131 ±0.001	6.9±0.4	63.65±0.14	≤0.04
L1M0127	0.0446±0.0006	N.D.	48.4 ±0.2	≤0.05
	0.0452±0.0005	3.5±0.5	48.25±0.09	≤0.04
L1S0128	0.0410±0.0005	≤53	40.2 ±0.1	≤0.05
	0.0398±0.0005	12.2±1.0	40.19±0.10	≤0.03
L1S0129	0.0524±0.0007	8.4±1	66.9 ±0.2	≤0.05
	0.0521±0.0006	15.1±0.9	65.59±0.15	≤0.04
L1M0130	0.0367±0.0006	≤4.9	48.9 ±0.2	≤0.04
	0.0381±0.0010	5.9±0.8	52.0 ±2.0	≤0.040
L1M0131	0.0366±0.0005	≤4.1	37.9 ±0.1	≤0.05
	0.0359±0.0006	3.9±0.4	38.92±0.10	≤0.04
L1M0140	0.0458±0.0006	≤6	46.8 ±0.1	≤0.06
	0.0448±0.0006	9.1±0.5	46.85±0.012	≤0.04
L1M0141	0.0275±0.0002	≤4.2	52.66±0.1	≤0.06
	0.0263±0.0010	4.6±0.9	53.0 ±2.0	≤0.07

Element Concentration (μg/g wet weight)				
Se	Br	Rb	Mo	Ag
0.494±0.005	2.26 ±0.02	8.0 ±0.7	0.56±0.04	≤0.006
0.520±0.005	2.45 ±0.03	6.3 ±0.3	0.52±0.04	≤0.006
0.661±0.005	4.58 ±0.03	10.2 ±0.9	0.55±0.04	0.018 ±0.002
0.613±0.008	5.19 ±0.05	7.1 ±0.3	0.48±0.05	0.016 ±0.004
0.599±0.004	2.43 ±0.15	6.1 ±0.6	1.21±0.06	≤0.006
0.515±0.004	2.76 ±0.03	5.8 ±0.3	1.26±0.05	≤0.007
0.45 ±0.01	1.67 ±0.02	9.8 ±0.6	0.38±0.04	0.009 ±0.002
0.492±0.005	1.76 ±0.02	9.3 ±0.4	0.53±0.05	≤0.008
0.640±0.006	1.42 ±0.09	7.9 ±0.6	0.99±0.05	≤0.009
0.652±0.08	1.61 ±0.02	8.9 ±0.5	0.96±0.04	≤0.007
0.643±0.006	2.12 ±0.02	8.2 ±0.8	0.21±0.04	≤0.008
0.621±0.004	2.45 ±0.03	6.7 ±0.3	0.20±0.04	≤0.007
0.566±0.005	1.18 ±0.01	9.4 ±0.7	0.44±0.05	≤0.005
0.554±0.005	1.37 ±0.02	7.31±0.18	0.40±0.05	≤0.006
0.473±0.005	1.08 ±0.02	5.5 ±0.4	0.70±0.04	≤0.009
0.493±0.005	1.12 ±0.02	4.9 ±0.2	0.97±0.05	≤0.007
0.520±0.020	1.127±0.019	5.4 ±0.3	0.69±0.04	≤0.006
0.486±0.004	3.29 ±0.03	4.9 ±0.4	1.00±0.05	≤0.006
0.472±0.004	3.71 ±0.04	3.36±0.18	1.01±0.05	≤0.005
0.494±0.004	1.23 ±0.01	9.6 ±0.7	0.80±0.04	0.0055±0.0016
0.546±0.020	1.44 ±0.02	8.4 ±0.5	0.82±0.04	≤0.006
0.574±0.005	1.35 ±0.02	12.8 ±1.0	0.88±0.06	0.014 ±0.002
0.539±0.005	1.46 ±0.02	9.5 ±0.4	0.97±0.06	$(8\pm2)\times10^{-3}$
0.504±0.005	2.10 ±0.18	5.2 ±0.4	0.39±0.03	≤0.006
0.462±0.002	2.71 ±0.03	5.44±0.14	0.25±0.04	≤0.004
0.494±0.005	1.99 ±0.03	7.4 ±0.5	0.44±0.3	≤0.008
0.535±0.005	2.11 ±0.02	7.4 ±0.3	0.48±0.04	≤0.006
0.674±0.006	2.40 ±0.15	8.8 ±0.6	1.07±0.05	≤0.008
0.687±0.005	2.77 ±0.03	9.6 ±0.4	1.08±0.05	≤0.007
0.496±0.005	2.03 ±0.12	6.4 ±0.6	0.72±0.04	≤0.007
0.553±0.020	2.35 ±0.04	7.7 ±0.4	0.88±0.05	≤0.005
0.442±0.003	2.76 ±0.03	6.4 ±0.4	0.40±0.04	≤0.008
0.439±0.004	3.09 ±0.03	5.50±0.16	0.35±0.04	≤0.007
0.568±0.003	2.71 ±0.04	6.6 ±0.4	0.41±0.04	≤0.007
0.548±0.004	2.67 ±0.03	6.2 ±0.3	1.18±0.05	≤0.007
0.375±0.002	35.6 ±0.4	3.5 ±0.2	N.D.	0.0087±0.0014
0.420±0.020	36.7 ±0.4	3.8 ±0.2	0.43±0.04	0.008 ±0.002

TABLE 2 CONTINUED

Sample Identification	Element Concentration (μg/g wet weight) Cd	Cs	La
L1S0091	1.23±0.07	(7.0 ±0.6)x10^{-}	N.D.
	1.38±0.07	(5.7 ±0.6)x10^{-3}	(8±2)x10^{-3}
L1S0092	1.28±0.08	(1.19±0.08)x10^{-}	N.D.
	1.47±0.08	(8.9 ±1.3)x10^{-3}	(5.7 ±0.3)x10^{-2}
L1B0100	2.39±0.10	(7.0 ±0.6)x10^{-3}	(6.2 ±0.4)x10^{-2}
	2.31±0.08	(6.4 ±0.6)x10^{-3}	(1.11±0.04)x10^{-1}
L1M0105	0.54±0.06	(1.23±0.08)x10^{-2}	N.D.
	1.02±0.07	(1.26±0.07)x10^{-2}	0.024±0.003
L1S0107	2.53±0.10	(6.8 ±0.8)x10^{-3}	0.208±0.006
	2.66±0.08	(6.4 ±0.6)x10^{-3}	0.204±0.004
L1S0110	2.19±0.08	0.013 ±0.001	N.D.
	2.33±0.08	0.013 ±0.001	0.049±0.003
L1M0118	2.9 ±0.1	(1.18±0.07)x10^{-2}	N.D.
	2.90±0.08	(1.31±0.07)x10^{-2}	0.039±0.003
L1M0119	1.62±0.07	(1.29±0.05)x10^{-2}	N.D.
	1.61±0.07	(1.15±0.07)x10^{-2}	0.126±0.004
	1.37±0.07	(1.23±0.05)x10^{-2}	0.037±0.004
L1B0121	≤0.2	(2.0 ±0.5)x10^{-3}	N.D.
	≤0.2	(3.2 ±0.5)x10^{-3}	≤0.01
L1S0123	1.93±0.07	(9.3 ±0.6)x10^{-3}	N.D.
	2.01±0.07	≤0.007	0.058±0.003
L1M0126	5.20±0.12	(1.06±0.08)x10^{-2}	0.160±0.004
	4.95±0.09	(1.10±0.08)x10^{-2}	0.160±0.004
L1M0127	0.63±0.06	(1.05±0.06)x10^{-2}	≤7x10^{-3}
	0.50±0.06	(1.20±0.05)x10^{-2}	≤0.012
L1S0128	1.33±0.06	(9.8 ±0.7)x10^{-3}	N.D.
	1.05±0.08	(1.07±0.07)x10^{-2}	0.025±0.003
L1S0129	2.87±0.11	(2.08±0.09)x10^{-2}	(2.8 ±0.3)x10^{-2}
	2.74±0.08	(1.98±0.08)x10^{-2}	(3.6 ±0.3)x10^{-2}
L1M0130	0.57±0.07	(8.1 ±0.7)x10^{-3}	0.021
	0.42±0.06	(7.0 ±0.4)x10^{-3}	0.028±0.003
L1M0131	0.73±0.07	(1.13±0.06)x10^{-2}	N.D.
	0.76±0.07	(9.9 ±0.7)x10^{-3}	(4.2 ±0.4)x10^{-2}
L1M0140	3.95±0.1	(6.9 ±0.6)x10^{-3}	N.D.
	1.00±0.07	(8.1 ±0.6)x10^{-3}	(4.2 ±0.3)x10^{-2}
L1M0141	≤0.4	0.0122±0.0005	N.D.
	≤0.4	0.013 ±0.001	≤0.012

Element Concentration (μg/g wet weight)				
Ce	Eu	Sm	Au	Hg
≤0.015	N.D.	≤9x10^{-4}	0.0003±0.0001	0.060 ±0.002
≤0.01	N.D.	≤1x10^{-3}	N.D.	0.058 ±0.002
(7.03±0.07)x10^{-2}	N.D.	≤1x10^{-3}	≤2x10^{-4}	0.109 ±0.003
(9.7 ±0.9)x10^{-2}	N.D.	N.D.	N.D.	0.200 ±0.005
0.058 ±0.004	N.D.	(1.20±0.04)x10^{-3}	≤2x10^{-4}	0.0565±0.0019
0.115 ±0.006	N.D.	N.D.	N.D.	0.182 ±0.004
(1.7 ±0.4)x10^{-2}	≤7x10^{-5}	N.D.	≤1.8x10^{-4}	0.070 ±0.003
(1.6 ±0.3)x10^{-2}	N.D.	≤0.001	N.D.	0.0456±0.0019
0.176 ±0.006	N.D.	(3.7 ±0.04)x10^{-3}	(2.5±0.5)x10^{-4}	0.095 ±0.003
0.170 ±0.010	N.D.	N.D.	N.D.	0.104 ±0.005
0.066 ±0.006	N.D.	N.D.	≤2x10^{-4}	0.029 ±0.003
0.065 ±0.005	N.D	≤0.0009	N.D.	0.082 ±0.003
0.060 ±0.005	N.D.	≤0.001	(4.7±0.6)x10^{-4}	0.114 ±0.002
0.052 ±0.004	N.D.	N.D.		0.178 ±0.003
0.048 ±0.004	≤1.5x10^{-4}	N.D.	(2.5±0.7)x10^{-3}	0.026 ±0.007
0.053 ±0.004	N.D.	N.D.	N.D.	0.0461±0.0018
0.052 ±0.004	N.D.	≤0.001	N.D.	0.038 ±0.005
≤0.02	N.D.	N.D.	≤2x10^{-4}	0.078 ±0.002
≤0.014	N.D.	≤0.002	N.D.	0.146 ±0.002
0.075 ±0.004	N.D.	N.D.	≤2x10^{-4}	0.059 ±0.002
0.076 ±0.004	N.D.	≤0.001	N.D.	0.075 ±0.002
0.223 ±0.007	N.D.	0.0051±0.0004	0.047 ±0.001	0.116 ±0.003
0.234 ±0.007	N.D.	N.D.	N.D.	0.203 ±0.007
≤0.014	N.D.	≤0.0012	≤1.5x10^{-4}	0.038 ±0.002
≤0.011	N.D.	N.D.	N.D.	0.0522±0.0018
(2.4 ±0.3)x10^{-2}	≤0.0002	N.D.	≤2x10^{-4}	0.039 ±0.002
(1.9 ±0.3)x10^{-2}	N.D.	≤0.001	N.D.	0.0253±0.0017
(3.4 ±0.4)x10^{-2}	N.D.	≤0.0014	≤7x10^{-4}	0.056 ±0.003
(4.5 ±0.4)x10^{-2}	N.D.	≤0.001	N.D.	0.051 ±0.002
<0.012	N.D.	≤0.0011	≤2x10^{-4}	0.053 ±0.002
(2.5 ±0.3)x10^{-2}	N.D.	N.D.	N.D.	0.051 ±0.002
(4.3 ±0.3)x10^{-2}	≤1.4x10^{-4}	N.D.	≤2x10^{-4}	0.099 ±0.002
(4.2 ±0.4)x10^{-2}	N.D.	N.D.	N.D.	0.083 ±0.002
(4.1 ±0.4)x10^{-2}	≤1.5x10^{-4}	N.D.	≤2x10^{-4}	0.071 ±0.002
0.0431±0.0005	N.D.	N.D.	N.D.	1.300 ±0.003
≤0.008	≤1x10^{-4}	N.D.	≤4x10^{-3}	0.521 ±0.006
≤0.01	N.D.	N.D.	N.D.	0.42 ±0.02

TABLE 3. ANALYSIS OF BOVINE LIVER (SRM 1577) BY INAA

Date	Na	Mg	Al	Cl	K
9/80A	2470±20	570±60	2.7±0.5		9920±220
9/80B	2470±20	580±50	3.2±0.4		9560±250
3/14/81	2290±20	600±160	N.D.	2750±30	9970±210
3/26/81	2300±20	550±110	N.D.	2810±30	9740±200
9/15/81	2400±30	710±100	$\leq$1.8	2990±40	10200±300
10/27/81	2680±20	520±70	3.6±0.5	2800±30	11300±200
9/ 5/80					
6/15/81					
9/23/81					
11/14/81					
Certified Values	2430±130	604±9		(2700)	9700±600

Date	Sc	V	Cr	Mn
9/80A		$\leq$0.07		9.64±0.11
9/80B		$\leq$0.06		10.1 ±0.1
3/14/81		$\leq$1.6		10.9 ±0.6
3/26/81		$\leq$0.7		10.1 ±0.4
9/15/81		$\leq$0.12		10.0 ±0.2
10/27/81		$\leq$0.09		10.8 ±0.5
9/ 5/80	N.D.		N.D.	
6/15/81	$\leq 2\times10^{-4}$		0.126±0.020	
9/23/81	$\leq 3\times10^{-4}$		$\leq$0.10	
11/14/81	$(2.3\pm0.7)\times10^{-4}$		$\leq$0.15	
Certified Values			0.088±0.012	10.3 ±1.0

TABLE 3 CONTINUED

Date	Fe	Co	Cu	Zn
9/80A				
9/80B			189±3	
3/14/81			203±22	
3/26/81			177±13	
9/15/81			198±4	
10/27/81			215±2	
9/ 5/80	249±3	0.260 ±0.003		133.1± 0.4
6/15/81	247±4	0.2412±0.0021		125.6± 0.4
9/23/81	254±3	0.250 ±0.003		132.0± 0.4
11/14/81	254±4	0.253 ±0.002		135.0± 0.3
Certified Values	268±8	(0.18)	193±10	130 ±13

Date	As	Se	Br	Rb
9/80A				
9/80B				
3/14/81				
3/26/81				
9/15/81				
10/27/81				
9/ 5/80	$\leq$0.15	0.99 ±0.03	15.08±0.11	20.4±0.2
6/15/81	$\leq$0.2	1.028±0.010	9.3 ±0.5	20.2±2.0
9/23/81	N.D.	1.12 ±0.14	10.64±0.11	21.8±1.0
11/14/81	$\leq$0.11	1.078±0.013	11.26±0.12	19.6±0.8
Certified Values	0.055±0.005	1.1 ±0.1		18.3±1.0

TABLE 3 CONTINUED

Date	Mo	Ag	Cd	Cs
9/80A				
9/80B				
3/14/81				
3/26/81				
9/15/81				
10/27/81				
9/ 5/80	4.6 ±0.2	0.080±0.015	$\leq$1.0	N.D.
6/15/81	2.7 ±0.2	0.058±0.004	$\leq$0.9	$(9.1\pm1.2)\times10^{-3}$
9/23/81	3.12±0.17	0.049±0.006	$\leq$0.8	$(1.22\pm0.19)\times10^{-2}$
11/14/81	3.28±0.18	0.062±0.006	$\leq$0.9	$(8.8\pm1.8)\times10^{-3}$
Certified Values	(3.4)	(0.06)	0.27±0.04	

Date	La	Ce	Au	Hg
9/80A				
9/80B				
3/14/81				
3/26/81				
9/15/81				
10/27/81				
9/ 5/80	N.D.	N.D.	N.D.	0.122±0.013
6/15/81	$\leq$0.02	$\leq$0.04	$\leq6\times10^{-4}$	0.375±0.006
9/23/81	$\leq$0.04	$\leq$0.05	N.D.	0.426±0.008
11/14/81	$\leq$0.03	$\leq$0.06	N.D.	0.376±0.011
Certified Values				0.016±0.002

NOTE: N.D. in Tables 2 and 3 indicates "not determined".

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SECTION 8

THE DETERMINATION OF NICKEL, COPPER, LEAD, CADMIUM, AND ZINC IN HUMAN LIVER SAMPLES BY VOLTAMMETRY

by

E. J. Maienthal and W. F. Koch

INTRODUCTION

Voltammetry, in particular cathode-ray polarography (1), has been used extensively for the analysis of a variety of materials, including biological samples, for trace metal content (2,3). This electrochemical method is based on the current-voltage relationship of reducible metal ions at a mercury-drop electrode when diffusion to the electrode is the rate-determining step. The potential of the mercury electrode is scanned from about 0.0 to -2.5 volts relative to a saturated calomel electrode. Species can be identified by the potential at which electrochemical reduction occurs. Species can be quantitated by the degree of reduction that occurs as indicated by the diffusion limited current. Concentration is directly proportional to this current and can be calculated through use of a theoretically-derived equation. However, due to the complexity of the equation, standard solutions and calibration curves are customarily used for quantitation. Standard solutions have a further advantage in that matrix effects can be compensated for by simulating the sample matrix in the standard solution. Standard addition techniques can also be employed.

EXPERIMENTAL

Several samples of the cryogenically ground liver specimens have been analyzed by the voltammetric technique. Great care was taken throughout the procedure to avoid contamination. Teflon® jars (which had been cleaned by soaking in 1:1 HCl, then 1:1 HNO_3, followed by a mixture of hot dilute $HClO_4$, H_2SO_4, and HNO_3) were used to transfer the samples from the cold storage facility to the chemical analysis laboratory. Samples were weighed directly into these tared jars. Empty jars were taken through the procedure to serve as blanks. All sample dissolution and preparation were performed in laminar flow hoods. All reagents used were of the highest purity; acids were purified by sub-boiling distillation.

DISSOLUTION PROCEDURE

The lids and interior sides of the Teflon® jars containing the samples were rinsed with about 10 mL of nitric acid into the jars. After standing for 12 hours at room temperature, the samples in the Teflon® jars were heated to about 90 °C on a hot plate until clear yellow solutions were obtained. The samples were then quantitatively transferred to 100-mL Teflon® beakers, rinsing with nitric acid. After addition of 5 mL of perchloric acid, the samples were fumed, washed down again with nitric acid, and fumed to dryness to completely oxidize the organic matrix. Solutions for the establishment of calibration curves were started through the procedure at this point. The walls of the beakers were rinsed with a few mL of hydrochloric acid and the samples again taken to dryness at 90 °C. The residues were dissolved in 1 mL of 10 mol/L hydrochloric acid and diluted to about 25 mL with distilled water in a separatory funnel. Iron, which will interfere with the voltammetric measurement, was extracted into 20 mL of isobutyl acetate and discarded. The aqueous layer from each separatory funnel was transferred back to Teflon® beakers. The organic material remaining from the extraction was destroyed by fuming to dryness with 10 mL of nitric acid and 2 mL of perchloric acid. The samples were redissolved in 0.5 mL of hydrochloric acid and 10 mL of water. A few grains of ascorbic acid were added to each to reduce any residual iron.

NICKEL EXTRACTION AND DETERMINATION

The samples, free of organic material and virtually free of iron were transferred to separatory funnels. One mL of 1 percent dimethylglyoxime was added to each.

The nickel complex was separated from the other metals with three extractions (15, 10, and 10 mL portions respectively) into chloroform. The three extracts were combined in a Teflon® beaker, evaporated to dryness at 90 °C and treated with nitric acid and perchloric acid to destroy the organic material. After rinsing the walls of the beakers with hydrochloric acid, the samples were evaporated to dryness. The nickel was redissolved in 1.0 mL of 1:40 sulfuric acid:water and 0.25 mL of pyridine. The resulting solution was transferred to a polarographic cell and deaerated with argon for 10 minutes. The nickel reduction peak was measured at -1.1 volt relative to a mercury-pool anode with a differential cathode ray polarograph. The second cell of the polarograph contained the blank solution.

COPPER, LEAD, CADMIUM, AND ZINC DETERMINATION

After the extraction of the nickel complex into chloroform, 10 mL of ammonium hydroxide and 7 mL of 0.1% sodium diethyldithiocarbamate (precleaned by chloroform extraction) was added to the aqueous portion in each separatory funnel. The copper, lead, cadmium, and zinc complexes were then extracted with 15, 10, and 10 mL portions of chloroform. The three chloroform extracts of each sample were combined in a Teflon beaker, evaporated to dryness, and treated with nitric and perchloric acids to destroy the organic material. After rinsing the interior sides of the beaker with hydrochloric acid, each sample was evaporated to dryness. The residue was redissolved in 2.0 mL of

ammonium acetate-acetic acid solution (pH = 4.7). After deaerating with argon, the reduction peaks were measured with a differential cathode ray polarograph using the second cell to contain the blank solution. The peaks occurred at -0.35 V, -0.7 V, -0.9 V, and -1.3 V relative to a mercury-pool anode for copper, lead, cadmium, and zinc, respectively.

RESULTS

The results of the determination of nickel, copper, zinc, cadmium, and lead in 24 human liver samples are summarized in Table 1.

The values for nickel are reported as "less than" values and are in the proximity of the minimum detection limits of the procedure. It is anticipated that additional research in the areas of background correction and reagent purification would lead to lower detection limits and more quantifiable values for nickel.

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TABLE 1. TRACE ELEMENT CONCENTRATIONS IN HUMAN LIVER SAMPLES AS DETERMINED BY VOLTAMMETRY

Sample Identification	- - Element Concentration (μg/g wet weight) - - Ni	Cu	Zn	Cd	Pb
L2M0062	≤0.04	4.73	71.7	2.76	0.25
	≤0.1	4.48	70.9	3.31	0.28
L1B0074		6.50	89.5	1.59	0.627
		6.44	67.0	1.66	0.670
L1S0080	≤0.02	3.75	81.7	0.275	0.352
	≤0.02	4.26	71.7	0.565	0.474
L1M0083	≤0.05	5.58	71.4	0.25	0.74
	≤0.05	5.21	69.5	0.28	0.72
L1S0086	≤0.05	7.85	40.6	0.15	1.2
	≤0.05	6.94	33.1	0.21	1.1
L1S0090	≤0.1	7.14	29.7	1.0	0.28
	≤0.1	11.8	40.6	0.84	0.79
L1S0091	≤0.03	4.72	32.7	0.76	0.20
	≤0.03	4.94	31.2	0.88	0.27
L1S0092	≤0.1	2.88	16.1	0.55	0.17
	≤0.1	9.88	26.0	0.85	0.79
L1B0100	≤0.06	7.39	43.1	2.06	0.440
	≤0.06	6.71	55.3	1.95	0.514
L1M0105	≤0.05	4.86	59.6	0.949	0.721
	≤0.05	4.65	58.9	0.758	0.716
L1S0107	≤0.05	6.23	59.0	1.42	0.58
	≤0.1	6.99	56.3	1.67	0.66
L1S0110	≤0.06	4.47	39.4	1.41	0.63
	≤0.1	3.66	38.6	1.67	0.70
L1M0118	≤0.06	3.00	30.0	2.64	0.40
	≤0.06	2.87	33.5	3.00	0.38
L1M0119	≤0.05	1.69	71.0	1.10	0.12
L1B0121	≤0.1	7.10	34.0	0.18	0.23
	≤0.1	8.25	23.0	0.15	0.28
L1S0123	≤0.06	5.47	55.3	1.4	1.03
L1M0126	≤0.06	5.28	63.0	2.68	0.357
L1M0123	≤0.04	2.62	46.6	0.455	0.14
L1S0128	≤0.05	6.75	39.2	0.953	0.434
L1S0129	≤0.06	6.98	46.1	1.27	0.15
L1M0130	≤0.05	5.73	48.8	0.345	0.429
L1M0131	≤0.05	3.63	37.8	0.65	0.22
	≤0.1	4.06	35.7	0.73	0.35
L1M0140	≤0.06	6.21	49.4	1.02	0.413
L1M0141	≤0.04	3.12	57.5	0.358	0.399

SECTION 9

RESULTS OF THE INORGANIC ANALYSIS OF THE FIRST YEAR HUMAN LIVER COLLECTION

by

R. Zeisler

SUMMARY OF RESULTS

The distribution of 31 trace elements in 36 human liver specimens is summarized in Figure 1. This figure is a plot of the more than 1,000 data points obtained for the analysis of human liver samples during the first year and also includes the concentration ranges reported for human livers by Iyengar et al., (1). These results are also summarized in Table 1 (e.g., mean value, median value, and range). Although many samples are prepared for analysis and storage by freeze drying, the pilot NESB program is currently reporting analytical results on the basis of fresh weight. An advantage of fresh weight is its direct relation to reality and it appears to be a basis which can be reliably obtained. By using the adopted sampling protocol which requires immediate sealing of the samples in Teflon® bags and deep freezing, the loss of moisture after sampling is minimized. Teflon® serves as an effective barrier for water (2), and the cryogenic conditions reduce the partial pressure of volatile species.

Accuracy and Precision of the Analytical Data

The accuracy of the applied methods has been demonstrated and the analytical imprecision can be minimized when the analytical procedures are optimized for the determination of a single element. For example, IDMS provides data of high precision and accuracy on thallium and lead with an estimated measurement error of <1 percent. AAS, NAA, and VOL have repeatedly demonstrated their capabilities in determining many of the priority elements with uncertainties ranging from 1 to 5 percent. However, a quantitation of the accuracy and precision of an individual result is virtually impossible unless it consists of many observations and statistics can be applied. The implemented analytical protocol provides only single observations for many elemental data points. However, conclusions about the accuracy of the measurements may be possible from a careful evaluation of those data points which have been obtained by multiple observations. Cadmium has been selected for this purpose because three analytical techniques were used and the elemental concentrations in the data set vary by a factor of 25 with the lowest values close to the detection limit obtained by the applied procedures.

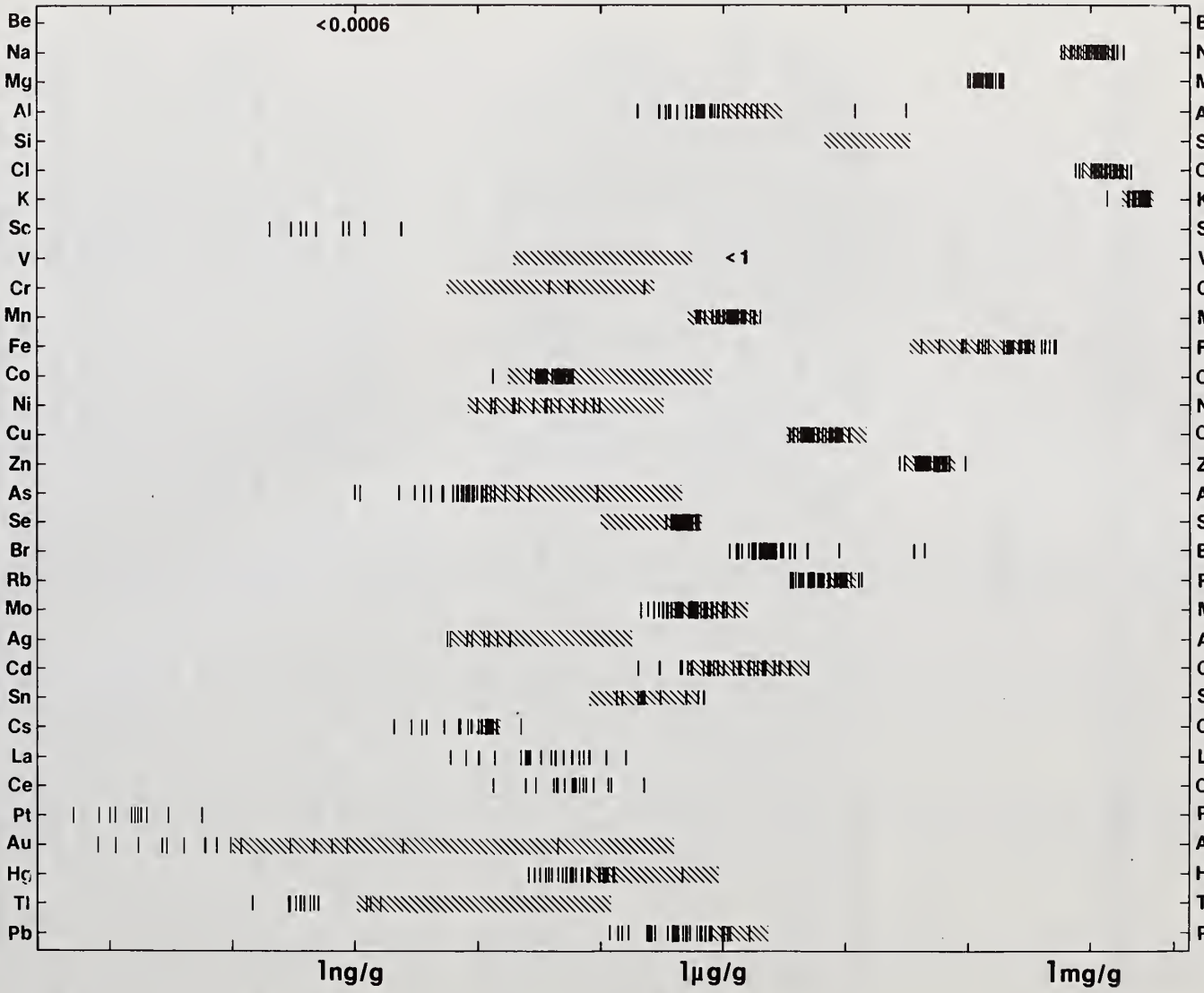

Figure 1. Elemental concentrations in 36 individual liver specimens and concentration ranges reported in the literature. NESB data represented by one line for each data point. Literature ranges represented by the shaded areas.

TABLE 1. TRACE ELEMENT LEVELS IN 36 HUMAN LIVER SPECIMENS (CONCENTRATION IN μg/g WET WEIGHT)

Element	Number of Samples	Mean	Standard Deviation	Median	Range
Be	0	---	---	---	<0.0006
Na	36	1091	343	1105	185-1890
Mg	35	146	26	140	100-195
Al	36	2	5	0.7	0.20 -31.1
Cl	36	1370	320	1370	760-2100
K	36	2520	350	2520	1380-3040
Sc	14	5.7×10^{-4}	6.1×10^{-4}	---	$<1.0\times10^{-4}$-0.0024
V	0	---	---	---	<0.02
Cr	3	0.11	0.11	---	<0.01 -0.234
Mn	36	1.2	0.4	1.2	0.60 -2.03
Fe	36	230	120	223	43-510
Co	36	0.042	0.010	0.045	0.0135-0.0605
Ni	18	0.037	0.027	0.022	<0.010 -0.100
Cu	36	6.3	2.0	5.7	3.50 -10.8
Zn	36	53	13	54	27.8 -96.1
As	34	0.012	0.016	8.9×10^{-3}	<0.001 -0.097
Se	36	0.50	0.080	0.50	0.350 -0.650
Br	36	4.7	8.9	2.2	1.11 -44.5
Rb	36	7.0	2.6	6.3	3.54 -13.5
Mo	36	0.57	0.25	0.54	0.21 -1.23
Ag	10	9.8×10^{-3}	4.0×10^{-3}	---	<0.005 -0.018
Cd	36	1.6	1.1	1.4	0.20 -4.96
Sn	9	0.35	0.22	0.22	0.135 -0.712
Cs	27	9.5×10^{-3}	4.1×10^{-3}	9.8×10^{-3}	2.0×10^{-3}-0.020
La	24	0.045	0.036	0.026	7.0×10^{-4}-0.160
Ce	18	0.066	0.49	0.041	$<1.4\times10^{-3}$-0.221
Pt	11	1.8×10^{-5}	1.5×10^{-5}	1.5×10^{-5}	5.0×10^{-6}-5.7×10^{-5}
Au	20	0.003	0.010	2.0×10^{-4}	8.0×10^{-6}-0.047
Hg	35	0.086	0.074	0.070	0.026 -0.47
Tl	13	6.1×10^{-4}	4.9×10^{-4}	3.9×10^{-4}	1.48×10^{-4}-16.7×10^{-4}
Pb	36	0.55	0.34	0.46	0.120 -1.660

The precision of the single determinations is a function of the concentration for all three techniques. Figure 2 was derived from the actual measurements of cadmium in the 36 human liver specimens. Clearly, concentrations above 1 μg/g can be determined reliably with less than 7 percent imprecision using any one of the methods. NAA and VOL significantly increase their precision with higher concentrations when compared to AAS. Above 1 μg/g the observed standard deviation of the data obtained by all three techniques is in the range from 0.5 to 10 percent. A point by point evaluation did not reveal a bias of any of the techniques to lower or higher values, therefore the data can be regarded as accurate within the limits of the precision. In the concentration range below 1 μg/g, the results have to be weighted by the much more favorable precision of AAS. The other two techniques provide data with deviations from the AAS value of up to 30 percent; thus the usefulness of these techniques is reduced to the possible discovery of gross errors. The cadmium determinations illustrate a problem encountered in environmental trace element measurements where large concentration ranges exist. In such cases a combination of different techniques is most likely to provide the most reliable data.

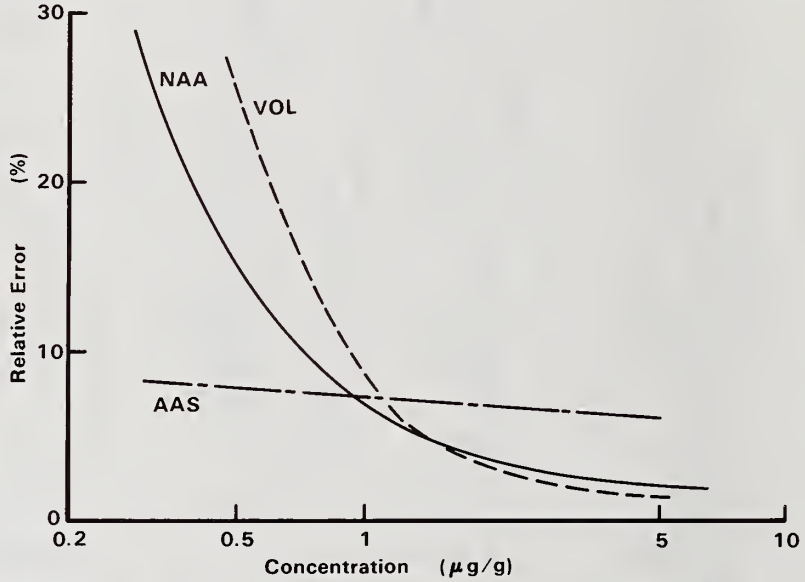

Figure 2. Imprecision of the analytical techniques under the constraints of the analytical protocol as observed for the determination of cadmium in human liver.

A similar evaluation was performed for the other elements where observations by different techniques were available. The respective ranges of elemental concentrations and corresponding relative errors of the techniques is summarized in Table 2. For each of these elements at least one method can provide measurements of most concentrations at a level of precision suitable for the storage evaluation i.e., imprecision <10 percent (see reference 2 for discussion of the storage evaluation study). For NAA the imprecision for most priority elements is between 1 and 7 percent for the observed concentrations, while AAS and VOL are providing data with 2 to 20 percent imprecisions depending on the concentration found. Only in the case of aluminum did the

currently applied techniques, (the combination of AAS and NAA) provide results with a 10 to 15 percent imprecision. However, good agreement was found between the methods especially for the few very high values of aluminum where the imprecision was less than 2 percent. In general, the limited quantity of material for multielement determination on individual samples did not excessively add to the uncertainties of the various techniques.

TABLE 2. COMPARISON OF DYNAMIC IMPRECISION OF FOUR ANALYTICAL TECHNIQUES

Element	Concentration Range (μg/g wet weight)	Percent Imprecision			
		AAS	IDMS	NAA	VOL
Al	0.3 - 2(31)	22- 5(1.6)	---	>30 -9(1.5)	---
Mn	0.6 - 2	25- 5	---	4.5-1.5	---
Cu	3.5 -11	7- 2	0.5	20 -6	6-1
Zn	28 -96	---	---	0.4-0.2	25-2
Se	0.4 -0.65	18-14	---	1.2-0.9	---
Cd	0.3 - 5	8- 6	---	23 -2	>30-1.5
Pb	0.12- 1.7	---	0.2	---	15-3

DISCUSSION OF SOME FINDINGS

Conclusions on environmental impacts, health related statements, etc. are not readily available from the small number of specimens analyzed, especially since the specimens in this first year of analyses were not selected to represent the population or parts of the population (see Table 4, Section 2 for information on the donors). However, some interesting findings can be pointed out to illustrate the potential utility of a specimen banking program in relation to monitoring environmental trends.

A significant result of this study is the very narrow range of concentration data for many essential trace elements (figure 3) as compared to the wide scatter of pollutant element concentrations (figure 4). 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, these broad ranges for the pollutant elements in the data may point to sources of pollution to which the individuals were exposed.

The range of the selenium data is even lower if individuals from the same geographical area are studied. Evaluation of the data by the NBS Statistical Engineering Division revealed a statistically significant difference in average Se values for samples from Baltimore (0.44 μg/g) compared to Minneapolis (0.51 μg/g) and Seattle (0.56 μg/g). Similar evaluations indicated that possible geographic differences may exist for several other

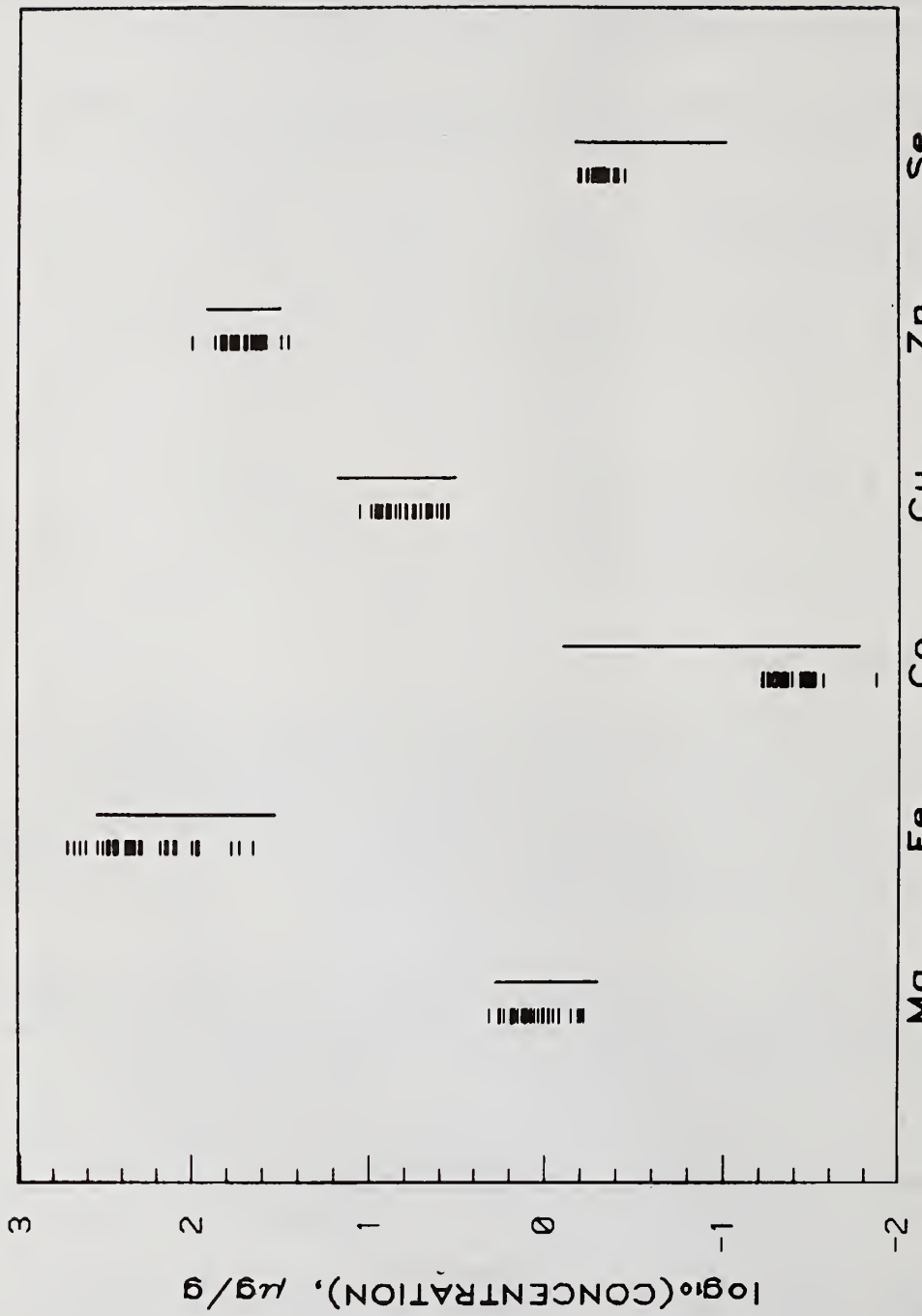

Figure 3. Concentrations of six essential trace elements in human liver. Solid lines indicate range of data found in literature.

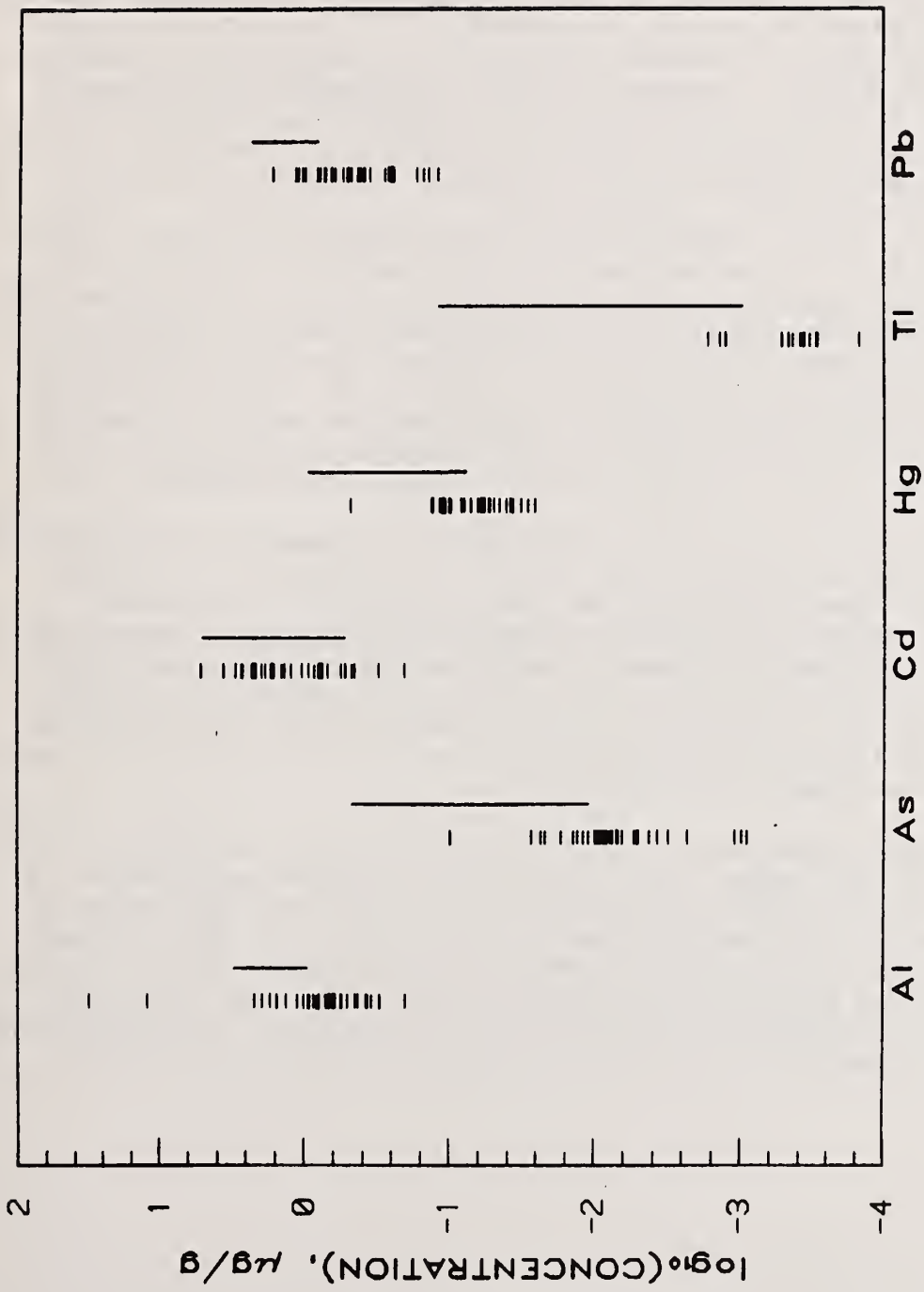

Figure 4. Concentrations of six pollutant trace elements in human liver. Solid lines indicate range of data found in literature.

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 possible correlations will increase significantly. Because of concern about possible analytical error, some data points which exceeded the previously reported ranges were also investigated. The highest iron value, 522 μg/g, was from a 40-year steel worker, while the highest zinc value, 96.1 μg/g, was found in the liver of an individual who worked in a galvanizing plant.

Many pollutant trace elements were found at concentration levels which are on the low side or below previously reported data (1). Specifically, levels of aluminum, arsenic, thallium, and lead are significantly lower than the concentrations reported in the literature (see figure 3). One possible interpretation of these differences is that the observed trends are the consequence of the careful contamination control in the Pilot NESB Program. The precautions taken to preserve the integrity of the samples, especially the minimization of possible sources of contamination during sampling and sample preparation, would naturally result in lower concentrations.

The selection of the specimens and possible low exposure of most of the donors to pollutant sources may offer another explanation for the low pollutant trace element concentrations. Recent significant and general improvements in the environmental concentrations and therefore reduced human exposure to those elements may have already resulted in lower human body burden. In particular for the case of lead, this interpretation of the results is supported by the significant decrease of lead levels in the air since about 1974 (compare for example Ref. 3 and 4) and the latest findings of lower blood lead levels in children (5,6).

Also a direct comparison of 1973 data from Baltimore residents (7) with the data in this study shows this trend within a specified geographical location. In 1973 the mean lead 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 the mean lead concentration of 8 livers was 0.58 μg/g with a range of 0.25-1.15 μg/g. However, there is a significant element of uncertainty in this comparison, since the applied analytical approaches cannot be compared. This uncertainty can only be overcome with a formal and documented monitoring and banking program.

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SECTION 10

DEVELOPMENT OF ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF ORGANOCHLORINE COMPOUNDS IN HUMAN LIVER SAMPLES

by

W. F. Kline, C. F. Allen, S. N. Chesler, L. R. Hilpert, and S. A. Wise

INTRODUCTION

In the Pilot National Environmental Specimen Bank Program (NESB), environmental samples will be analyzed for both inorganic and organic constituents. During the first year of sample collection in this program, the human liver samples were analyzed only for trace elements, and analytical methods for selected organic pollutants were evaluated for implementation for the second year samples.

In contrast to trace element analyses, screening for large numbers of organic compounds is difficult because of the requirements of specific extraction, digestion, and/or isolation procedures for various compound classes. As a result, two classes of organic compounds, organochlorine compounds and polycyclic aromatic hydrocarbons (PAH), have been selected for inclusion in the current analytical protocol for the pilot NESB program. Additional classes of compounds will be added to the program as resources increase and analytical expertise for these groups is developed. For the human liver samples the major focus of the organic analyses is the determination of organochlorine compounds (i.e., organochlorine pesticide residues) due to their widespread use and their abundance in human tissues. Because of the extremely low levels of PAH expected in human liver samples (possibly due only to sample contamination), PAH will be measured only in selected liver samples. However, for the analyses of the second specimen type, mussels, the determination of PAH will be a major concern in addition to the organochlorine compounds.

EXPERIMENTAL

Sample Preparation

The human liver samples are analyzed for organochlorine compounds following the general scheme of solvent extraction, liquid chromatographic cleanup, and capillary gas chromatographic analysis with selective and sensitive electron capture detection. An extraction/cleanup procedure used for liver analysis by investigators in the Pilot Environmental Specimen Bank Program of

the Federal Republic of Germany was adopted for use in the NBS pilot program (1). A 2 g sample of the liver homogenate (prepared by cryogenic homogenization as described in Section 3) was thoroughly mixed at room temperature with approximately 50 g of anhydrous sodium sulfate in an agate mortar and pestle. The resulting mixture was a dry powder. The liver/sodium sulfate mixture was placed in a 22 mm x 350 mm glass column with about 1 cm of sodium sulfate on the top and bottom of the sample. Solutions containing the internal standards (pentachlorobenzene, aldrin, and/or decachlorobiphenyl) were added at the top of the sample column. The liver/sodium sulfate mixture was then extracted by eluting 200 mL of a 2:1 mixture of hexane:acetone (all solvents were commercially available "distilled in glass" or "pesticide analysis" grade) through the column. The extract was concentrated to about 50 mL using a rotary evaporator. An aliquot (1 mL) was removed, evaporated to dryness using a rotary evaporator, and the residue weighed to determine the percent extractable fat (lipid). The bulk of the extract was transferred to the top of a 22 mm x 350 mm glass column packed with 25 g of 60-80 mesh Florisil (activated by heating at 400 °C overnight, then deactivated by adding 3 percent by weight of water). The chlorinated hydrocarbon pesticide residues were eluted from the column in the initial fraction of 150 mL of 20 percent methylene chloride in hexane. This fraction was concentrated in a rotary evaporator and then analyzed by gas chromatography (GC) with electron capture detection or by gas chromatography-mass spectrometry (GC-MS).

Quantitation of Organochlorine Pesticide Residues by Gas Chromatography (GC)

For the GC analyses with electron capture detection, the chromatographic separations were performed on a fused silica SE-52 capillary column (30 m x 0.25 mm i.d.). The samples were injected at an initial temperature of 200 °C and the temperature was then programmed at 3 °C/min to 290 °C. Standards of the compounds of interest were run under identical conditions to determine retention times and electron capture detector response. Quantitation was based on comparison to internal standards of pentachlorobenzene, aldrin, and decachlorobiphenyl.

Quantitation of *p,p'-DDE* in Human Liver Extracts by GC-MS

For the GC-MS determinations the GC separations were performed on a 30 m x 0.25 mm i.d. fused silica SE-30 wall-coated, capillary column which was interfaced directly to the ion source of a quadrupole mass spectrometer. The column was temperature programmed from 150 °C to 275 °C at a rate of 4 °C/minute. The injection port and GC-MS interface were maintained at 275 °C. Under these conditions the aldrin (internal standard added during sample preparation) and *p,p'-DDE* (1,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene) were eluted as symmetrical peaks at approximately 13 and 18 minutes, respectively.

Selected ion monitoring detection was used to enhance sensitivity and insure selectivity by minimizing any co-eluting interferences. Two characteristic, intense ions were monitored for both the *p,p'-DDE* and the aldrin internal standard. In each case the ions monitored contain chlorine atoms which yield characteristic isotope patterns. Ions at m/z 246 and 248 were monitored for the *p,p'-DDE* and ions at m/z 263 and 265 were monitored for

aldrin. Ion intensity ratios for each analyte in the samples were compared to ion intensity ratios for standards of the pure compounds to insure that there were no co-eluting interferences. A mass spectrometer dwell time of 100 ms was used to insure at least 20 data points across each chromatographic peak.

A solution of pure aldrin and *p,p'-DDE* was run under identical conditions as the sample extracts to determine relative response factors in the selected ion monitoring mode. Determinations of the *p,p'-DDE* concentration in the liver extracts were made based on the selected ion record peak areas, the aldrin (internal standard) spike concentration, and the experimentally determined relative response factors.

RESULTS AND DISCUSSION

The analytical methodology for the determination of organochlorine compounds in human liver has been developed and applied to several samples from the Pilot National Environmental Specimen Bank Program. The gas chromatogram of one of the samples is shown in Figure 1A. The major chlorinated compound in the samples analyzed was identified by GC and MS as *p,p'-DDE* which is a major metabolite of *p,p'-DDT* (1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane). Quantitative results for the determination of *p,p'-DDE* in three samples are summarized in Table 1. Table 1 also includes the results of an interlaboratory sample split with the U.S. EPA Health Effects Research Laboratory (2). Trace quantities (5-30 ng/g wet weight) of the following organochlorine species were also present: hexachlorobenzene (HCB), β-isomer of hexachlorocyclohexane (β-BHC), *trans*-nonachlor, dieldrin, *p,p'-DDD* (2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane), and *p,p'-DDT*. These compounds will be measured in ∿30 samples/year during the pilot specimen banking program. Heptachlor epoxide and oxychlordane are also present but coelute as one peak under these GC conditions. Trace quantities of polychlorinated biphenyl (PCB) isomers were found in the sample shown in Figure 1A. Total concentration of PCB isomers will also be determined on selected samples.

TABLE 1. DETERMINATION OF *p,p'-DDE* IN HUMAN LIVERS BY GC-MS

	- - - - -(μg/g wet weight) - - - - -		
Sample I.D.	GC[a]	GC/MS	GC (EPA)[d]
B0025	0.72 ± 0.04	0.77[b]	
	0.75 ± 0.04	0.81[b]	
M0136	0.23 ± 0.01	0.23[c]	0.26
S0129	0.35 ± 0.01	--	

[a]Uncertainty based on four determinations, reported as one standard deviation from the mean.
[b]Single determinations.
[c]Mean of two determinations, range of ±7 percent.
[d]Single determination.

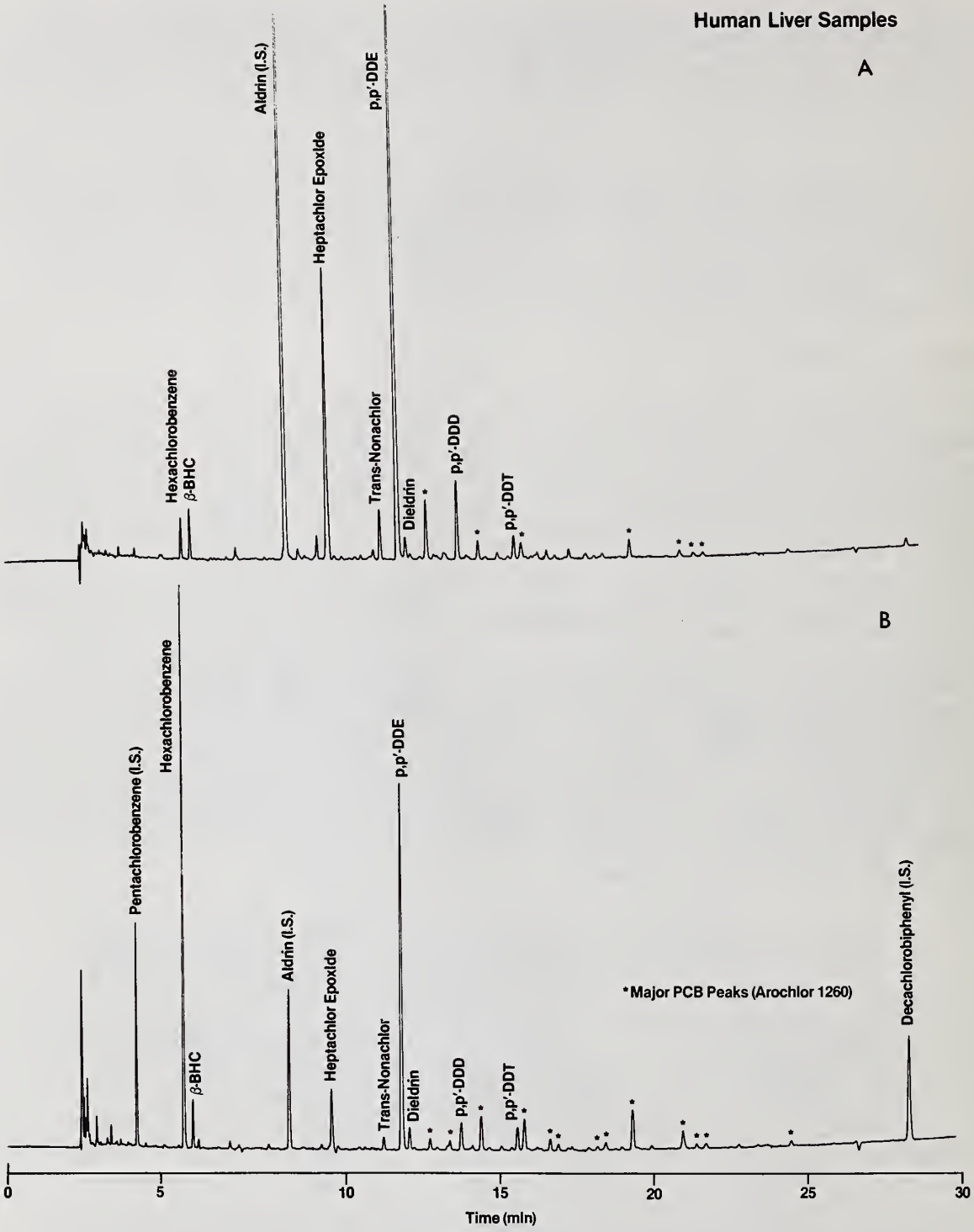

Figure 1. Gas chromatograms from the analysis of human liver samples. (A) Liver sample from NBS/EPA Pilot Program, (B) Liver homogenate from German Pilot Environmental Specimen Bank Program.

Several studies have reported data for selected pesticide residues (primarily HCB, DDE, and dieldrin) in human liver samples (3-6). Kraul and Karlog (3) reported ranges of DDT and metabolites (predominately DDE) at 0.62-22 μg/g extractable fat (mean of 6.0) and dieldrin at 0-1.07 μg/g extractable fat (mean of 0.29) in human livers for 77 samples from Denmark. Bjørseth et al., (4) found mean levels of HCB and DDE of 0.15 and 1.87 μg/g extractable fat for 10 liver samples from Norway. The results of the analyses samples from the Pilot NESB program will add considerable information to the existing data base for pesticides in human livers.

A sample of a human liver homogenate from the German Pilot Environmental Specimen Bank was analyzed as part of an interlaboratory comparison of methods. The gas chromatogram from the analysis of the German liver homogenate is shown in figure 1B and the quantitative results are summarized in Table 2. The quantitative results from the analysis of a liver sample from the NBS pilot program are also given in Table 2 for comparison. Of particular interest in comparing the two samples is the large quantity of hexachlorobenzene present in the German Sample as compared to the U.S. sample. In addition, when quantitation is on an extractable fat basis, the U.S. sample is higher in concentrations of several compounds. Analyses of several liver samples from the U.S. pilot program indicate that the levels of HCB are approximately a factor of 10 lower than the German homogenate. Acker and Schulte (7) reported higher levels of HCB in human fat from Germany when compared to fat samples from the Norwegian study (4), 6.4 vs. 0.11 μg/g extractable fat.

TABLE 2. CONCENTRATIONS OF SELECTED ORGANOCHLORINE PESTICIDE RESIDUES IN HUMAN LIVER SAMPLES[a]

	German Homogenate[b]		U.S. Sample[c]	
Compound	ng/g (wet weight)	ng/g extractable fat	ng/g (wet weight)	ng/g extractable fat
Hexachlorobenzene	413 ± 20	3110 ± 260	8 ± 3	190 ± 66
α-BHC	---	---	0.9 ± 0.1	23 ± 2
β-BHC	88 ± 9	670 ± 120	44 ± 12	1100 ± 330
trans-nonachlor	8 ± 1	60 ± 15	27 ± 6	670 ± 170
p,p'-DDE	305 ± 9	2310 ± 315	195 ± 59	4840 ± 1590
Dieldrin	22 ± 4	170 ± 40	83 ± 38	2030 ± 960
p,p'-DDD	34 ± 5	260 ± 60	---	---
p,p'-DDT	25 ± 5	190 ± 40	25 ± 6	606 ± 163

[a]Measurements based on three samples, uncertainty is ±1σ calculated standard deviation based on 2 degrees of freedom.

[b]13% average extractable fat.

[c]4% average extractable fat.

The gas chromatograms shown in figure 1 contain aldrin, pentachlorobenzene, and/or decachlorobiphenyl as internal standards for quantitation. Recent results indicate that quantitation based on aldrin provides more reproducible results than pentachlorobenzene or decachlorobiphenyl (see Table 3). In addition, aldrin is rarely found in biological samples since it is readily converted to dieldrin.

TABLE 3. CONCENTRATIONS OF SELECTED ORGANOCHLORINE PESTICIDES IN HUMAN LIVER HOMOGENATE FROM THE GERMAN PILOT ENVIRONMENTAL SPECIMEN BANK[a]

Compound	Internal Standard Aldrin ng/g (wet weight)	Internal Standard Decachlorobiphenyl ng/g (wet weight)
Hexachlorobenzene	413 ± 20	427 ± 60
β-BHC	88 ± 9	90 ± 13
trans-nonachlor	8 ± 1	8 ± 2
p,p'-DDE	305 ± 9	316 ± 31
Dieldrin	22 ± 4	23 ± 5
p,p'-DDD	34 ± 5	35 ± 7
p,p'-DDT	25 ± 5	26 ± 4

[a]Measurements based on three samples, uncertainty is ±1σ calculated deviation based on two degrees of freedom.

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APPENDIX I

DEVELOPMENT OF A RADIOCHEMICAL SEPARATION PROCEDURE FOR TIN

R. R. Greenberg

Although tin has been shown to be essential for mammalian nutrition (1), it can be highly toxic in some forms, i.e., the alkyl tin species (2). Unfortunately, tin cannot be readily determined in biological materials because of the naturally occurring low levels. For example, very few biological reference materials with known tin concentrations are available. Relatively little reliable data exist concerning the tin concentrations in human and animal tissues and fluids.

Neutron activation analysis can be used to determine tin in most biological materials at the naturally occurring levels if a radiochemical procedure is used to separate tin from interfering elements and if the background level of radiation (noise) is reduced. Both the ^{113}Sn and ^{117m}Sn isotopes can be used to quantify tin if the interfering ^{160}Tb (for ^{113}Sn) and ^{47}Sc (for ^{117m}Sn) isotopes are removed. Removal of these interferences was accomplished with a separation procedure based on the extraction of SnI_4 into toluene. The sample is dissolved with nitric, sulfuric, and hydrofluoric acids and heated to fumes of sulfuric acid. The sample is cooled and hydrofluoric acid is again added. The sample is again heated to sulfuric acid fumes and a large fraction of the selenium present is driven off as volatile fluorides. This hydrofluoric acid treatment is repeated and the sample allowed to cool. A few drops of hydrogen peroxide are added to insure that all tin is in the upper, +4, oxidation state and excess peroxide is destroyed by the addition of water and boiling. If necessary, the solution is filtered to remove insoluble oxides and sulfates. The solution is transferred to a separatory funnel and potassium iodide is added. The tin is then extracted into toluene as the iodide (SnI_4). The aqueous fraction is discarded and the toluene fraction is washed twice with a potassium iodide-sulfuric acid solution. Tin is then back-extracted into a basic, aqueous solution of Na_2EDTA. The sample is counted and the tin concentration is determined using either the ^{117m}Sn isotope or the ^{113m}In daughter of ^{113}Sn. Tin recovery for this procedure is highly quantitative ($\geq$99.5 percent).

This procedure has been evaluated by analyzing several NBS SRM's. The results obtained are compared with literature values in Table 1. Analysis of human liver samples is currently in progress and the results of the first 10 samples are summarized in Table 2. Due to the long halflives of the radionuclides used for the tin determination, this radiochemical procedure can be applied after the instrumental multielement neutron activation analysis.

Thus, no additional samples are needed in the NESB program and the data are obtained from the very same subsample.

TABLE 1. TIN CONCENTRATIONS IN STANDARD REFERENCE MATERIALS (ng/g)[a]

SRM	Found	Literature[b]
Orchard Leaves (1571)	290 ± 17[c]	294 ± 16 290 ± 20 4100
Citrus Leaves (1572)	235 ± 12	---
Bovine Liver (1577)	20 ± 3	21 ± 3 10

[a]Weight basis — as received (not dried).

[b]Reference 3.

[c]Uncertainties are ±1s sample standard deviation, >5 degrees of freedom.

TABLE 2. CONCENTRATION OF TIN IN HUMAN LIVER SPECIMENS (ng/g)

Sample Identification	Wet-Weight Basis
L1S0090	301 ± 18[a]
L1S0092	164 ± 11
L1B0100	224 ± 14
L1S0110	211 ± 15
L1M0118	135 ± 8
L1B0121	501 ± 12
L1M0126	670 ± 18
L1M0127	712 ± 21
L1M0131	213 ± 8
L1M0140	221 ± 12

[a]Uncertainties are ±1s based on counting statistics of a single determination.

The implemented radiochemical procedure is an exceptionally clean separation. The resulting reduction in background radiation is better than a factor of 1000, and no scandium or terbium can be detected using other, more intense, γ-rays, indicating decontamination factors of >20,000 for scandium and >1000 for terbium. The only element observed to accompany tin in the procedure is selenium for which decontamination factors of up to 10^5 are observed.

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APPENDIX II

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ULTRATRACE DETERMINATION OF PLATINUM IN BIOLOGICAL MATERIALS VIA NEUTRON ACTIVATION AND RADIOCHEMICAL SEPARATION

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A neutron activation analysis scheme based upon a radiochemical separation of the activation products has been developed. The method utilizes the inherent sensitivity of the activation reaction $^{198}Pt(n,\gamma)^{199}Pt$ and counting of the daughter nuclide ^{199}Au. This nuclide is radiochemically separated from interfering activities by homogeneous precipitation as elemental gold. The remaining interference of the secondary reaction $^{197}Au(n,\gamma)^{198}Au(n,\gamma)^{199}Au$ from gold in the samples is quantitatively assessed and corrected. During this process accurate gold concentrations in the samples are obtained at ultratrace levels. The analysis scheme is applied to gold and platinum determinations in biological Standard Reference Materials and human liver specimens. Gold and platinum are determined at concentrations of $5 \cdot 10^{-11}$ g/g, and at higher levels.

Introduction

Concern continues about the possible impact on the biosphere from increasing use of platinum in industrial and medical applications, and interest is increasing in the development and application of analytical methods to determine platinum in environmental and biological materials. Platinum has not yet been shown to accumulate in the environment, and adverse effects have not been established despite the intense interaction of the element and its compounds with biological systems. However, it has been assumed that platinum will be detected in measurable quantities in the environment with the end of the last decade, as platinum may accumulate to higher concentrations, and more sensitive methods are developed.

Two possible major sources contributing to environmental hazards and human body burden are the noble metal catalysts employed to reduce automobile emission and the administration of platinum-containing drugs in cancer chemotherapy. Although only a small quantity of the metal is used in an automobile catalytic converter, the annual consumption by the automotive industry is estimated to be about 18 700 kg (600 000 troy ounces) of platinum.[1] The therapeutic utilization

of platinum complexes is still in its beginning stages. However, increasing medication and pharmacological research with therapeutic doses of the complexes up to 80 mg/kg body weight introduces the element into the environment possibly in form of highly bioactive compounds.

Procedures have been developed for many analytical techniques, including neutron activation analysis (NAA), to determine platinum, but originally most have been concerned with the analysis of geological and industrial materials containing trace amounts of the element, or the evaluation of reagents and techniques applied in industrial processes. In a few instances the methods have been expanded to applications in the biological and environmental fields,[2,3] and elevated platinum levels have been determined successfully in environmental and biological matrices.[4] NAA has been applied to determine the element in tissues and blood after administration of antitumor drugs containing platinum coordination compounds of the cis-$Pt(L)_2Cl_2$ type (L = N-coordinated amino or peptide ligands).[5,6] However, previous attempts to measure baseline concentrations have failed in many investigated matrices because the reported sensitivities of the respective techniques were several ng/g, while the actual levels were at least one order of magnitude lower.

Quantitative analysis of elemental concentrations below 1 ng/g almost always requires rigorous minimization of analytical blanks, or at least the rigid control of the blank. The application of NAA techniques provides a blank-free analytical step leaving only pre-irradiation handling as a controllable source of analytical blanks. To take full advantage of the capabilities of NAA and the method's intrinsic sensitivity for the determintion of platinum at levels below 1 ng/g, a newly-developed NAA scheme with radiochemical separation of the activation products is investigated in this study. The technique is applied to the determination of naturally occurring concentrations of platinum in biological Standard Reference Materials (SRM's) and in human liver tissue.

NAA method

The different platinum isotopes undergo a multitude of nuclear reactions with reactor neutrons. For analytical purposes, the most useful reactions are: $^{196}Pt(n,\gamma)^{197}Pt$ and $^{198}Pt(n,\gamma)^{199}Pt(\beta^-)^{199}Au$. The nuclear data for the respective reactions and the common interferences for the analysis via the use of these reactions are summarized in Table 1.

As demonstrated in Table 1, the use of either one of the reactions requires radiochemical separation from the interferences to achieve accurate determinations at low levels. In addition, with the expected extremely low levels of platinum in

Table 1
Nuclear data for platinum and gold analysis and for major interferences

Element	Isotope	Half-life	Gamma-energy, keV	Activity*
Analytical interest				
Pt	^{197}Pt	18.3 h	77.35	$1.6 \cdot 10^{-2}$
			191.31	$3.9 \cdot 10^{-3}$
	^{199}Au	3.15 d	158.37	14.7
			208.20	3.2
Au	^{198}Au	2.697 d	411.80	101.2
Potential interferences				
Hg	^{197}Hg	64.7 h	77.35	$6.7 \cdot 10^{-9}$
Pt	$K_{\beta'2}$ X-ray		77.8	fluorescence
Au	$K_{\beta'1}$ X-ray		77.9	fluorescence
Bi	$K_{\alpha1}$ X-ray		77.1	background
Ca	^{47}Sc	3.41 d	159.40	$2.1 \cdot 10^{-7}$
Sn	^{117m}Sn	14.0 d	158.40	$6.2 \cdot 10^{-4}$
Lu	^{177}Lu	6.74 d	208.34	$2.2 \cdot 10^{-3}$
Au	^{199}Au	3.15 d	158.37	15**
			208.20	3.3**

*Gamma emission of respective energy per nanogram of the element and per second, after one hour irradiation time at a thermal neutron flux of $5 \cdot 10^{13}$ $n \cdot s^{-1} \cdot cm^{-2}$, and three days decay time.

**Experimental value for irradiation facility RT-3 at the NBS Reactor.

biological materials, radiochemical efforts should be aimed at "background and interference-free" counting of the respective nuclide to achieve best possible limits of detection, i.e., the separation should be selective for the product nuclide. The use of the isotope ^{197}Pt imposes problems which cannot be easily controlled. The addition of platinum carrier during the radiochemical separation increases the contribution of the interference by the Pt $K_{\beta'2}$-emission. The numerical correction of this effect is severely hampered by the difficult determination of the respective Pt-X-ray peaks in the spectrum. The Pt–X-rays interfere with the Au–X-rays produced in the contacts of the detector and other background radiation. Limits of detection for radiochemical NAA via ^{197}Pt are reported in the range of several ng/g.[3]

The determination of platinum via the daughter nuclide ^{199}Au has the greatest inherent sensitivity. The specific activity produced during irradiation and subsequent decay of ^{199}Pt is considerably higher than for ^{197}Pt, and the higher rela-

tive emission rate of the main gamma line provides additional sensitivity (Table 1). The longer half-life allows a more rigorous radiochemical procedure with several steps to provide interference-free counting with low background. The well known interference by ^{47}Sc at the 158.35 keV line can be controlled by a careful radiochemical separation. Any possibly remaining ^{47}Sc activity can be detected by the use of a high-resolution detector. The 159.40 keV gamma energy would distort the platinum gamma line and show up as a doublet or shoulder in the spectrum. The quality of the separation can be further ascertained by the search for ^{46}Sc activities. A more serious problem can occur when the 208.2 keV line of ^{199}Au is used. The interfering ^{177}Lu is often disregarded but can lead to serious overestimation of platinum concentrations. Other elements likely to follow gold in the radiochemical separation are noble metals, rare earth elements, and hafnium, which might cause increased background in the respective energy range.

When complete separation is achieved, the only remaining interference to be considered is the second order $^{197}Au(n,\gamma)^{198}Au(n,\gamma)^{199}Au$ reaction from the naturally occurring gold in the sample matrix. Second order interferences have been discussed in the literature[7] and the magnitude of these interferences have been tabulated for specific irradiation condition conditions.[8] The second order reaction is a function of $\Phi^2\ t^2$ for $t \ll T$ of the respective nuclide (Φ = neutron flux, t = irradiation time, T = half life) and can become a severe interference for the determination of platinum with relatively high gold levels, a situation that substantially raises the lower limit of detection for platinum. In biological materials where the concentrations of gold may not be significantly higher than that of platinum, this interference can be quantitatively assessed and corrected. However, this correction depends on an experimental determination with limited accuracy and precision. The error in the determination of the ^{198}Au to ^{199}Au ratio, both nuclides are formed from the naturally occurring ^{197}Au, may become the governing factor in the determination of platinum. Considering the $\Phi^2\ t^2$ dependence of the interfering reaction, the detection limit for platinum may not be lowered due to better counting statistics. Due to the inaccuracy in determining the ^{198}Au to ^{199}Au ratio optimum irradiation times can be estimated to achieve the lowest relative statistical error for given platinum to gold ratios in the analytical sample [Eq. (1)].

$$\sigma_{rel} = K \left(\frac{\sqrt{m_{Pt}\, t + m_{Au}\, t^2}}{m_{Pt}\, t} + \frac{m_{Au}\, t^2 E}{m_{Pt}\, t} \right) \qquad (1)$$

where σ_{rel} – relative statistical error for the 159.35 keV peak;
K – constant containing nuclear parameter from activation equation, and specific neutron flux conditions;
m_{Pt}, m_{Au} – mass of platinum and gold in the sample;
t – irradiation time; and
E – non-random error for the ^{198}Au to ^{199}Au ratio.

The non-random error in the determination of the ^{198}Au to ^{199}Au ratio is caused by several effects as discussed below. A realistic estimate of E = 15% is used in Eq. (1) to generate the optimization diagram (Fig. 1) for the relative sensitivity of the platinum determination as a function of the statistical error of the corrected 158.35 keV peak and as a function of gold concentration and irradiation time.

The inaccuracy and imprecision of the ^{198}Au to ^{199}Au ratio determination are mainly caused by two effects. The variations in the neutron flux energy spectrum and intensity over the sample volume have larger than usual effects on the activation rate because of the considerable resonance activation integrals and the Φ^2 dependence of the interfering reaction. The second effect is the distortion of the gamma spectrum at the 158.35 keV line by the ^{198}Au backscatter "peak". This distortion is shown in Fig. 2. The peak area determinations are very difficult and even with 100 000 counts in the 158.35 keV gamma line the uncertainty of this measurement is at least 2 to 3%.

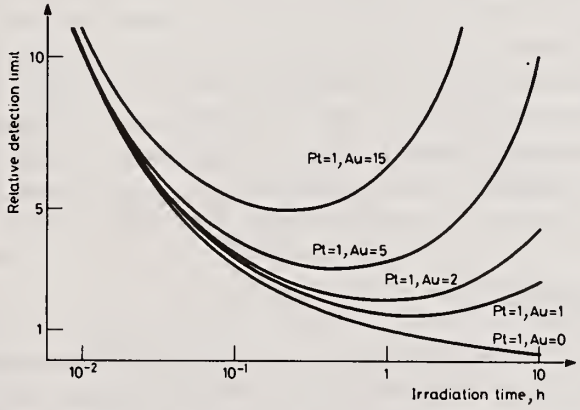

Fig. 1. Platinum detection limits in dependency of Pt to Au ratio and irradiation time at a neutron flux of $5 \cdot 10^{13}$ n · cm^{-2} · s^{-1} (RT-3 position of NBS Reactor). The minimum for Pt = Au = 1 corresponds to a detection limit of about $20 \cdot 10^{-12}$ g/g in a biological matrix

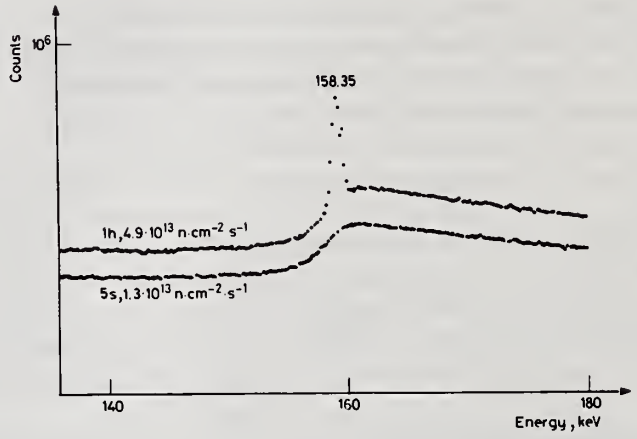

Fig. 2. Interfering activity from $^{197}Au(n,\gamma)^{198}Au(n,\gamma)^{199}Au$. Spectra obtained from pure gold standards

Experimental

Sample preparation

All samples have been homogenized and freeze-dried. Approximately 1.5 g of each material is pressed into a pellet of about 1 cm^3 volume. A blank consisting of cellulose powder is treated the same way. To assure representative sampling, up to three pellets (5 g total weight) are used for the determinations in Standard Reference Materials. Standards are prepared by dissolving the pure metals and pipetting calibrated solutions on filter paper (Whatman 41), drying and pelletizing. Samples and standards are then sealed in precleaned bags made from linear polyethylene and packaged in fixed geometry in the irradiation rabbit. The sample preparation is carried out under clean room conditions.

Irradiation and counting

Irradiations have been carried out in the pneumatic tube facilities of the NBS Research Reactor at a flux of $5 \cdot 10^{13}$ n $\cdot$ s^{-1} $\cdot$ cm^{-2} for time intervals of 15 min to 1 h. After 24 h decay the samples and standards are unpacked and processed. They are ready for counting 3 to 4 days after irradiation. The samples and standards are counted in close geometry on high-resolution Ge(Li) detectors (18% relative efficiency, FWHM 1.9 keV at 1332 keV). The spectral data are collected with

the Nuclear Data* 6600 multichannel analyzer system. Special effort, including hand-integration, has been used for the determination of small peak areas.

Radiochemical procedure

The principle of this newly-developed radiochemical separation scheme is to dissolve the sample matrix completely, to equilibrate the produced ^{199}Au and ^{198}Au activities with the gold carrier, and to separate the gold from the other matrix activities in as pure a form as possible. Fifty ml of a 2 : 1 mixture of conc. nitric acid/sulfuric acid containing 80 mg of gold carrier and 10 μg scandium is heated gently to dissolve the tissue samples. The plant materials require initial addition of hydrofluoric acid to dissolve silicate components. After eight to fifteen hours at elevated temperatures (~90 °C), hydrochloric acid is added with additional nitric acid if necessary to form aqua regia and to equilibrate the gold completely. The temperature of the solutions is then raised to boil off nitric and hydrochloric acids. Blackening of the solution indicates remaining organic material which is subsequently destroyed by adding portions of nitric acid until all organic material has vanished. Aqua regia is used again to ensure equilibration. The temperature of the solution is then slowly brought up to over 250 °C, boiling off the more volatile acids. At temperatures above 250 °C, gold compounds, especially $AuCl_3$ and Au_2O_3, decompose and an auto-reduction to elemental gold takes place. This process is sufficiently slow to allow the gold to homogeneously precipitate in the form of fine particles or leaflets which agglomerate into nugget or leaflike precipitates without the inclusion of matrix activities. This process takes several hours. After its completion, the gold precipitate is thoroughly washed with HNO_3, H_2O, HCl and H_2O in that order. The precipitate is dried on a hot plate and weighed for yield determination. The chemical yield for this separation is generally 95–98%. The precipitate is sealed in a polyethylene bag and rolled out to a thin foil of about 1 cm^2 area for counting.

The plant materials yield some insoluble white precipitates with gold precipitate. These precipitates seem to be metal oxides. They contain high rare earth and hafnium activities. The contaminating activities cannot be separated by washing. Consequently, the gold precipitate is dissolved in aqua regia. The solution is filtered, sulfuric acid added, and the gold is precipitated again. With another 2–5% loss of yield, a clean gold sample is obtained for counting.

*Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 2
Determination of ^{199}Au to ^{198}Au ratio
(Concentration in standards 10^{-9} g/g)

Detector	Apparent Pt ^{199}Au	Au in standard ^{198}Au	Ratio
1 h irradiation			
γx	5.90 ± 0.07*	5.958	0.99 ± 0.15**
66	6.26 ± 0.17	5.918	1.06 ± 0.18
90	6.63 ± 0.20	5.782	1.14 ± 0.16
95	6.00 ± 0.10	6.122	0.98 ± 0.15
15 min irradiation			
66	0.92 ± 0.06	4.299	0.21 ± 0.03
90	1.03 ± 0.10	4.390	0.23 ± 0.04

*Error due to counting statistics (1 σ).
**Experimental error (1 σ), five degrees of freedom for 1 h irradiations and three degres of freedom for 15 min irradiations.

Results and discussion

^{198}Au to ^{199}Au ratio determination

Each sample irradiation has been conducted with a set of pure gold standards. The data from those irradiations for the ^{198}Au to ^{199}Au ratio determination are compiled in Table 2. The measured standard deviation of the pooled data express the difficulties encountered in the precise determination of this ratio. However, this ratio needs to be determined as accurately and precisely as possible, and improvement can be expected with the planned setup of a Compton suppressor system. The 411.80 keV peak of ^{198}Au is already determined with high accuracy and precision. The analytical scheme thus provides the possibility for a highly accurate and precise determination of gold at ultratrace levels. The detection limit of gold in the investigated materials has been found to be less than 10^{-15} g/g.

Biological Standard Reference Materials

Three different materials have been analyzed: Orchard Leaves (SRM 1571), Citrus Leaves (SRM 1572), and Bovine Liver (SRM 1577). The platinum and gold concentrations determined in this study are given in Tables 3 and 4 and compared with data found in the literature.[3,9] The wide scatter of gold data in Bovine Liver can be explained by inhomogeneity in the material. This inhomogeneity has been observed in this study, and much more consistent results can be obtained with increased sample sizes (up to 5 g).

Table 3
Platinum and gold in Orchard Leaves (SRM 1571)
(Concentration 10^{-12} g/g)

Sample No.	Irradiation time	^{199}Au*	Interference*	Au	Pt
1	1 h	1780 ±250**	1440 ±200**	1344 ± 1**	(340 ±320)**
2	1 h	1505 ±100	1500 ±250	1496 ± 1	(0 ±270)
3	15 m	660 ±330	310 ± 40	1404 ± 3	(350 ±320)
4	15 m	636 ±185	300 ± 40	1387 ± 6	310 ±190
5	15 m	428 ± 75	280 ± 40	1530 ± 1	150 ± 80
X ± 1s				1430 ± 80	~200
Literature				970 ± 90	89 200 ±15 200[3]
				1800 ±1000	89 200 – $1.2 \cdot 10^6$ [9]

*Calculated as platinum concentrations.
**Errors are random errors or sum of random and nonrandom errors [Eq. (1)] for a single determination.

Table 4
Platinum and gold in Bovine Liver (SRM 1577) and Citrus Leaves (SRM 1572)
Bovine Liver
(Concentration 10^{-12} g/g)

Sample No.	Pt	Au
1	28 ± 22*	68.9 ± 0.3*
2	99 ± 23	53.2 ± 0.3
3	94 ± 23	41.9 ± 0.1
4	58 ± 18	69.1 ± 0.1
Literature	ND	200–30 000

Citrus Leaves
(Concentration 10^{-12} g/g)

Sample No.	Pt	Au
1	100 ± 30*	105.8 ± 0.2*
2	40 ± 30	105.7 ± 0.2
3	50 ± 40	119.0 ± 0.5

*Errors are random errors or sum of random and nonrandom errors [Eq. (1)] for a single determination.

The comparison of the data for platinum in Orchard Leaves demonstrates the difficulties encountered in the determinations of this element. The data in Table 3 demonstrate the effect of shortened irradiation time to lower the detection limit for platinum in the presence of relatively high gold concentrations. Despite this optimization we could only obtain a positive identification for platinum in Orchard Leaves at about $200 \cdot 10^{-12}$ g/g taking into account the counting statistics of the 158.35 keV peak and the uncertainty for calculating the interference by gold. However, this level appears to be more correct than previously reported concentrations, which were orders of magnitude higher. The radiochemical separation of interferences is crucial, e.g., disregarding the rare interference from lutetium would have given a false platinum concentration of about $40 \cdot 10^{-9}$ g/g. The accuracy of gold determinations by activation analysis has been demonstrated previously.[10] The good agreement of the gold data with previous determinations by activation analysis[9] suggest also good accuracy for the platinum data, since both isotopes ^{198}Au and ^{199}Au have the same chemical behavior during the radiochemical separation and are counted simultaneously. The new SRM Citrus Leaves has a much lower gold level, therefore we have been able to determine platinum at the ultratrace level (Table 4). It would be of interest to analyze a similar material collected recently to compare with these SRM's which were collected more than ten years ago. Possibly an increase of platinum in the environment could be detected.

During collection and preparation of these materials, extreme caution has to be exercised to avoid contamination with gold. The SRM Orchard Leaves was probably contaminated during its production, fortunately homogeneously, since

Table 5
Platinum and gold in human liver specimens
(Concentration 10^{-12} g/g)

NESB ID	Pt	Au
B0005	ND	2652 ± 1*
B0014	54 ± 13*	64 ± 1
B0041-A	114 ± 23	71.9± 0.2
B0041-B	127 ± 60	143.1± 0.3
B0042	240 ± 70	298 ± 1
S0049	ND	496 ± 2

ND: not detectable.

*Errors are random errors or sum of random and nonrandom errors [Eq. (1)] for a single determination.

the observed gold level is abnormally high compared to its crustal abundance and the other trace elements in the material. The Bovine Liver data suggest that gold might be an inhomogeneously distributed contaminant. The platinum determination is occasionally impossible because of unexpected high interference from gold. The platinum data have too large errors to judge on the distribution of this element.

Human liver specimens

The results for five selected specimens out of the National Environmental Specimen Bank program are presented in Table 5. The gold data fall well in the range of reported results,[11] no data for platinum have been reported previously. These first results on the liver specimens are an indication for the current baseline level. If ratios of platinum to gold in the liver are compared with its crustal abundance ratio (Pt : Au = 2 : 1), no elevanted platinum levels can be detected in this limited number of specimens. Further analysis of randomly selected samples from the National Environmental Specimen Bank will provide a first baseline for platinum in human liver.

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ADDENDUM TO APPENDIX II

TABLE 6. CONCENTRATIONS OF PLATINUM IN HUMAN LIVER SAMPLES

Sample Identification	Concentration (pg/g wet weight
L1B0014	15
L2B0041	30
L2B0042	57
L1M0083	17
L1S0086	5
L1S0092	18
L1B0121	8.1
L1M0127	20
L1S0128	16
L1M0130	10
L1M0131	11

APPENDIX III

DETERMINATION OF ORGANOMERCURY CATIONS IN TISSUE SAMPLES USING LIQUID CHROMATOGRAPHY WITH AMPEROMETRIC DETECTION

by

William A. MacCrehan

INTRODUCTION

Mercury has long been recognized as an important environmental toxin. Of the various forms of mercury, the alkylmercury cations have been found to be the most toxic (1). Thus, the determination of the levels of various organomercury species in biological tissues is important in addition to the measurement of total mercury concentration. Other workers have reported the presence of methylmercury in aquatic samples (2,3) and in human tissues (4) and ethylmercury in plants (5). It would be particularly valuable to measure the different mercury species in available reference materials and to establish baseline values in different tissue samples.

ORGANOMERCURY DETERMINATION

The current method for organomercury determination in tissue samples involves a laborious digestion, extraction, derivatization, and sample clean-up, followed by gas chromatography with electron capture detection (4,6). An outline of this method is shown in Table 1A.

As an alternative to this gas chromatographic method, a simplified procedure based on liquid chromatography with electrochemical detection (LCEC) has been developed. The new sample preparation is outlined in Table 1B. Organomercury compounds in tissues are thought to be bound to protein sulfhydryl groups (7). Acidification with strong acid liberates the organomercury cations from the weak-base complexes:

$$\text{R-Hg-S-protein} + H^+ + Br^- \longrightarrow \text{RHg-Br} + \text{H-S-protein}.$$

The reagent under investigation consists of 1 moL/L HCl, 1 mol/L KBr and 0.05 percent v/v of an n-alkyl thiol (C_nSH). A concentration of HCl was selected which was strong enough to protonate the weak-base protein thiol but not so strong as to cause the acid hydrolysis of the organomercury compounds. The Br^- is added to help competitively complex the mercury cations. The addition of the thiol to the extraction reagent was required as an antioxidant to prevent the formation of Br_2 from the reaction of Br^- with atmospheric O_2.

Otherwise, the Br_2 formed rapidly oxidizes the organomercury compounds to inorganic mercury (II).

TABLE 1. OUTLINE OF METHODS FOR THE DETERMINATION OF METHYLMERCURY IN TISSUES

A	B
— weigh 0.5 to 2.0 g sample	— weigh 250 mg lyophilized sample
— add 45% NaOH, 1% Cysteine	— add 750 µL 1 mol/L HCl, 1 mol/L KBr, 0.05% C_nSH
— heat at 100 °C for 0.5 hour	— heat at 40 °C and sonicate for 0.5 hour
— add 8 mol/L urea, 3 mol/L HCl, 0.5 mol/L $CuSO_4$	— filter centrifugally
— shake 5 minutes	— add 50 µL concentrated NH_4OH
— extract with benzene twice with centrifugation, transfer	— purge with Ar and analyze by LCEC
— add 0.01 mol/L $Na_2S_2O_3$ shake, followed by centrifugation, transfer to a new tube	Total time 0.75 hour
— add benzene, extract, transfer	
— add Na_2SO_4-Florisil, transfer	
— analyze by GC-EC	
Total time 3.5 hours	

An important aspect of the sample preparation is the digestion and filtration of the lyophilized tissues. For the new method a commercially available centrifugal filtration apparatus was employed. This apparatus permits the digestion and filtration to be performed without transfer. It was necessary to modify this assembly, however, by replacing the original plastic sample and receiver containers with borosilicate glass (see Appendix IV for details).

RESULTS AND DISCUSSION

A new separation of organomercury species has been developed for this work. Previously, neutral complexes of the organomercury cations were formed with 2-mercaptoethanol (2-ME) for LC separations (8,9). However, for measurements on tissue samples, the additional selectivity of differential pulse electrochemical detection was required (9,10). In the new method, the selectivity of the chromatographic separation was increased so that simple amperometric detection could be employed. This was accomplished by the use of n-alkyl thiols as complexing agents for the organomercury cations. These reagents form even less polar, neutral complexes with the cationic mercury species than the 2-ME thus providing greater chromatographic selectivity. In

the reversed-phase separation, the complexes are retained much longer than the polar matrix constituents which are co-extracted by the aqueous sample reagent. The complexation reaction takes place "in-situ" during the chromatographic separation:

$$RHg\text{-}Br + H\text{-}S\text{-}(CH_2)_nCH_3 \xrightarrow{pH\ 5} RHg\text{-}S\text{-}(CH_2)_nCH_3 + H^+ + Br^-.$$

The effect of the chain length of the n-alkyl thiols on the retention of ethyl- and methylmercury in a reversed-phase separation is shown in Figure 1. As expected the longer alkyl chains increase retention.

The LC separation of methyl-, ethyl-, and inorganic Hg(II) is shown in Figure 2A. This separation is achieved using an octadecyl modified silica (5 μm) column and an 85 percent methanol/water eluent containing the C_7 thiol. The detector is a thin layer electrochemical cell with a 1.2 mm gold amalgam working electrode and an applied potential of -825 mV.

Preliminary results of the new method are shown in Figure 2B. Samples of NBS RM 50 Albacore tuna were prepared by the acid extraction procedure and analyzed by LCEC. Preliminary quantitative results for methylmercury in the two samples agreed with that found by the previously reported method (9).

Future work will focus on expanding the sample preparation to include the recovery of inorganic mercury(II) as well as the alkyl species which will allow a simultaneous determination of all the expected forms of mercury in tissues.

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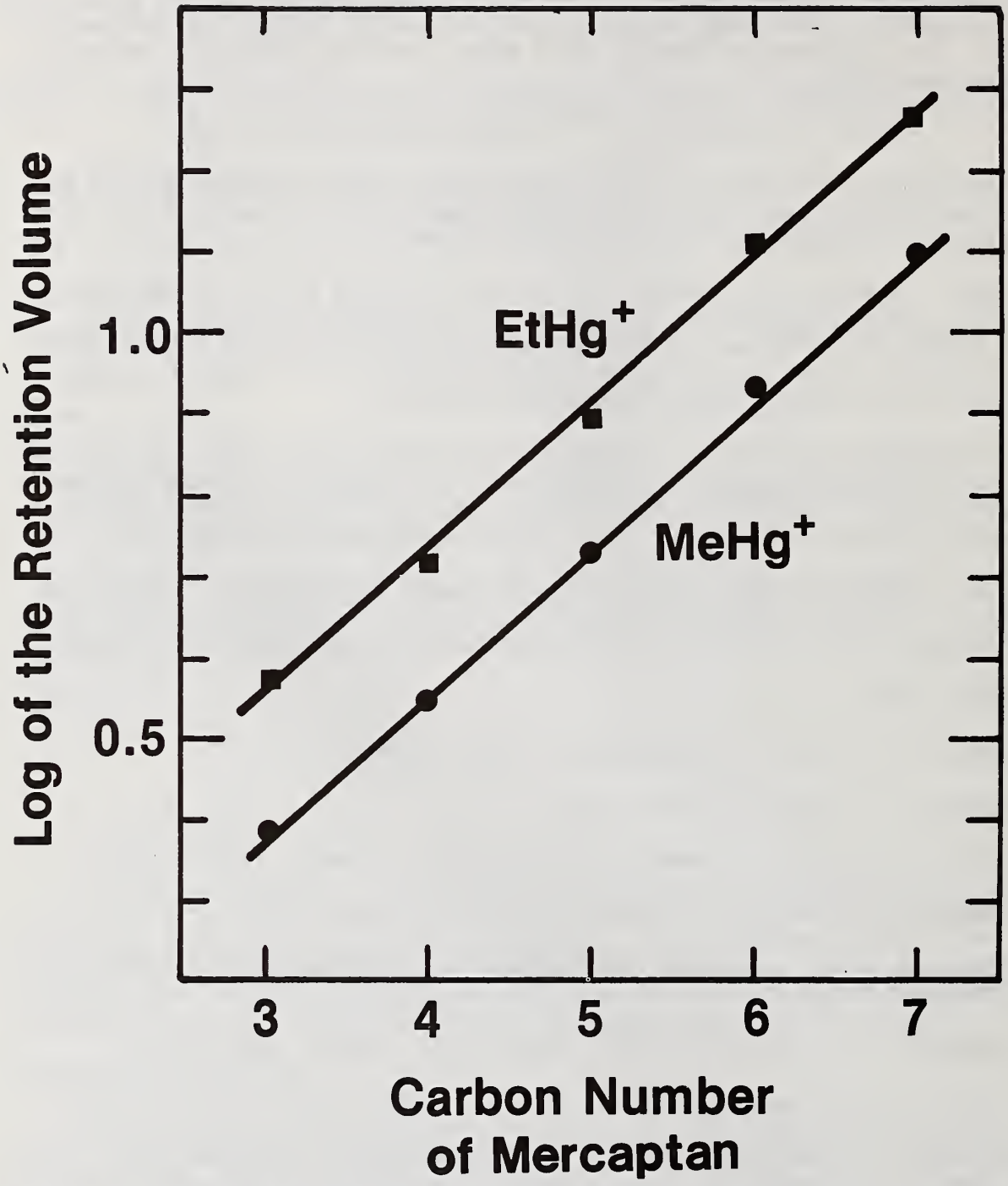

Figure 1. Effect of the alkyl chain length of the n-alkyl thiol on the retention of ethylmercury and methylmercury. Conditions: column - octadecyl modified silica, 5 μm particles, solvent - 72% MeOH/H_2O with 0.05% v/v of each thiol.

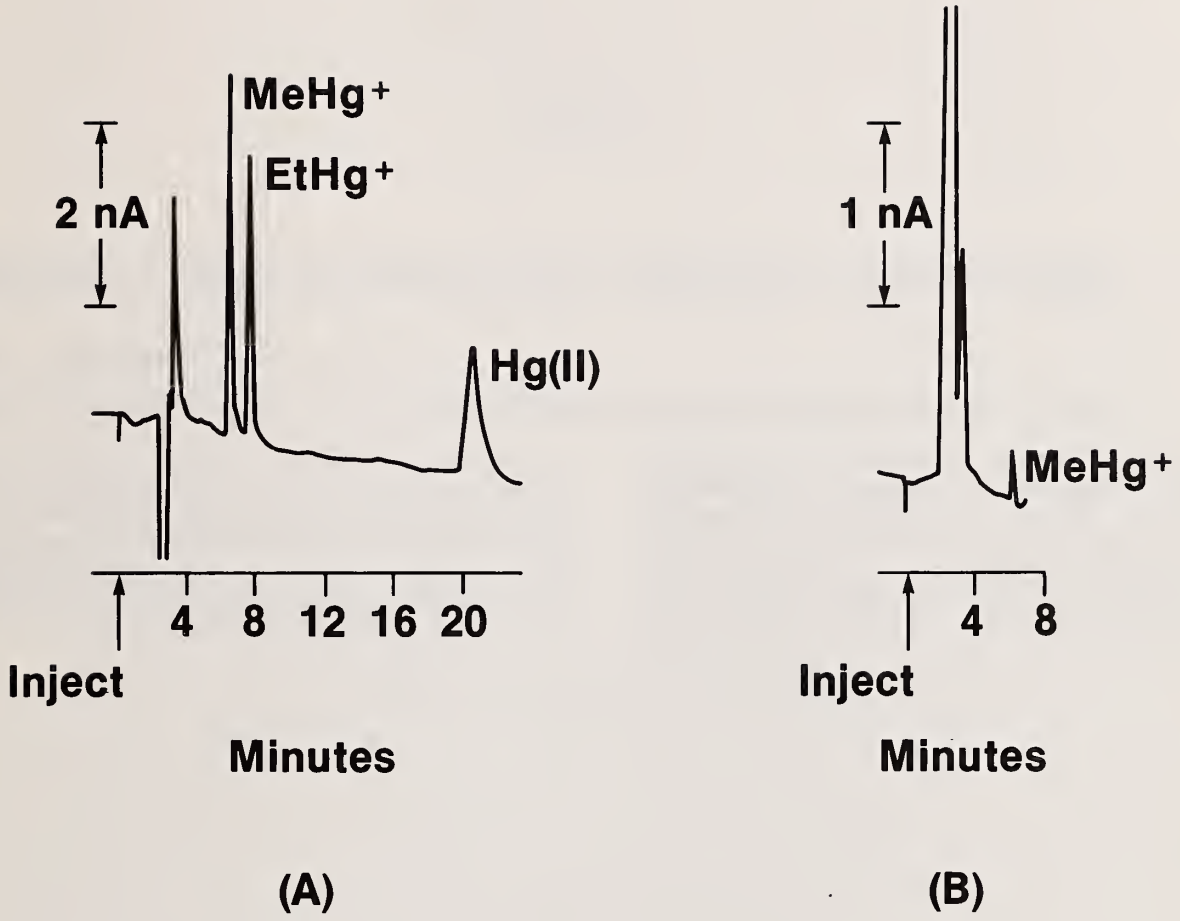

Figure 2. Reversed-phase LC separation of mercury species. Conditions: column-Spherisorb ODS 5 μm, 0.4 x 25 cm; eluent-85% methanol/water, 0.05 moL/L NH_4OAc, 0.05% n-$C_7H_{15}SH$ at 1.0 mL/min; detector-amperometry at a gold amalgam electrode (1.2 mm diameter button) at -825 mV.

A. Sample amounts 1.0 x 10^5 mol/L of each (about 45 ng).

B. Detection of methylmercury in tissue sample (NBS RM-50).

APPENDIX IV

Modification of Centrifugal Filtration Device for Elimination of Sorption Losses

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The preparation of many liquid samples for analysis frequently requires filtration to remove suspended solid matter. This is particularly true for samples prepared for liquid chromatography, where particles in the micrometer range can clog protective column frits. Several devices are commercially available for the filtration of liquid samples in the sub-milliliter range. Two approaches that we have used are syringe–membrane filters and centrifugation with withdrawal of the supernatant liquid. Although both approaches are effective for removing particles, each has disadvantages. For example, syringe–membrane filters are cumbersome to use for routine work, and considerable sample volume may be lost as the filter becomes clogged. Withdrawal of the clear supernatant liquid after centrifugation is difficult, and some liquid is always

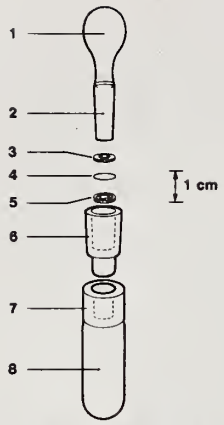

Figure 1. Glass-modified centrifugal filtration apparatus: (1) borosilicate glass sample compartment, (2) 10/18 inner joint cut to a length of 9.75 mm, (3) silicone washer, (4) filter membrane, (5) filter support, (6) original plastic filter holder, (7) 10/18 outer joint cut to a length of 17.25 mm, (8) borosilicate glass receiving container.

retained in the interstitial volume of the "plug".

A new approach to the problem of small volume filtration is to combine the action of membrane filtration and centrifugation into one system. One commercially available microfilter device (Bioanalytical Systems MF-1 Microfilter) consists of a capped, upper sample compartment, a filter membrane and support, and a lower receiving tube. The sample to be filtered is placed in the sample compartment, the appropriate membrane-filter size and material are chosen, the apparatus is assembled, and the sample is centrifuged at high speed. The force generated drives the liquid through the filter and the filtrate is collected in the receiving tube. The microfilter is designed to be useful for the filtration of volumes in the range of 50–1000 μL. The material from which this particular assembly is constructed is a polyethylene–polypropylene copolymer.

The particular application for which filtration was required was the extraction of organomercury cations from lyophilized tissue. The extraction involves digestion and ultrasonic agitation at 50 °C for 0.5 h using a reagent that consists of 1 mol/L HCl, 1 mol/L KBr and 0.1% v/v 3-mercaptopropanol (3-MP). The sample is digested, filtered, and sampled in the microfilter apparatus, without any transfers. The results of the new method will be described in a subsequent publication.

While the microfilter efficiently filters out the solid material with high recovery of the liquid, significant loss of organomercury cations was observed. Methyl- and ethylmercury were found to be retained on the plastic container material, probably as the neutral bromo or mercapto complexes.

Glass-Modified Microfilter. Since no loss of organomercury complexes was observed to occur on borosilicate glass in this concentration range, new sample and receiver compartments were constructed from this material. The modified microfilter assembly is pictured in Figure 1. Standard borosilicate inner and outer 10/18 joints were cut on a glass saw to fit the filter support compartment. The ends of the tubes were closed, and a small bulge was blown on the end of the sample compartment to increase the volume to about 2 mL. The glass was carefully annealed to avoid stress cracking upon centrifugation.

The original commercial filter support was still used even though it was fabricated from the same plastic as the other

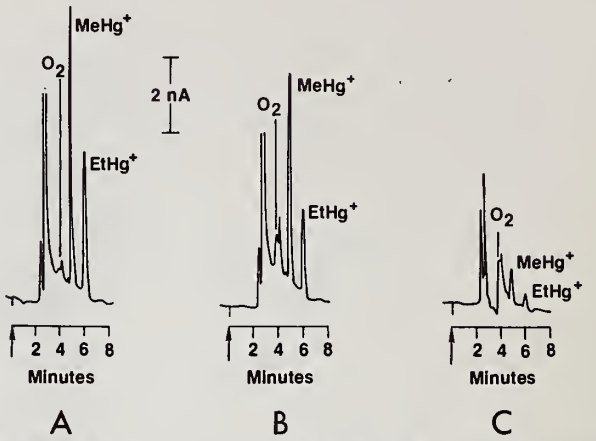

Figure 2. Organomercury determination. Conditions: column, commercially available C-18 bonded phase silica (5 μm spherical particles) 0.4 × 25 cm; solvent, 80% MeOH/H_2O, 0.1 mol/L NH_4OAc pH 5, 0.02% 3-mercaptopropane; flow rate, 1.0 mL/min; detector electrode, Hg/Au 1.2 mm diameter; potential, −0.75 V; current offset, 20 nA; (A) glass–modified microfilter; (B) original plastic microfilter; (C) methanol leach of middle chromatogram filter after water wash. Starting concentration of methylmercury ($MeHg^+$) and ethylmercury ($EtHg^+$) were 5×10^{-8} mol/L.

compartments. This was possible because of the short contact time and small plastic surface exposed to the sample on this part of the apparatus.

Recovery Study. The performance of the glass-modified filtration apparatus was compared to the original plastic containers by using the filters for the digestion, ultrasonic agitation, and filtration of a standard solution of methyl- and ethylmercury in the micromolar concentration range. The first chromatogram in Figure 2 shows the results obtained after filtration through the glass-modified filter. The center chromatogram is from an identical solution prepared by use of the original plastic filter. The third chromatogram shows a methanol solution obtained by rinsing the plastic filter with distilled water and then leaching the plastic (used for the center chromatogram) with 200 μL of solvent. The partial recovery of sorbed organomercury compounds can be seen in this last chromatogram. The recoveries obtained for the glass-modified filter, expressed as a mean plus or minus the relative standard deviation in percent for five samples was 98 ± 3% for methylmercury and 100 ± 4% for ethylmercury compared to 75 ± 5% (methylmercury) and 56 ± 21% (ethylmercury) for all-plastic filters.

Cleaning. The plastic parts of the filter holder can be cleaned for reuse by soaking in 30% H_2O_2 overnight. Any remaining tissue and the organomercury compounds are removed by this treatment. No deterioration of the polyethylene/polypropylene copolymer was noted after 10 such cleanings. The glass sample compartment and receiving tube can be cleaned by soaking in concentrated nitric acid overnight.

RECEIVED for review September 10, 1981. Accepted December 18, 1981. To describe adequately materials and experimental procedures, it is occasionally necessary to identify commercial products by manufacturer's name or label. In no instance does such identification imply endorsement by the National Bureau of Standards nor does it imply that the particular products or equipment is necessarily the best available for that purpose.

APPENDIX V

NEUTRON CAPTURE PROMPT GAMMA-RAY ACTIVATION ANALYSIS OF HUMAN LIVER SAMPLES

by

David F. Anderson

A series of human liver samples were analyzed by instrumental neutron capture prompt γ-ray activation analysis (PGAA) at the University of Maryland-NBS reactor facility. The lyophilized samples (in powder form) were formed into 1.25 cm diameter pellets, packaged in thin Mylar film and irradiated in the thermal neutron beam (thermal flux = 2 x $10^8 n/cm^2 \cdot s$, Cd/Au ratio 55/1). Full descriptions of the system and its capabilities can be found in References (1-3).

Eight liver samples from specimen L1B0014, one from L2S0035, and one sample from NBS SRM 1577 Bovine Liver were evaluated and the results are presented in Table 1. For L1B0014, the individual samples' maximum statistical errors (not shown in Table 1) for γ-ray counting and elemental sensitivity was <1 percent for H and K, 1.5-3 percent for C, N, S, Cl and Cd, and >3 percent for B, Na and P. Using the mean and σ values for H and K, the homogeneity of the eight samples is then 2.7 ± 0.1 percent.

The elemental concentrations in SRM 1577 found for H, N, Na, P, S, K, and Cd were in agreement with NBS certified and literature values. The carbon determination appears to be slightly low (~4 percent), while the Cl is ~12 percent high, compared to several literature values (2,4,5), but these values have significant error (up to 11 percent) as reported. The PGAA data are included in the paper presented at the Conference on Modern Trends in Activation Analysis, Toronto, Canada, June, 1981 (6).

TABLE 1. PROMPT GAMMA-RAY ACTIVATION ANALYSIS (PGAA) RESULTS FOR HUMAN LIVER SAMPLES

	L1B0014 Subsamples							
Element	1	4	5	6	10	12	14	17
H (%)	7.42	7.44	7.48	7.08	7.31	7.38	7.00	7.50
B (μg/g)	1.78	1.70	1.82	1.81	1.69	--[a]	--[a]	--[a]
C (%)	53.6	51.3	51.0	48.3	48.4	54.1	52.4	56.3
N (%)	10.73	10.52	10.45	10.32	10.43	11.02	10.60	11.67
Na (%)	0.25	0.26	0.30	0.31	0.26	0.27	0.25	0.29
P (%)	0.97	1.13	1.12	1.15	1.09	1.21	1.03	1.03
S (%)	0.782	0.787	0.775	0.716	0.756	0.789	0.764	0.795
Cl (%)	0.388	0.395	0.395	0.378	0.389	0.405	0.382	0.407
K (%)	1.069	1.098	1.088	1.034	1.053	0.103	1.028	1.118
Cd (μg/g)	4.78	4.84	4.83	4.49	4.73	4.84	4.52	4.90
Sample Mass (g)	0.92014	0.97486	0.97244	0.95312	0.96260	0.87620	0.99784	0.90496

[a]Different system setup, B background too high for reliable determination.

Mean±σ	L2S0035	SRM 1577
7.33 ±0.19	9.85±0.11	7.01 ±0.01
1.76 ±0.06	---[a]	---[a]
51.9 ±2.8	66.2 ±3.0	48.7 ±0.4
10.72 ±0.44	13.51±0.09	10.39 ±0.12
0.27 ±0.02	0.94±0.02	0.25 ±0.04
1.09 ±0.08	---	1.0 ±0.3
0.77 ±0.03	1.00±0.01	0.76 ±0.04
0.392±0.010	0.85±0.01	0.291±0.001
1.07 ±0.03	1.15±0.02	0.95 ±0.01
4.74 ±0.16	9.61±0.16	0.22 ±0.04
---	0.3039	1.33886

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APPENDIX VI

PUBLICATIONS OF THE U.S. EPA/NBS NATIONAL ENVIRONMENTAL SPECIMEN BANK

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11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)

This work was performed under a joint EPA/NBS research program to develop state-of-the-art protocols for the sampling, storage, and analysis of biological and environmental-type matrices as part of a Pilot National Environmental Specimen Bank program. The purpose of this report is to summarize the experience and results obtained for the first sample type, human liver. The sample collection protocol was evaluated with respect to costs and suitability of donor selection criteria. An analytical protocol was implemented for the determination of trace elements in the liver samples using the techniques of atomic absorption spectrometry, isotope dilution mass spectrometry, neutron activation analysis, and voltammetry. Individual sections of this report describe in detail the procedures used and the results obtained for each of these analytical techniques. The analytical results for the determination of 31 trace elements in 36 liver specimens are presented and discussed. Analytical methodology for the measurement of organochlorine compounds is also described.

This report is submitted in partial fulfillment of EPA Interagency Agreement EPA-81-D-X0105 by the National Bureau of Standards.

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)

atomic absorption spectrometry; cryogenic homogenization; gas chromatography; human liver; inorganic analysis; isotope dilution mass spectrometry; neutron activation analysis; organic analysis; organochlorine pesticides; specimen banking; trace elements; voltammetry

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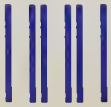

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