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To cite this Article Wise, Stephen A. , Koster, Barbara J. , Parris, Reenie M. , Schantz, Michele M. , Stone, Susan F. and Zeisler, Rolf(1989) 'Experiences in Environmental Specimen Banking', International Journal of Environmental Analytical Chemistry, 37: 2, 91  $-$  106

To link to this Article: DOI: 10.1080/03067318908026889 URL: <http://dx.doi.org/10.1080/03067318908026889>

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*Inrern. J. Enuiron. Anal. Chem.,* **Vol. 37. pp. 91-106 Reprints available directly from the publisher**  Photocopying permitted by license only

# **EXPERIENCES IN ENVIRONMENTAL SPECIMEN BANKING**

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For the past 10 years, the National Institute of Standards and Technology (NIST), has been involved in environmental specimen banking activities. These activities have resulted in the development of collection, storage, processing, and analysis procedures for long-term archiving of a variety of environmental specimens including human liver, fish muscle and liver, oysters, mussels, sediment, and seal tissues. In this paper, we describe some of the experiences and results from these efforts as related to environmental trend monitoring and the potential value of a specimen bank for future retrospective analyses.

**KEY WORDS:** Specimen banking, environmental monitoring, trace elements, polychlorinated biphenyls (PCBs), marine pollution.

### INTRODUCTION

The concept of environmental specimen banking, i.e., the long-term storage of biological specimens for retrospective analysis, has received increased attention in recent years as an important complement to traditional environmental pollution monitoring. A bank of environmental samples, which are collected and archived, provides well-preserved and documented samples for retrospective analysis as analytical techniques improve or as concerns about as-yet unidentified pollutants arise. Several international workshops'-4 were held in the 1970's and early **1980's**  to discuss the need for and the requirements of specimen banking activities. During this period several countries established and implemented formal specimen bank programs.<sup>4</sup> In 1979, the National Bureau of Standards [presently the National Institute of Standards and Technology (NIST)], in conjunction with the U.S. Environmental Protection Agency (EPA), established a pilot Environmental Specimen Bank Program to investigate the feasibility of long-term storage of environmental samples. Human liver specimens were selected as the first sample type to be included in this pilot study, and approximately *550* human liver

Presented at the 18th International Symposium on Environmental and Analytical Chemistry, Barcelona, 5-8 September, 1988.

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specimens have been collected since 1980 as part of this project. During the past decade of NIST involvement in specimen banking activities, we have gained extensive experience in the requirements, problems, and value of specimen banking. In this paper, several of these aspects of environmental specimen banking are presented.

## SPECIMEN BANK PROJECTS AT NIST

In recent years the specimen bank activities at NIST have expanded beyond the pilot program with human livers to include samples from the marine environment (sediments, oysters, mussels, and fish tissue), Alaskan marine mammal tissues, human serum, and total human diet samples. These activities are associated with several different projects that are supported by various government agencies. These specimen banking activities, which are listed in Table 1, are known collectively as the National Biomonitoring Specimen Bank (NBSB). The title reflects the inclusion of specimens of nutritional and medical importance in addition to those specimens archived for environmental purposes. These various projects have provided a wide range of experience in the collection, processing, long-term storage, and analysis of different samples types. These projects are described briefly below and a summary of the current inventory of the specimens included in the NBSB is provided in Table 2.

## *Pilot Environmental Specimen Bank*

The goals and the development of the EPA/NIST pilot Environmental Specimen Bank Program have been described in detail in a previous publication, $<sup>5</sup>$  and the</sup> results and experiences of the early years of this project were reviewed in **1984.6**  This pilot effort was originally intended to focus on four types of environmental specimens: human liver, marine specimens, food specimens, and an atmospheric accumulator. However, since other government agencies involved in projects related to the marine environment and food specimens have joined the NBSB program, the EPA/NIST pilot specimen bank project has focused primarily on the establishment of a human liver bank and research related to specimen banking. The existing database on the stored human liver specimens (see discussion below) and the nearly **450** unanalyzed liver specimens in the bank represent a substantial resource for EPA for the investigation of environmental pollution trends.

## *National Status and Trends Specimen Bank*

In the **1985** the National Oceanic and Atmospheric Administration (NOAA) incorporated specimen banking into their National Status and Trends (NS&T) Program. The NS&T program is a national monitoring program designed to quantify the current status and long-term trends in the concentration of selected contaminants and biological indicators of contaminant effects in U.S. coastal and





**Table 1** Projects within the National Biomonitoring Specimen Bank at the National Institute of Standards and Technology Projects within the National Biomonitoring Specimen Bank at the National Institute of Standards and Technology

PCBs = polychlorinated biphenyls; PAHs = polycyclic aromatic hydrocarbons. **'PCBs** = **polychlorinated biphenyls, PAHs** = **polycyclic aromatic hydrocarbons** 

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estuarine environments.' The NS&T program consists of two separate monitoring activities: **[l]** the Benthic Surveillance Project, in which sediment and fish tissues (muscle and liver) are collected annually from approximately 50 coastal sites, and [2] the Mussel Watch Project, in which sediment and bivalve molluscs (mussels and oysters) are collected from over 150 coastal sites. Each year samples from approximately 20% of these sites are collected specifically for archiving in the specimen bank; thus, samples from all of the original Benthic Surveillance and Mussel Watch sites will be archived after a five-year period.

### *Alaskan Marine Mammal Tissue Archival Project*

The Alaskan Marine Mammal Tissue Archival Project was initiated in 1987 with the goal of establishing a representative collection of tissues from Alaskan marine mammals (e.g., seals, walruses, and whales) for future contaminant analyses and documentation of long-term trends in environmental quality. Since most marine mammals are at or near the top of the food chain, chemical analysis of their tissues may be useful in determining whether bioaccumulation of contaminants associated with human industrial activities is occurring in the marine food chains in the Arctic. In addition, some of the native population of Alaska depend upon such animals for a substantial portion of their diet. Therefore, the contaminant levels found in marine mammals may have health implications for the human population occupying these regions. A detailed discussion of the project including the rationale for the selection of specimens and the sample collection protocols has been published.<sup>8</sup>

Specimens of blubber, kidney, liver, and muscle tissue were collected from northern fur seals during the subsistence harvest on the Pribilof Islands in **1987.**  Tissue samples from a second species, ringed seals collected at Point Barrow, Alaska, were added to the archive in 1988. Additional species and/or new sites will be included in the project each year.

# *NCI Cancer Chemoprevention Program and IAEAIUSDAIFDA Nutrients in Human Diet*

Two additional projects, the Cancer Chemoprevention Program and the Nutrients in Human Diet Project, are not specifically directed toward specimen banking as are the EPA and NOAA projects; however, they do have minor specimen banking components associated with them. Both of these projects focus on nutrients rather than on environmental contaminants as in the three major projects described above. **As** part of a quality assurance program for the NCI's Cancer Chemoprevention Program, NIST serves as a reference laboratory for NCI-supported laboratories involved in the determination of micronutrients in human serum (e.g., vitamins A, C, and E and  $\beta$ -carotene). Large batches of human serum samples containing measured amounts of these analytes are prepared and distributed as proficiency testing samples for the various laboratories. Since the long-term stability of these nutrients in serum is unknown, selected well-characterized specimens are stored under various conditions to determine storage stability. The Nutrients in Human Diet Project is a joint program among several agencies to obtain comparative data on dietary intakes of nutritionally important minor and trace elements in a number of countries.' As part of this effort, samples of the total human diet composites, collected as part of the FDA "market basket" survey,<sup>10</sup> are banked for long-term storage.

# RESULTS AND EXPERIENCE FROM ENVIRONMENTAL SPECIMEN BANKING

# *Development of Procedures/Techniques for Non-contaminating Collection and Processing of Biological Samples*

*Sample collection procedures:* The purpose of a specimen bank is the long-term preservation of specimens that are representative of the state of a site or organism immediately prior to collection. Therefore, a major concern of specimen banking efforts is that the samples be collected, processed, and stored under conditions that avoid or minimize contamination of the specimen or any other changes in their chemical composition. Most environmental samples contain extremely low levels of inorganic and organic contaminants. Since these specimens are intended for long-term storage, which implies cost associated with maintaining the specimens, extreme caution must be exercised to ensure that the samples are not contaminated. Detailed sample collection protocols have been developed and implemented for each of the specimen types in the various projects, i.e., human liver;<sup>5</sup> sediments, fish tissue, and mussels/oysters;<sup>11</sup> and marine mammal tissues.<sup>8</sup>

The basic philosophy for the development of these protocols focuses on the use of non-contaminating materials for any contact with the sample. For example, when samples require dissection (e.g., in the case of human liver, marine mammal, or fish tissues), a titanium-bladed knife is used to avoid contamination from environmentally important trace elements (e.g., Ni, Cr, and Fe) found in conventional cutting instruments. During sample preparation, contact with the specimen is generally limited to clean, dust-free Teflon surfaces and the specimens are stored in Teflon bags or jars. After the specimens are placed in the storage containers, they are frozen in liquid nitrogen as soon as feasible and transported to the specimen bank facility at NIST where the samples are stored in liquid nitrogen vapor freezers at  $-150^{\circ}$ C. Each of the collection protocols for the different specimen types was developed in conjuction with individuals involved in the sampling to achieve a suitable non-contaminating procedure within the bounds of practicality. As part of the sampling protocol, information describing the sample and the sampling site are recorded; this information is maintained, both in hard copy and in a computer database, as part of the documentation for each sample in the specimen bank.

*Cryogenic homogenization:* The preparation of homogeneous sample aliquots from the bulk sample is a major requirement for specimen banking. Identical (i.e., homogeneous) sample aliquots are necessary to allow for valid comparison of analytical techniques and evaluation of the stability of specimens during storage. To address this requirement, we developed a cryogenic homogenization procedure using Teflon disk mills.<sup>12</sup> These mills are capable of homogenizing 150-g sample aliquots to provide homogeneous frozen samples with greater than  $90\%$  of the particles less than 0.46 mm in diameter and with subsampling errors due to inhomogeneity estimated at less than  $2\%$ . Since the initial evaluation and report describing this procedure,<sup>12</sup> we have used it successfully for the homogenization of a variety of specimen types including: human liver and adipose tissues, mussel and oyster tissues, fish tissues (liver and muscle), honey bees, seal tissues (liver, kidney, muscle, and blubber), chicken tissue, and total human diet composites. The cryogenic procedure uses Teflon mills to minimize contamination and eliminates the risk of potential changes in the sample associated with thawing and re-freezing. A similar approach has been used in the Environmental Specimen Bank program in the Federal Republic of Germany where a "continuous flow" aparatus has been developed for the preparation of large quantities of homogenous frozen material.<sup>13</sup>

### *The Value of Specimen Banking*

The specimen bank projects at NIST have provided several examples of the advantages of such activities including: [ 13 providing baseline environmental data for monitoring pollutant trends over time and among different sites, **[2]** providing the opportunity for retrospective analysis of samples from the past, and **[3]**  evaluating the stability of biological samples during storage. Examples of the value of specimen banking activities are discussed below.

*Baseline Environmental Data:* As part of the specimen banking activities, we analyze approximately 20% of the archived specimens to determine selected organic and inorganic constituents. These analyses provide accurate baseline data for the following purposes: **[l]** for use in evaluating the stability of the specimens during long-term storage, **[2]** for comparison with data obtained from other laboratories analyzing similar samples, which were collected at the same time from the same site as part of a monitoring program, **[3]** for comparison with data from samples to be collected in the future for monitoring long-term trends in pollution at a particular site, and **[4]** for comparison with data from different sites for realtime monitoring. An important aspect of our specimen banking activities is that they provide both inorganic and organic baseline data on the same specimens.

Of the 550 human liver specimens collected since **1980, 96** specimens have been analyzed to provide data on about **30** trace elements per specimen. These **96**  specimens were analyzed in three groups of samples collected in **1980, 1982,** and **1984.** The **1980** samples were from three locations, i.e., Baltimore, MD; Minneapolis, MN; and Seattle, WA; whereas the **1982** and **1984** were primarily from Seattle. The complete inorganic data set for the **36** samples from **1980** has been published<sup>5, 14</sup> and portions of the 1982 and 1984 inorganic data sets have been reported.<sup>15</sup> In addition, organic analyses of aliquots from 50 of the same samples have been performed for the determination of chlorinated pesticides and polychlorinated biphenyls (PCBs).16

During the three years of collection and analysis of marine specimens as part of the NOAA NS&T program, we have analyzed sediment, fish muscle, and fish liver samples from **12** sites in the Benthic Surveillance Project and sediment and mussels/oysters from **18** sites in the Mussel Watch Project. Baseline data for approximately 30 trace elements in the fish muscle and liver samples and **45** trace elements in the mussel, oyster, and sediment samples have been determined in these 30 U.S. coastal sites.<sup>17,18</sup> Organic analyses have provided data for selected polycyclic aromatic hydrocarbons (PAHs), PCBs (individual congeners), and chlorinated pesticides for samples from these same **30** sites." From the Alaskan Marine Mammal Tissue Archival Project, we have analyzed muscle, blubber, kidney, and liver tissues from the **1987** collection of northern fur seal *(Callorhinus ursinus)* samples. Similar measurements will be completed on tissue specimens from the **1988** collection of ringed seals *(Phoca hispoda).* 

*Environmental trend monitoring:* One observation from the inorganic baseline data for the human livers<sup>5,6</sup> is that many of the pollutant trace element concentrations are on the low end of or below previously reported ranges.<sup>20</sup> Specifically, the levels of Al, As, Sn, and Pb are lower than previously reported concentrations of these elements in human liver from **1940-1972.20** 

The data for Pb concentrations in the human liver specimens illustrate the use



**Figure 1** Concentrations  $(\mu g/g \text{ wet weight})$  of lead in human liver, as determined in the **NBSB program, compared with literature ranges from various time periods. Numbers above data sets indicate number** of **liver specimens analyzed. Wide horizontal bars in each data set indicate the mean concentration value for that data set.** 

of baseline data in monitoring environmental trends in pollutant levels. The concentration of Pb was determined in the three sets of human liver samples representing specimen collections in **1980, 1982,** and **1984.** These data are illustrated in Figure 1 and are compared with data from earlier studies oi Pb in human livers reported in the literature.<sup>20-23</sup> The mean Pb concentrations for the 1980, 1982, and 1984 sample collections were 0.55, 0.39, and 0.47  $\mu$ g/g, respectively. The mean values from our studies are lower than the mean values for the compiled literature concentrations from **1940-197220** and **1972-1984** (excluding our data sets)23 (see Figure **1);** and the individual values are generally on the low side of the reported literature ranges for Pb in human liver. One of the **1973**  studies" illustrated in Figure **1** was for liver samples from Baltimore, MD, as were some of the **1980** samples from our study, thereby providing a direct geographical comparison. In **1973** the mean Pb concentration in **22** liver samples from Baltimore was  $2.5 \mu g/g$  with a range of  $1.0-6.3 \mu g/g$ ;<sup>21</sup> whereas in 1980 the mean Pb concentration of eight Baltimore samples from our study was  $0.58 \mu g/g$  with a range of  $0.25 - 1.15 \mu g/g$ .

The dramatic decrease in mean Pb concentrations in our 1980-1984 results **as**  compared to earlier data reported in the literature could be attributed to improvements in analytical methodology, improvements in the control of sample contamination in our studies, and/or a decrease in the Pb levels in the environment. Comparing the Pb data from the specimen bank project for 1980-1984, there is a slight decrease in the mean Pb levels between the 1980 and the 1982/1984 data sets. However, the majority of the individual results for the 1982 and 1984 sets are lower than the mean values. This decrease in Pb levels is best illustrated by comparing the median values for the three collections, 0.46, 0.34, and  $0.26 \mu g/g$  for 1980, 1982, and 1984, respectively. These data more accurately reflect the decrease in Pb levels in the environment from 1980 through 1984 which may be attributed to the decrease in the use of leaded gasoline and lead-containing paints.

*Real-time monitoring:* The generation of baseline data on a fraction of the banked samples provides limited real-time monitoring, particularly when the specimen banking is part of an on-going, systematic monitoring program such as the NOAA NS&T specimen bank project for marine specimens. The results from the analyses of fish liver specimens from 12 sites for the determination of selected trace elements (V and Ag) and organic contaminants (PCB 101, PCB 138, 4,4'-DDE, and 4,4'-DDT) are summarized in Figure 2. These results illustrate the monitoring potential available by analyzing banked specimens from selected sites and the different pollution patterns indicated by different analytes. Some analytes are general indicators of urban activity, e.g., Ag and PCBs, as indicated by their high levels in Santa Monica Bay (near Los Angeles, CA) and Boston Harbor, MA; whereas other analytes may be more indicative of high regional inputs such as 4,4'-DDE at all of the California coastal sites and 4,4'-DDT at St John's River, FL. Even though the levels of 4,4'-DDE are high on the California coast, indicating past inputs of 4,4'-DDT into the environment, the levels of 4,4'-DDT are very low except for a large input at St John's River, FL. Since the use of 4,4'- DDT in the U.S. has been limited since the early 1970's, the high concentration of 4,4'-DDT in the St John's River fish specimens would suggest inputs from the Caribbean or South America carried by the ocean currents. The level of V in the fish liver specimens is a good indicator of petrogenic inputs into the environment which are not limited necessarily to urban sources, e.g., high levels in Elliott Bay and Buzzards Bay as well as Santa Monica Bay and Boston Harbor.

A number of PCB congeners, in addition to PCB 101 and 138, have been measured in these fish liver specimens and it appears that the relative ratios of some of these congeners may be species specific. For example, in Figure 2 the relative ratios of PCB 101 and 138 for the Elliott Bay and Nisqually Reach samples (both English sole) are different from all the other specimens (Atlantic and white croaker, spot, winter and starry flounder, and hornyhead turbot). $24$ 

By analyzing different tissues from the same animal or different specimen types from the same site (e.g., sediment and bivalves or fish), we can determine which



**Figure 2 Concentrations (pg/g dry weight)** of **selected inorganic and organic contaminants in fish liver specimens for selected US. coastal sites.** 

specimen tissue or sample type is the most appropriate for long-term archiving. Since storage space in a long-term archive is limited, tissues that tend to concentrate the analytes of interest are preferred. In the NOAA NS&T program, both tissue and sediments have been collected and in the case of fish both the muscle and the liver tissue have been archived. Preliminary comparisons of the fish muscle and liver tissue indicate that the muscle levels of the **PCBs** and pesticides are 2-10 times lower than those in the livers. Thus, even though liver samples are more difficult to obtain, i.e., more than 200 fish livers may be required to obtain sufficient sample for banking (about  $150g$ ), the liver samples are the most appropriate for banking. Similar comparisons of contaminant levels in the various tissues are underway for the Alaskan marine mammal samples. A similar argument can be made for banking tissues rather than sediments; even though the sediments



Figure 3 Concentration of arsenic (ng/g wet weight) in human liver specimens collected in 1980 **compared with literature ranges reported in 1978 and 1985.** 

are easy to obtain in sufficient quantities, the sediment levels are generally an order of magnitude lower. In addition, the sediments may not accurately reflect the impact of the contaminants on the organisms as indicated by the high levels of Ag found in the fish liver specimens from Dana Point and Santa Monica Bay, CA, whereas only the sediment from Santa Monica Bay contained a high level of Ag.

### *Opportunity for Retrospective Analyses*

An important justification for specimen banking is that analytical methods are continually improving and thus the availability of banked samples allows researchers to apply these "improved" procedures to specimens from the past. During our nearly nine years of banking human liver specimens, we have witnessed a number of such advances and/or improvements in analytical procedures. The determination of As in the human liver specimens is an example where the analytical procedures lacked sufficient sensitivity at the time the samples were initially collected. Based on the literature data available in 1980 for the concentration range of As in liver (see Figure 3), we used atomic absorption spectroscopy (AAS) to analyze the samples. However, the concentrations of As in the samples were below the expected levels and very near the detection limit of the **AAS** technique. Since 1980 we have developed a more sensitive radiochemical neutron activation analysis (RNAA) procedure for the determination of  $As.<sup>15</sup>$  In our efforts to determine the stability of banked specimens (see discussion below), we re-analyzed the human liver specimens from the 1980 collection during 1988 using the RNAA procedure that was not available when the samples were

originally analyzed in 1980. The results are summarized in Figure 3 and the more recent literature range reflects the lower concentrations of As found in human livers.

In the case of our organic analyses of the human livers, we have implemented significant improvements in our procedures since 1980 that allow quantitation of individual PCB congeners, whereas in the analysis of the 1982 liver collection, only selected chlorinated pesticides (e.g., **4,4'-DDE,** dieldrin, and trans-nonachlor) were measured. For the analysis of the 1984 collection, an LC isolation procedure was implemented to isolate the chlorinated pesticides and PCBs into two separate fractions prior to GC analysis.

An excellent opportunity for retrospective analyses exists for the determination of ultra-trace levels of Pt in banked human liver specimens. Recently, interest in the level of Pt in the environment has increased due to the use of Pt in the catalytic converters on many automobiles. In 1980 when the human liver samples were collected, sensitive methods capable of determining the low levels of Pt in human livers  $(10-70 \text{ pg/g})$  were not available. Since then we have developed methods for the accurate determination of these levels in liver and have reported baseline data on 12 samples from the 1980 collection.<sup>25,26</sup> The opportunity now exists to apply these sensitive techniques to a significant number of samples that were collected since 1980 to verify whether Pt concentrations are increasing or decreasing in the human population.

# *Assessment of Long- Term Stability of Environmental Specimens*

One goal of the EPA pilot Environmental Specimen Bank project was to determine the stability of specimens at various conditions such as freeze-dried and stored at room temperature, and fresh frozen and stored at  $-25^{\circ}$ C,  $-80^{\circ}$ C, and  $-150^{\circ}$ C. To address the question of storage stability, aliquots (about 6-8 g) of the homogenized human liver samples were stored under the above conditions. After seven years of storage under two of the conditions  $(-25^{\circ}C \text{ and } -150^{\circ}C)$  and reanalyzed for comparison with baseline data obtained in 1980 (for inorganic constituents only) and for direct comparison of the two storage conditions. The results for the determination of Se in 24 samples from each of the two conditions are compared to baseline data in Figure **4.** No changes in Se concentrations during storage are indicated by the data. The slight deviation in the slope of the regression lines is within the boundaries for the uncertainty of the analytical measurements. Some of the "outliers" in this set can be related to "outliers" in the results for other elements determined<sup>27</sup> and are probably due to weight differences in the freeze drying process. Similar results indicating no changes during storage were observed for additional trace elements measured.<sup>27</sup>

Aliquots of the same samples were also analyzed for the determination of pesticides and selected PCB congeneners. Preliminary results for the determination of  $4,4'-DDE$  and PCB 153 in nine subsamples stored at  $-25$  and  $-150^{\circ}C$  are shown in Figure 5. As with the trace element results, no significant changes were observed between the samples stored at  $-25^{\circ}$ C and  $-150^{\circ}$ C as indicated by the

.



**Figure 4 Comparison of selenium results from the analysis of human liver specimens in 1981 and**  re-analysis after seven years storage at  $-25$  °C and  $-150$  °C. Concentrations are reported in ng/g wet **weight.** 

slope of the regression lines. Unfortunately, no organic baseline data for the 1980 liver samples were available to determine whether analyte concentrations had changed at both of the storage conditions since the initial storage.

Even though the chemical analyses of the samples stored at the  $-25^{\circ}$ C and  $-150^{\circ}$ C indicated no significant changes in composition, there was physical evidence of changes in the sample aliquots. At  $-25^{\circ}$ C the aliquots of frozen liver homogenate had formed ice crystals under the container lids and on the sample surface (i.e., the moisture in the samples had separated), and the homogenates were no longer powdery but were clumped. The samples stored at  $-150^{\circ}$ C were still powdery, as they had been at the time of homogenization. The subsample weights were stable over the storage interval for both storage conditions. The separation of moisture from the samples stored at  $-25^{\circ}$ C would necessitate the use of the total subsample for any analytical determinations. In addition, the color of the sample aliquots stored at  $-25^{\circ}$ C was different from those stored at  $-150^{\circ}$ C. Samples stored at  $-80^{\circ}$ C did not show any formation of ice crystals or any noticeable color changes. Complete details and results of the re-analysis of liver specimens stored for seven years will be reported elsewhere. Re-analyses of banked samples will continue in the NBSB projects to further asess the effects of long-term storage at different conditions. We are currently banking all of our samples at  $-150^{\circ}$ C to



**Figure 5 Comparison** of **results for the determination of 4.4'-DDE and PCB 153 in human liver**  subsamples stored at  $-25$  °C and  $-150$  °C for seven years. Concentrations in ng/g extractable fat.

avoid the physical changes noted above and because of the relative maintenancefree, low-cost operation of the liquid nitrogen vapor freezers.

### **CONCLUSIONS**

Almost ten years of practical experience in specimen banking within the National Biomonitoring Specimen Bank have demonstrated that the concept of long-term storage of environmental specimens is feasible. Although the total scientific value of the banked samples is not fully known at this time, the current uses of the banked samples and the implementation of the concept has already contributed to major monitoring programs in the **US.** and abroad. Even though the types of specimens and the number of samples collected are limited, the NBSB can serve as a valuable resource for the assessment of long-term trends of pollutants affecting human and environmental health, in particular for those pollutants that have been unnoticed thus far or that could not be measured in the past.

### *Acknowledgements*

This work was supported in part by the Office of Health Research, Office of Research and Development of the Environmental Protection Agency; National Ocean Service, Office of Oceanography and Marine Assessment, Ocean Assessments Division of the National Oceanic and Atmospheric Administration; and the Minerals Management Service, Department of Interior.

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