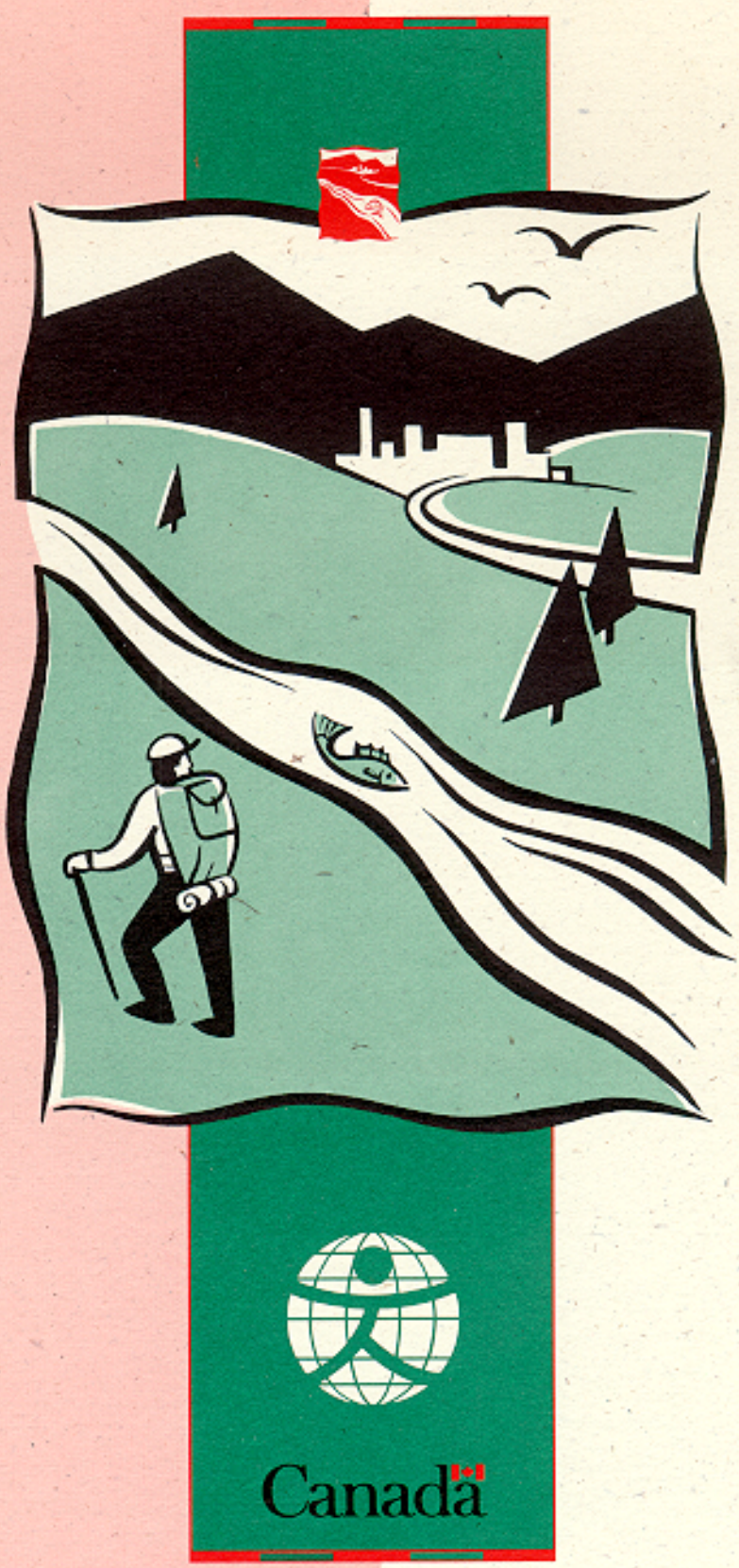
FRASER RIVER ACTION PLAN



ASSESSMENT OF THE INTEGRITY OF CHEMICALS IN ENVIRONMENTAL SAMPLES OVER AN EXTENDED PERIOD OF TIME

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Disclaimer

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ABSTRACT

The stability of trace organic and inorganic analytes in environmental samples stored prior to analysis is related directly to the effectiveness of the methods used to preserve the samples. In most analytical laboratories, freezing is the storage method of choice for environmental samples, although little is known about the effects of freezer temperature and storage duration on the stability of target contaminants. A study was thus undertaken to seek out the available stability data, to evaluate the usefulness of the data in generating storage protocols, and to recommend areas needing further study.

This study looks at the sample storage conditions currently used in analytical laboratories, evaluates published and unpublished storage study data, and documents anecdotal information gathered in an extensive literature search and through personal communications with scientists. The primary focus of the study is on the stability of a selected list of semi-volatile organic contaminants, organotin compounds, and trace metals in sediments and tissues. A less rigorous look is taken at the stability of these compounds in vegetation and water/wastewater.

Little stability data was found for most analytes, with the exception of chlorinated pesticides and PCBs in tissues, organotin compounds in sediments, and trace metals in all matrices. Most analytes were found to be stable in samples frozen at -20ºC, but the duration of stability at this temperature was less well understood. The report includes a summary of current storage stability data, takes a look at long term storage in specimen archives, provides information on current storage protocols, and makes recommendations for analyte/matrix combinations requiring further study.

Résumé

Il existe une corrélation directe entre la stabilité des substances organiques ou minérales à l'état de traces contenues dans des échantillons provenant de l'environnement et qui sont stockés pendant un certain temps avant d'être analysés, et l'efficacité des méthodes employées pour leur conservation jusqu'au moment de l'analyse au laboratoire. La plupart de ceux-ci préfèrent congeler les échantillons prélevés dans l'environnement; mais l'efficacité de la conservation au froid et les effets de la durée de la période de conservation sur la stabilité des contaminants à étudier sont peu connus. Nous avons donc réuni les renseignements connus sur la stabilité des contaminants et nous avons examiné dans quelle mesure ils peuvent servir à la préparation de protocoles de stockage; enfin nous indiquons des questions qu'il faudrait étudier de manière plus détaillée.

Nous examinons les conditions de stockage des échantillons présentement employées par les laboratoires d'analyse, nous évaluons des données publiées ou non et nous documentons des renseignements à caractère anecdotique que nous avons recueillis à l'occasion de notre recherche documentaire exhaustive et au cours d'échanges avec des scientifiques. L'objectif premier de l'étude était de déterminer la stabilité d'une liste déterminée de contaminants d'intérêt pour Environnement Canada, notamment de composés organiques semi-volatils, d'organo-étains et de métaux à l'état de traces dans les sédiments et dans les tissus animaux. Nous nous sommes penchés de façon moins rigoureuse sur la stabilité de ces composés dans la végétation et dans l'eau ou les eaux usées.

Exception faite des pesticides chlorés et des BPC dans les tissus animaux, des organo-étains dans les sédiments et des métaux à l'état de traces dans tous les substrats, il existe peu de renseignements sur la stabilité de la plupart des échantillons à analyser. La plupart de ces substances à doser sont stables lorsque les échantillons les contenant sont congelés à -20°C, dependant la durée de la stabilité à cette température est moins bien documentée. Nous incluons dans le rapport un résumé des données courantes sur la stabilité au stockage, nous examinons le stockage à long terme des collections d'échantillons, nous décrivons sommairement les protocoles de stockage en vigueur et nous formulons des recommandations d'étude de combinaisons de substances à doser et de substrats.

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ABBREVIATIONS

AA	atomic absorption spectroscopy
AMMTAP	Alaska Marine Mammals Tissue Archival Project
AOX	adsorbable organic halides
ASTN	American Society for Testing and Materials
B[a]p	benzo[a]pyrene
CB	chlorinated biphenyl
CCIW	Canadian Centre for Inland Waters
CHC	chlorinated hydrocarbon
CWS	Canadian Wildlife Service
DBT	dibutyl tin
DDD	6,6'-dithiobis-2-naphthalenol
DDE	(pp'-dichlorodiphenyl) dichloroethylene
DDT	(pp'-dichlorodiphenyl) trichloroethane
DFO	Department of Fisheries and Oceans, Canada
EOX	extractable organic halides
GESB	German Environmental Specimen Bank
GLFSB	Great Lakes Fisheries Specimen Bank
GLRSB	Great Lakes Regional Specimen Bank
HCB	hexachlorobenzene
Hg	mercury
ICP	Inductively Coupled Plasma
IJC	International Joint Commission
LN_2	liquid nitrogen
MBT	monobutyltin
MFO	mixed function oxygenase
MMS	Minerals Management Service, USA
NBSB	National Biomonitoring Specimen Bank, U.S.A.
NCASI	National Council of the Paper Industry for Air and Stream Improvement
NIES	National Institute of Environmental Studies, Japan
NIST	National Institute of Science and Technology, USA
NMMTB	National Marine Mammals Tissue Bank, USA
NOAA	National Oceanic and Atmospheric Administration, USA
NWRI	National Water Research Institute, Canada
OPR	Office of Protected Resources, NOAA, USA
PAH	polycyclic aromatic hydrocarbon
Pb	lead
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-para-dioxin
PCDF	polychlorinated dibenzo-furan
PCR	polymerase chain reaction
QA/QC	quality assurance/quality control
RT	room temperature
SRM	standard reference material
TBT	tributlytin
TOX	total organic halides
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey

1. INTRODUCTION AND BACKGROUND

Contamination of our environment by toxic anthropogenic chemicals has presented a problem of escalating proportions since the time of the industrial revolution. However, only in recent years has man taken a serious interest in examining the effects of these chemicals on the environment, quantifying their presence, and attempting to reduce their discharge onto the land, and into the air and water.

One of the critical components in looking at contamination in our environment is the chemical analysis of soils, biota, water and air to determine the magnitude and extent of the contamination. Today, large numbers of samples are taken in the field and transported to public and private laboratories for analysis of a wide variety of analytes, ranging from trace elements to organic pesticides. Samples must be taken in such a way as to be representative, and to allow their integrity to be maintained once they have left their locations of origin in the field. Methods of transportation of the samples to a laboratory for analysis and preservation of the samples until they can be analyzed are crucial considerations in planning field sampling programs.

Since many environmental samples are subject to change once they have been taken from the field, they must be stored in such a way as to minimize these changes. A number of preservation techniques are used to retard microbial degradation and oxidation of target analytes, and minimize losses of volatile components in environmental samples. These techniques include controlling pH, poisoning, drying, isolation from the atmosphere, refrigeration, and freezing. The most important factors, however, in the storage of environmental samples are time and temperature (Mudroch 1991) and these two considerations are the primary focus of this report.

There has always been uncertainty over holding times for environmental samples destined for organic or inorganic chemical analysis. The ASTM defines holding time as "... the period of time during which a water sample can be stored after collection and preservation without significantly affecting the accuracy of analyses..." (Mudroch 1991). Although this definition can reasonably be applied to samples other than water, little research has been done to quantify holding times for sample matrices containing specific chemical contaminants. Even large regulatory agencies have not always based their protocols for sample storage on documented studies. However, since there is always a period of time between collection and analysis of environmental samples, there is a need to define storage conditions and maximum holding time in order to preserve the integrity of the target analytes. For the benefit of field scientists and research laboratories, choice of storage conditions should be based on rigorous and well-documented studies.

The storage of environmental samples over periods of months, years, or even decades may

be desirable or unavoidable for retrospective studies, specimen archiving, or due to analytical laboratory backlogs. While some holding times have been specified by organizations such as the USEPA (Klemm 1990a and 1990b, Tetra Tech 1986), Environment Canada, and many of the provinces (Gaskin 1988, McQuaker 1989, Gaskin 1991, Anon. Env. Can. 1983, Hart 1991, Kalnins 1991, British Columbia 1994), much variability still remains in storage practices used by analytical chemistry laboratories. Many laboratories base their storage practices on poorly documented in-house studies or recommendations arrived at by workshop participants during project planning. This sample storage information is thus not accessible to other groups.

This report assembles and evaluates existing information from laboratories, researchers, and the scientific literature and is intended to provide the information necessary to determine whether further storage studies are needed, and in which areas. It also makes recommendations based on the information gathered in the study.

2. OBJECTIVES OF STUDY

The objectives of this study are as follows:

a. To access, compile, and evaluate existing information on the degradation of chemical contaminants in environmental samples stored over various periods of time.

b. To document data gaps where the information required for specific chemicals is inadequate for proper evaluation, and to make recommendations for additional studies to generate the required information.

c. To prepare a report documenting the above information.

3. STUDY METHODOLOGY

3.1 Scope of the Study

The list of analytes considered was taken from the current Standing Offer for Analysis which Environment Canada, Pacific Region, has with Axys Analytical Services Ltd. Sample matrices of interest to Environment Canada included primarily sediment (soil) and biota (tissue and vegetation). Water and wastewater matrices were given secondary consideration due to the small volume of samples normally taken by Environment Canada.

3.2 Survey of Unpublished Findings

Much of the data on the stability of environmental samples under different storage conditions are unpublished. For this reason, a number of scientists in the field were personally contacted in order to uncover this "grey" literature or raw data. A list of these individuals is given in Appendix I.

3.3 Literature Search

The University of Victoria and University of British Columbia library catalogues were searched with such key words as "specimen", "sample", "stability", and so on. In addition, published material and references were provided by many of the scientists consulted in the survey portion of this study.

A comprehensive literature search for specific analytes was not carried out as there are an almost unlimited number of analytes, a huge number of specimen types, and a wide range of analytical techniques for each combination. Similarly, searching such terms as "storage" turned up thousands of entries.

The "Chemical Abstracts" computerised database operated by Chemical Abstracts Service (CAS), a division of the American chemicals Society, was searched with key words such as "sample#", "stabil?", and "storage" (Appendix IV). A consultant may make and provide one copy of information obtained from CAS services to Environment Canada, provided that the CAS copyright notice is placed on the material. Environment Canada can subsequently use the literature search material under CAS guidelines, which are provided in Appendix III. The "Medline" computerized database was similarly searched (Appendix V).

A final survey of recent literature (1994/95) was carried out to look for studies which had not yet been abstracted. Information on sampling and storage techniques was obtained from a number of reports which studied concentrations of contaminants in environmental samples. This information was used to give an overview of currently accepted storage techniques and, while copies of the papers are provided with the report, the many individual references are not reported in the bibliography.

4. RESULTS

4.1 Literature Search

The computerised searches resulted in an extensive set of abstracts on the topics of stability and storage. However, the great majority of these articles referred to pharmacological, medical, photographic, or petroleum distillate products. Articles referring to environmental samples were almost exclusively in reference to specimen banks. Edited versions of the literature search results are compiled in Appendices IV and V for the use of Environment Canada. These abstracts are not intended for distribution and the most relevant titles have been included in the bibliography.

4.2 Analyte Specific Sample Storage Information

Information from published and unpublished sample storage studies along with anecdotal information is summarized according to analyte. Most storage studies were carried out at 4, -20, -80, and -150ºC while a few reported freezer temperatures of -28 and -40ºC, various freeze drying techniques, or room temperature storage.

4.2.1 Chlorophenols and Chlorinated Anisoles

4.2.1.1 Sediments

A sample storage stability study, carried out by the National Council of the Paper Industry for Air and Stream Improvement Inc. (NCASI), suggests a maximum storage time of at least five months for phenolic compounds in wastewater treatment plant sludges kept frozen at -15ºC (Louch 1993). The forty phenolic compounds studied included chlorinated and non-chlorinated phenols, guaiacols, syringols, catechols, vanillins and syringaldehydes. Analysis of centrifuged sludge sub-samples was carried out at 0, 20, 37, 65 and 156 days.

4.2.1.2 Tissues

A recent paper reports that duck mussels from a lake in Finland were stored whole at -20ºC prior to analysis for chlorophenols, hydrocarbons and fatty acids (Pellinin 1994). As was found to be typical of most reports in the recent literature, no reference was made to storage duration.

4.2.1.3 Vegetation

No data.

4.2.1.4 Water and Wastewater

In their literature survey, Neaves and Fong found that large volumes of data were available for drinking/surface waters. Samples were found to be stable for up to 15 weeks at 4ºC when they are preserved with chloroform or H_2SO_4. No further study was recommended (Neaves 1990). The Canada Centre for Inland Waters recommends storage of drinking water samples preserved with chloroform or sulphuric acid for up to 3 weeks (Koester 1993).

Recent unpublished studies by NCASI on the stability of wastewater samples indicate that chlorophenolics were not stable enough at pH > 2 to allow shipping at ambient temperatures from the sampling site to the laboratory (Lafleur, pers. comm.). Stability was achieved only at pH $\leq$ 2.

4.2.2 Chlorinated Paraffins

No data.

4.2.3 Polychlorinated Dibenzodioxins and Dibenzofurans (PCDD/Fs)

4.2.3.1 Sediments

An international inter-laboratory study of fortified and unfortified soil standards for the 17 toxic PCDD/F isomers included storage stability as one of its Data Quality Objectives (Keith 1992). Analyses after 3 months at room temperature, and again after 3 subsequent months at 4ºC indicated sample stability within Certificate of Analysis requirements. Additional stability data is expected to be published by Radian Corporation at a later date.

Long-term agricultural experiments in the UK are being used to quantify inputs of PCDD/Fs, PCBs, and heavy metals into agricultural soils from sewage sludges (Stewart 1995). Samples from sewage treatment works were air dried and ground prior to analysis and/or archival storage at room temperature. Stability under these conditions was assumed in the retrospective analysis of samples dating from 1942 to 1960. This important sample archive has the potential for repeat analyses and may provide information on the stability of PCDD/Fs in the future.

A review of current literature indicates that moderate storage conditions are being employed for PCDD/Fs in sediment and soil samples although no references were found to studies of storage stability in environmental samples. In a typical study, sediments from two US lakes were stored in the laboratory at 4ºC and dried overnight at 40ºC prior to analysis (O'Keefe 1994).

4.2.3.2 Tissues

An anecdotal report from the Institute of Marine Biosciences in Ottawa tells of undocumented studies on the stability of standard reference material fish tissue used for PCDD/F and PCB measurement (Guevremont, pers. comm.). Samples are processed by grinding to a fine homogenate, ampouling, baking in steam, and sealing in glass. They are subsequently stored for at least one year.

4.2.3.3 Vegetation

No data.

4.2.3.4 Water and Wastewater

From the Analysis of Drinking Water for Trace Organics comes the following quotation: "...For hydrophobic analytes such as PCDDs and PCDFs, it may not be necessary to refrigerate samples before analysis ... however, no definitive study has been reported to examine this factor..." (Koester 1993).

No data for wastewater and limited data for drinking/surface water were found by Neaves and Fong who recommended the addition of $Na_2S_2O_3$ if the presence of chlorine was suspected, and refrigeration at 4ºC (Neaves 1990).

According to a recent supplement to the USEPA method for PCDD/Fs in drinking water, samples can be stored for up to 90 days at ambient conditions without preservative, with analysis to follow within 40 days of extraction (Koester 1993, USEPA 1990).

4.2.4 Polychlorinated Biphenyls (PCBs)

4.2.4.1 Sediments

A study carried out at the National Water Research Institute in preparation for the establishment of a Great Lakes Sediment Bank found that total PCB concentrations in sediment samples stored at -20ºC or freeze dried at 20ºC remained stable over a period of 6

months (Bourbonniere 1986, Mudroch 1991). The samples which were freeze dried at 20ºC were found to have stable PCB concentrations for 6 years, while loss of frozen samples due to freezer failure precluded storage data past 6 months. Further details of the study can be found in Section 4.2.4.2.

In a long term monitoring project in the UK, samples of agricultural soils have been archived since the mid 1800s. Since the 1940s, sample handling procedures have remained constant with soils being air-dried, ground and sieved, then sealed in glass containers and stored at room temperature. More contemporary samples are being stored at 4ºC. These samples provide an excellent opportunity for retrospective analysis of trace contaminants, and two recent studies report trends in PCB contamination with time (Alcock 1993, Sanders 1994). Alcock reviewed the possibilities for PCB loss during storage and explained why photo-oxidation and microbial degradation were not expected to contribute significantly to analyte loss. Volatilization during air-drying was discounted because a reduction in low molecular weight PCB congeners, which would be expected to accompany volatilization, was not seen. In fact, a predominance of lower molecular weight congeners was seen in earlier samples. This circumstantial evidence does not, however, conclusively prove that volatilization did not occur in some or all of the samples.

Eric Crecelius of Battelle Pacific Northwest Laboratories reported that a field sampling manual resulted from a project carried out on sediments from Puget Sound (Crecelius, pers. comm.). Sediments were stored at -18ºC and analyzed every 6 months for three years. No change was observed for PCBs, PAHs, and chlorinated pesticides over the full three year period. Data was not available.

Jim Smith, a chemical environmental consultant, reported two court cases which hinged on the analysis of PCBs in stored soil samples (Smith, pers. comm.). Two dried soil samples, stored at room temperature for one and 6 years, showed no significant differences in PCB concentrations on reanalysis.

4.2.4.2 Tissues

A report describing the long term effects of frozen storage on total PCB and organochlorine pesticide concentrations in lake trout whole fish homogenates is currently in preparation at the Great Lakes Fisheries Specimen Bank (GLFSB) in Burlington, ON (Kiriluk 1995). Samples stored at -20, -80 and -196ºC were analyzed fresh, after 1, 3, and 11 months, and again after 2, 3, and 4 years. The study used a homogenate of two lake trout captured in 1986 from which five replicate sub-samples were taken for each storage condition. Total PCB concentrations were found to be significantly higher in the fresh samples than they were in the samples stored for 1, 3, and 11 months at all temperatures. No significant difference was found between samples stored at -20 and -80ºC while samples stored at -196ºC had

slightly lower PCB concentrations. At 48 months, samples stored at all temperatures had significantly lower total PCB concentrations. Results for samples stored for 24 and 36 months are being re-evaluated for apparent analytical anomalies. A detailed final report is in preparation.

An interesting study of temporal trends in PCB and p,p'-DDE concentrations in lake Ontario is nearing completion at the Canadian Department of Fisheries and Oceans Bayfield Institute (Huestis 1995). Samples of lake trout of uniform age dating back to 1977, all archived at -20ºC, were re-analyzed for total PCBs, PCB congeners and p,p'-DDE. These results were compared to the results of analyses carried out immediately following collection each year throughout the study. Re-analyzed total PCB concentrations were found to vary randomly in relation to the historical total PCB levels. While a trend toward decreasing PCBs concentrations over the 16 year study period was evident, there was no obvious trend in PCB degradation with storage over that time.

In an experiment carried out at NIES in Japan, homogenized mussel tissue was spiked with PCBs, organochlorine pesticides, hydrocarbons, phthalate esters, and other organic contaminants and stored at -20 and -85ºC (Ambe 1993). No significant changes in PCB or chlorinated hydrocarbon concentrations were noted at either temperature over the 2 year study.

Alaska Marine Mammal Tissue Archival Project (AMMTAP) routinely analyses seal, whale, and sea lion blubber, primarily for PCBs but including organochlorine pesticides (Becker 1993).

When a pilot Environmental Specimen Bank was set up in 1979 by the US EPA at NIST, one of its goals was to determine the stability of specimens under various storage conditions: freeze dried and stored at room temperature, and fresh frozen and stored at -25 and -150ºC (Zeisler 1992). A set of 24 samples stored at -25 and -150ºC were analyzed for chlorinated pesticides and selected PCB congeners. No significant differences or changes in concentrations were observed over the 7 year duration of the study for PCB 180 and 4,4'-DDE in homogenized human liver samples. The only difference noted between the samples held at the two different storage temperatures was the formation of ice crystals under the lids of samples stored at -25ºC. The authors recommend the use of a complete sample for any analytical determination. All samples at NIST are now stored in liquid nitrogen freezers at -150ºC.

In one of the few comprehensive storage studies found for biological tissues, carried out at Canada's GLFSB, samples of whole coho salmon homogenates and bulk zooplankter (Mysis relicta) were stored at -20, -40, -80 and -196ºC or prefrozen in liquid nitrogen and stored at -20 or -40ºC (Hyatt 1986). Drying at +60ºC was also investigated. Analysis of samples for

PCBs, chlorinated pesticides and lipids provided a wealth of information on storage of tissue samples for organic contaminants.

Pre-freezing at -196ºC did not have a significant effect on PCB concentrations in salmon homogenates. Lipid levels remained constant for one year at -20ºC and 2 years at -40ºC, but decreased significantly at -20ºC after 2 years. PCB concentrations decreased gradually over two years at -20ºC (average 21% loss), but did not change significantly at -40ºC. Zooplankter samples stored at -20 and -40ºC and freeze dried at +60ºC followed by storage at room temperature showed no changes in concentration over one month. However, after 35 months of storage, concentrations were all below detection limits (0.09 ug/g). The need for immediate storage or processing of Mysis relicta was confirmed with experiments which showed a loss of up to 66% of PCBs when storage was delayed for one day after collection. (Samples were stored at -196ºC for approximately 6 months.) Unexpectedly, samples stored at -80ºC on processing day retained significantly higher concentrations of PCBs (26%) than those stored at -196ºC (Hyatt 1986).

Section 4.2.3.2 describes the preparation and storage of PCB standard reference material (ampouled fish tissue homogenates) which was found to be stable for at least one year (Guevremont, pers. comm.).

4.2.4.3 Vegetation

No data.

4.2.4.4 Water and Wastewater

Analysis of drinking/surface water data suggested two effective storage methods (Neaves 1990). Without preservation, samples were found to be stable for 6 weeks at 4ºC, and with chloroform preservation, the storage time could be extended to at least 15 weeks. Limited data was found for wastewater and indicated variable recoveries at 4ºC without preservation.

4.2.4.5 Other

Samples of human milk stored for 15 years at -20ºC in the Mother's Milk Centre in Stockholm were recently re-analyzed for total PCBs and pesticides (Noren 1993). Calculated on the basis of fat, and re-analyzed by methods used previously, it was shown that total PCB concentrations did not decline over the lengthy storage period. Congener-specific PCB analysis of archived samples indicated a change in PCB profile over the years.

Musial and Uthe (1988) stored sealed ampoules of cold-extracted herring oil, fortified with Arochlor 1254, and control oils at -20ºC for a period of 8 years, analyzing samples at 1, 9, 16,

41, 49, 82, and 94 months (Musial 1988). Concentrations of PCBs in both fortified and control oils did not change appreciably during the first 16 months and small variations during the balance of the study were not considered significant.

4.2.5 Chlorinated Pesticides

4.2.5.1 Sediments

An anecdotal report on the stability of chlorinated pesticides in sediments showed that no changes in concentration occurred over 3 years at -18ºC (Crecelius, pers. comm.) Details are given in section 4.2.4.1.

In preparation for establishing the Great Lakes Sediment Bank, scientists at Canada's National Water Research Institute compared the stability of HCB, total PCBs, DDE, Mirex, and DDD in sediment samples from 2 sites in Lake Ontario, under three storage conditions: frozen at -20ºC, freeze dried at 20ºC, and freeze dried at 37ºC following freezer storage at -20ºC for two months (Bourbonniere 1986). During the freeze drying process, half the water was removed at 37ºC and the remaining half at room temperature. After storage for 6 months, good agreement was found between samples frozen at -20ºC and samples freeze-dried at 20ºC (within experimental error of 20%). Samples freeze-dried at 37ºC showed lower concentrations of some analytes and the authors noted an anomaly of Mirex concentrations increasing with increased storage and handling temperatures.

In a separate time series experiment, sediment samples were analyzed at 0, 2 and 6 months. Significant loses in concentration over 6 months were noted for Mirex (35%), DDE (30%), and DDD (43%) in both frozen and freeze dried (20ºC) sediment samples, with two thirds of the total losses occurring in the first two months. Total PCBs and HCB concentrations did not change significantly over the 6 month study period. Freezer failure precluded analysis of samples at one year. The freeze-dried samples were analyzed once again after 6 years in storage and were found to have changed little between 6 months and 6 years (Bourbonniere 1986).

4.2.5.2 Tissues

A report currently in preparation at GLFSB which documents storage of lake trout homogenates at -20, -80, and -196ºC (see Section 5.1 4.2) indicates few significant differences in organochlorine pesticide concentrations at -20 and -80ºC (Kiriluk 1995). Pesticides included α-and γ-hexachlorocyclohexane, hexachlorobenzene, α- and γ-chlordane, dieldrin, endrin, p,p'- and o,p'-DDT, p,p'-DDE, p,p'-DDD, Mirex, and photomirex. There appeared to be lower concentrations of organochlorine pesticides in samples stored at -196ºC for all of the time periods. Lipid content was lower for all samples stored at -196ºC

while moisture content was significantly higher. The authors note that little baseline data is available to compare concentrations in fresh, unfrozen samples with samples which have been stored frozen. Observed decreases in DDT concentrations were explained by dehydrochlorination which led to corresponding increases in concentrations of DDT metabolites (primarily p,p'-DDE). The authors conclude that, based on this study, long-term storage at liquid nitrogen temperature does not offer any advantages over storage in commercial electrical freezers (-80 or -20ºC). Both of the lower temperatures appeared to provide adequate means of preventing the degradation of homogenized whole fish tissue by bacterial or enzymatic activity.

The reanalysis of Lake Ontario trout samples described in Section 4.2.4.2, showed that concentrations of p,p'-DDE found on reanalysis of samples up to 16 years old were higher than the concentrations found at the time of sampling (Huestis 1995). This was explained in terms of changes in analytical protocols and techniques and changes in quantification methods. (e.g., the authors used 80 PCB congeners to generate total PCB values, a practice which was not possible 10 years ago.) Sample degradation was assumed to be unlikely based on previous studies and on the random variation shown between historical and reanalyzed samples (Norstrom 1985).

Over a three year period, scientists from the Canadian Wildlife Service analyzed fresh herring gull egg homogenates, freeze dried whole body herring gull homogenates, and freeze dried chicken egg homogenates for 23 organochlorine compounds (Norstrom 1985). Concentrations of heptachlor epoxide, oxychlordane, dieldrin, HCB, p,p'-DDE, Mirex, and PCBs in herring gull egg homogenates remained stable during storage at -18 and -28ºC. In general, significant evaporative losses of the more volatile compounds, and dehydrochlorination of DDT, DDD and γ-HCH occurred in all of the freeze dried homogenates stored at room temperature. The authors recommend against the use of freeze drying for preservation of tissue samples for organochlorine compounds.

See section 4.2.4.2 for a report of a study carried out at NIST in which homogenized human liver samples stored at -20 and -150ºC did not show any decrease in p,p'-DDE concentrations over 7 years (Zeisler 1992).

A study of the storage stability of organochlorine pesticides in coho salmon homogenates, investigated storage at -20 and -40ºC. Of the 10 pesticides studied, only Mirex showed a significant decrease in concentration at -20ºC over one year. Losses over 2 years at -20ºC were significant for Mirex, photomirex, and p,p'-DDD, while storage at -40ºC alleviated all but the Mirex concentration changes. γ-Chlordane, oxychlordane, and heptachlor were at or near detection limits, and concentrations of p,p'-DDT, α-chlordane, HCB, and dieldrin fluctuated randomly, thus precluding analysis of the data. The report concludes that storage temperatures of -20 and -40ºC are not adequate to maintain the integrity of many chlorinated

hydrocarbon residues in coho salmon homogenates on prolonged storage (> 2 years). Pre-freezing in liquid nitrogen did not affect sample integrity.

Phase II of the project studied the stability of PCBs and organochlorine pesticides in the zooplankter Mysis relicta stored at -20, -40ºC or freeze dried at +60ºC followed by storage at room temperature (Hyatt 1986). It was concluded that HCB was the only chlorinated hydrocarbon to be affected by storage conditions over a period of 20 months, and this effect was noted only in the freeze dried sample.

Phase III of the project looked at the storage of Mysis relicta samples at -80 and -196ºC and compared storage initiated on collection day with storage initiated the following day. Major reductions in all chlorinated hydrocarbon concentrations (50 to 80%) were found when zooplankter samples were analyzed one day after collection as opposed to on collection day. This phenomenon appears to be related to lipid loss which occurs very quickly after these fragile organisms are captured. There was some evidence for loss of chlorinated hydrocarbons when samples were immersed in liquid nitrogen (-196ºC) as opposed to being cooled in liquid nitrogen vapour or stored at -80ºC. The authors recommend storage of Mysis at -80ºC immediately upon collection (Hyatt 1986).

Section 4.2.4.2 includes a description of a study carried out at NIES which showed no changes in chlorinated hydrocarbon concentrations for samples stored at -20 and -85ºC over two years (Ambe 1992).

4.2.5.3 Vegetation

Very limited data found by Neaves and Fong indicated that storage with no preservation produces variable results (Neaves 1990).

4.2.5.4 Water and Wastewater

As a component of the recent National Pesticide Survey, the USEPA studied the preservation and stability of well water samples and extracts over a 14 day period (Munch 1992). One litre samples were preserved with 10 mg of $HgCl_2$ and analyzed immediately and after 14 days storage at 4ºC. Additional samples were extracted and the extract analyzed after 14 days. Of the 25 chlorinated non-polar pesticides which can be extracted by EPA Method 515.1 (GC/ECD), only heptachlor showed reduced concentrations in some samples (22%) held for 14 days. Other pesticides studied included nitrogen phosphorus containing pesticides, chlorinated acids, triazines and related pesticides, N-methyl carbamoyloximes and n-methyl carbamates, ethylene thiourea, and brominated hydrocarbons, and data on these analytes are included in the report.

4.2.5.5 Solid Phase Extraction of Water Samples

The process of pumping water through a solid phase extraction (SPE) medium at the sampling site and storing the contaminant-containing medium, rather than the water, provides a relatively new storage alternative for water samples. In a joint US-Russian project in Lake Baikal, 180 L water samples were pumped through glass fiber filters and then through columns of extraction resin (XAD-2) to remove organochlorine compounds (Kucklick 1994). This extraction process was carried out on board ship. Resin columns, containing dissolved organics, were capped and stored at 4ºC, and filters, containing suspended particulates, were frozen at -20ºC. Both media were extracted for toxaphenes, total DDT, and total PCBs. An unrelated study by Japanese researchers in Lake Baikal also utilized XAD-2 resin extraction columns to extract organochlorine pesticides and PCB's from Lake Baikal water (Iwata 1995). In water samples taken elsewhere by the same group, formalin was passed through the resin extraction columns prior to storage (Iwata 1994).

Storage studies on alkanes (Green 1987), using resin columns containing XAD-2, have shown that these compounds are relatively stable at room temperature on these extraction media. No evidence of changes in alkane composition occurred on XAD-2 or C-18/silica gel columns through which 4 L of seawater spiked with crude oil had been passed (Green 1987). Column samples were stored (a) capped at room temperature for 14 weeks, and (b) open under water in the ocean for 8 weeks. Addition of oleophilic bacteria did not affect storage of the column samples but completely degraded the hydrocarbons in water samples in shorter time periods.

The stability of pesticides on extraction disks (C18 impregnated Teflon) has also been investigated (Senseman 1992, Moye 1993). Empore C-18 extraction disks were used to extract 12 pesticides (triazines, alachlor, captan, etc) from spiked water samples and the disks were stored at 4ºC, -20ºC, and a combination of 4ºC for 24 hours followed by -20ºC (Senseman 1992). Disk samples, along with a water sample stored at 4ºC, were analyzed at 0, 3, 30, 90, and 180 days. In all cases, each pesticide stored on an extraction disk at -20ºC gave better recovery than the water sample. Some microbial growth was evident on the disks stored at 4ºC for 90 and 180 days.

Moye (1993) investigated the influences of humidity, light, atmosphere, temperature, time, and pesticide loading on the storage of exposed Empore extraction disks (containing 36 pesticides). The study showed that for many pesticides, Empore disks could be stored wet at ambient temperatures for up to 30 days without prohibitive decreases in analyte concentrations. A wealth of data was generated by this comprehensive study.

While exhaustive testing of storage conditions for SPE media has not yet been done, this method of storage may allow more flexible conditions to be accepted for the storage of water samples.

4.2.6 Polycyclic Aromatic Hydrocarbons (PAHs)

4.2.6.1 Sediments

An unpublished study of storage of marine sediments at -18ºC showed no evidence of PAH losses over 3 years (see Section 4.2.4.1) (Crecelius, pers. comm.).

Two new natural matrix Standard Reference Materials (SRMs) were certified at NIST for PAHs in marine sediment and mussel tissue in 1989 and 1990 (Wise 1993). Samples of both materials were analyzed immediately, and again after one and 2 years to assess the stability of the PAHs. Bulk sample material was cryogenically homogenized and provided as a frozen, powder-like substance sealed in a glass vial. All 18 PAHs remained stable in concentration over the 2-year study period. Storage temperature was not given.

4.2.6.2 Tissues

A natural matrix SRM for PAHs in mussels was found to be stable over a two year period (see Section 4.2.6.1) (Wise 1993).

Two recent papers on PAH concentrations in the tissues of aquatic organisms report the common practice of freezer storage. Baltic clams and clam worms (Foster 1988), and livers of bream (Siddall 1994) were frozen immediately upon capture and were stored at -18ºC until analyzed. Storage duration was not reported.

4.2.6.3 Vegetation

No data.

4.2.6.4 Water and Wastewater

Several PAHs, including benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene, which were found to be unstable in chlorinated water, were stabilized by the addition of $Na_2S_2O_3$ (Koester 1993).

The most favourable preservation technique for most PAHs was proposed to be acidification to pH <2 and storage at 4ºC for up to 33 (Neaves 1990). Some losses of PAHs were noted for all

preservation methods.

4.2.6.5 Atmospheric Particulates

An experiment to determine the stability of benzo[a]pyrene (B[a]p) in atmospheric particulate samples was undertaken at the National Institute for Environmental Studies in Japan (Ambe 1992). Filter samples, collected by high volume air samplers, were stored in the dark at 20 and -20ºC under air or argon for a period of four years, with analyses occurring at 0, 6 mo, 1, 2, and 4 years. B[a]p concentration decreased 35% at 20ºC and 12% at -20ºC over the study period, while no difference was found between air and argon atmospheres. The rate of decrease in B[a]p concentration was twice as great during the first 6 months of the study than it was during the second 6 months, and losses decreased even more over the following 2 years. At -20ºC, little change in concentration occurred over 6 months.

4.2.7 Halogenated Diphenyl Ethers

No data.

4.2.8 Phthalate Esters

4.2.8.1 Sediments

No data.

4.2.8.2 Tissues

Section 4.2.4.2 describes the storage of phthalate esters in mussel tissue at -20 and -85ºC (Ambe 1992, Ambe 1993). The only losses in concentration over 2 years occurred when the samples were stored at -20ºC in plastic bottles rather than glass containers.

4.2.8.3 Vegetation

No data.

4.2.8.4 Water and Wastewater

No data.

4.2.9 Organotin Compounds

A survey of the recent literature describing analysis of water, sediments, and biota for organotin compounds revealed that water samples were typically acidified to pH 1 or 2 and stored at 4ºC, while samples of sediment and biota were stored at -20ºC. Some sediment and biota samples were freeze dried, homogenized, and stored in the dark at 4 to 5ºC. No mention was made of storage time prior to analysis.

4.2.9.1 Sediments

In a storage study carried out at the University of Bordeaux, researchers looked at the stability of tributyltin in waters and sediments (Quevauviller 1991). Sub-samples of sediment were stored at 4 and -20ºC for up to 120 days and, just prior to analysis, either oven dried at 50ºC, freeze dried, or air dried. Neither the method of storage nor the method of drying was found to affect the stability of tributyltin in sediments at two different organotin concentrations. TBT degradation products, monobutyl- and dibutyltin, were often subject to variations (mostly losses), and freezing followed by oven drying at 50ºC was shown to be the most suitable treatment to preserve all three organotin compounds.

As the result of investigations into the adsorption properties of TBT, environmental scientists were cautioned to "... seriously question their collection, storage, and analysis procedures in an endeavour to minimize adsorption processes..." (Carter 1989). The authors found that significant adsorption took place on all materials except polycarbonate. Materials tested included glass, silanized glass, waxed glass, nylon, polyethylene, Teflon, polypropylene, polyvinylchloride, Perspex, and polycarbonate.

As a component of a method validation study, the effects of storage of sediments on the stability of four butyltin species (TBT, DBT, MBT, and TTBT) were observed (Uhler 1989). Samples were stored at -20ºC in the dark, and were analyzed initially and weekly for a period of one month. A second experiment studied the storage stability of extracted, derivatized butyltin species. In both experiments, all four analytes remained stable for the study period. There appeared to be no advantage to extracting the butyltins prior to storage.

An international intercalibration study on the determination of butyltins in mussel tissue and sediments was reported at the OCEANS Conference in 1987 (Stephenson 1987). Representative environmental samples of mussels and sediments were collected, frozen, homogenized, aliquoted, and refrozen prior to distribution to seven participating laboratories in the USA, UK, and France. Samples were shipped frozen in dry ice but no indication of freezer storage temperature was given. Each laboratory analyzed the samples for TBT after 3 and 6 month storage periods. Concentrations after 6 months were found to be not statistically significantly different from samples analyzed prior to storage.

In a presentation to the OCEANS 86 conference, frozen sediment samples from San Diego Bay were shown to maintain stable levels of TBT, DBT, and MBT over a period of 140 days. In contrast, samples stored in the dark at 15ºC showed decreases in TBT and increases in MBT over the same period while DBT concentrations remained stable (Stang 1986).

4.2.9.2 Tissues

Section 4.2.9.1 describes an international intercalibration study in which TBT concentrations in mussel tissue were found to remain stable for up to 6 months in the frozen state (Stephenson 1987).

4.2.9.3 Vegetation

No data.

4.2.9.4 Water and Wastewater

A long term national monitoring program for butyltins in the environment is currently underway in the USA (Keithly, pers. comm.). Samples of sediments, mussels and waters from around the country are analyzed for TBT, DBT, MBT, and TTBT within the required one month maximum holding time. An opportunity for a storage stability study arose recently when a laboratory contamination problem necessitated the reanalysis of water samples after two different holding durations. No significant difference was found between samples analyzed 190 days apart. Water samples were held on ice, dry ice or frozen until shipped but no indication was given of laboratory storage conditions.

The stability of organotin compounds in water was found to be related to the amount of suspended material in the water (Quevauviller 1991). Tributyltin concentrations in filtered seawater, acidified to pH 2 and kept in the dark, were found to be stable for 4 months at both 20-25ºC and 4ºC. MBT and DBT were stable at 4ºC but showed some losses at 25ºC. Poor

recovery of butyltins characterized water with high suspended particulate loads.

Unfiltered natural sea water was fortified with tributyltin leachate from anti-fouling paint and stored frozen at -20ºC for a period of approximately two years (Valkirs 1990, Valkirs 1987). Analyses of randomly selected duplicate samples were carried out at 10 time intervals during the study, and at the end of the storage period, TBT concentrations were found to have dropped by about 50%. Since a previous study indicated the precision of the analytical method to be 15%, this figure was used to show that a significant loss had apparently occurred after 90 to 170 days (depending on the type of regression analysis employed). During the course of the study, a white crystalline precipitate was observed in samples stored 2 months or more. The authors suggest that the crystals were $CaCO_3$ and that they may have removed TBT from solution.

A homogeneous water sample from Southern Chesapeake Bay was acidified, stored in the dark at 5ºC, and analyzed for tributyltin (TBT) after 0, 1, 2, 3, 4, and 13 weeks (Huggett 1986). No loss of TBT was detected over the study period. In a parallel experiment, extracted and derivatized TBT was shown to be stable at 5ºC for at least 10 weeks.

A report on the occurrence of organotin compounds in Canadian waters and sediments cites unpublished information from within Environment Canada to confirm stability of acidified water samples at 4ºC for at least 3 months (McGuire 1986).

4.2.10 Metals

4.2.10.1 Sediments

A typical sample handling and storage procedure was described in a report on the determination of metals in surface and core sediments from Manila Bay (Prudente 1994). Samples were dried at room temperature, sieved to 2 mm, ground to 0.5 mm, homogenized, and stored at room temperature in sealed polyethylene bags until analyzed for Fe, Mn, Zn, Cu, Pb, Ni, Cd, and Co. Most reports did not indicate duration of storage, thus assuming sample stability over an indefinite period of time.

While air drying is commonly used for sedimentology, it is generally not recommended for chemical studies (Mudroch 1991). Changes in metal availability, complexation, and speciation have been shown to occur on air drying, although it is preferable to drying at elevated temperatures for retention of volatile metals such as mercury.

Freeze drying appears to be the preservation method of choice for trace metals in sediments, although research results vary in the potential for mercury loss (Mudroch 1991). According to Eric Crecelius of Battelle Pacific Northwest Laboratories, metals in freeze dried soils are stable for decades, while short term storage at -18ºC is convenient and effective (Crecelius, pers. comm.).

4.2.10.2 Tissues

LaFleur compared the effect of room temperature storage, with prefreezing at -20ºC, or -196ºC prior to freeze drying muscle and liver samples. No differences in concentration of methylmercury chloride were noted amongst the samples (Mudroch 1991).

Scientists at NIST evaluated the storage of zinc, selenium, and arsenic in fresh frozen human liver samples over a 7-year period at storage temperatures of -25, -80 and -150ºC (Zeisler 1988). Samples were cryogenically homogenized prior to storage and freeze dried prior to analysis. Samples stored at -25ºC, showed evidence of ice crystals under the lids and on the sample surfaces. Although this was not evident in samples stored at the lower temperatures. Trace element data showed that fresh frozen samples could be stored at -25ºC for extended periods of time without changes in concentration. If available, storage at -80ºC and lower was preferred to avoid segregation of ice from the samples. Freeze dried bovine liver SRM did not change in physical appearance during long term storage at room temperature. All trace metals in the SRM were stable, with the exception of some volatile arsenic species which appeared to have been lost during storage.

AMMTAP analyses and archives seal, whale, and sea lion livers for 36 inorganic constituents including many trace metals (Becker 1993), but no indication is given of plans to re-analyze the samples.

Archiving of human organs for trace element analysis is the main aim of a biological specimen bank in Sweden (Gerhardsson 1993). Samples of human lung, liver, kidney, bone, brain, fat hair, heart muscle, skin and stomach of deceased workers from a copper and lead smelter and control areas are stored at -20ºC. They are analyzed for Sb, As, Cd, Cr, Co, La, Se, Pb and Zn on collection and it is not know whether there are plans for reanalysis.

In an unpublished study carried out at Le Centre de Toxicologie du Quebec, spiked samples of serum and urine were found to be stable for Al, Cd and Hg over periods of up to 2 months without freezing (LeBlanc, pers. comm.):

Element	Matrix	Duration (days)	Temp.
Al	Serum	20	RT
Cd	Urine	62	4ºC
Hg	Urine	21	RT

In the CRC Handbook of Technologies for Aquatic Sediment Sampling, the authors found "...little variation in concentrations of selected trace elements in tissue samples that were stored for one year at -80ºC compared to the fresh samples..." (Mudroch 1991).

4.2.10.3 Vegetation

No data.

4.2.10.4 Water and Wastewater

Eric Crecelius of Battelle Pacific Northwest Laboratories assures us that metals in water can be stored acidified at room temperature (Crecelius, pers. comm.). No references were given and no time frame was indicated.

Mercury in sea water at ng/L concentrations was found to be very stable and could be stored in Teflon bottles for up to a month without preservation (Lu 1982, Matsunaga 1979). For longer storage periods, acidification was recommended to avoid volatilization.

4.2.10.5 Miscellaneous

Trace metals contamination in Slovakia was investigated by analyzing samples of soil, air, water, plants, animals, and human tissues (Trnovec 1993). Samples were not stored following collection; instead, they were extracted with nitric acid and the extracts stored for 5 to 10 years. Reanalysis of selected samples confirmed that there was no loss of metal concentration over this time period.

4.3 An Important Sample Storage Literature Study

A very useful document discussing the stability of environmental samples in storage was prepared in 1990 for the Ontario Ministry of the Environment (Neaves 1990). The authors reviewed the literature and assembled an extensive collection of storage data to support their recommendations for further study of chlorinated and non-chlorinated organics in environmental matrices (Table 1). Where adequate data permitted, statistical analysis of

analyte recovery against temperature, concentration, preservative, and time were performed. They comment on the lack of good stability data and suggest that priorities must be set for filling in the gaps in stability information. Planned follow-up research has not yet been initiated (Hobson, pers. comm.).

Table 1. Recommendations for further study of sample storage conditions arising from a literature survey carried out for the Ontario Ministry of the Environment (Neaves 1990)

Compound Class	MATRIX				
	Sediment	**Biota (Tissue)**	**Vegetation**	**Drinking /Surface Water**	**Waste Water**
Acid Extractables (Phenolics)	TR: -20ºC ≤ 15 weeks F (0)	TR: -20ºC ≤ 15 weeks C (24)	TR: -20ºC ≤ 15 weeks F (0)	Chloroform or H_2SO_4 3 weeks N (937)	$Na_2S_2O_3$ 4ºC 7 days analyze 40 days F (0)
Base-Neutral Extractables (PAHs, etc.)	PR: -20ºC 3 days C (36)	TR: -20ºC under air-Ar 2 years F (2)	TR: -20ºC 3 days F (0)	pH 2, 4ºC 33 days N (442)	PR: formaldehyde 4ºC 56 days C (72)
PCDD/Fs	TR: -20ºC analyze: soon as possible F (0)	TR: -20ºC analyze: soon as possible F (0)	TR: -20ºC analyze: soon as possible F (0)	PR: EPA $Na_2S_2O_3$ 4ºC 7 days analyze 45 days C (31)	TR: EPA $Na_2S_2O_3$ 4ºC 7 days analyze 45 days F (0)
Neutral Chlorinated Organics (PCBs, pesticides)	TR: -85ºC 2 years F (14)	PCBs: -20ºC 1 year. Others: -40ºC 4 weeks N (264)	TR: 4ºC Extract: soon as possible. Analyze: 8 weeks to 5 years F (14)	1. 4ºC for 6 weeks and analyze, or 2. chloroform and 15 weeks N (844)	TR: Chloroform 4ºC 7 days analyze 14 days F (11)

F = Full study required
C = Study required for confirmation of recommendations
N = No further study required
( ) = Number of data points upon which recommendation is based.
PR = Provisional recommendation (insufficient data for statistical analysis)
TR = Tentative recommendation (no data and no existing criteria/based on behaviour of other compounds in the same matrix)

4.4 Non-Analyte Specific Sample Storage Information

Dixon Landers of the USEPA in Oregon recommended storage of sediment samples, for organics and metal speciation, at temperatures at which they occur in the field (e.g., 0 to 4ºC simulates a deep lake). He recommended freezing tissues as soon as possible, and air drying lichen and moss followed by storage in a cold room for years (Landers, pers. comm.).

Stephen Wise of NIST indicated that specimen storage at liquid nitrogen temperatures should be adequate for 50 to 60 years (Wise, pers. comm.). He reported instances of samples being inseparable from their aluminum foil packaging after long term storage at -25ºC, but suggested that for storage of less than one year, freezer temperature is probably not important.

In an overview of Mussel Watch programs around the world, details of sample collection and storage were given, but no mention as made of storage times (Sojo 1990). In most Mussel Watch programs, samples were frozen on site, but in some cases samples were transported to the laboratory live and acclimated in seawater aquaria. In the laboratory, samples were thawed, homogenized, then either analyzed or refrozen at -20ºC. No information was given on the effect which re-freezing had on concentrations of trace metals or organics in mussel tissue, or on any specific storage requirements of the program.

Arctic researchers have pointed out the need for consistency in all aspects of monitoring. "... Collection, storage, and analytical conditions should be exactly the same for all samples in order for comparisons between samples to be valid. Samples preserved under different conditions are not directly comparable unless the possibility of different pollutant derivation or loss can be reasonably excluded ..." (Becker 1993).

A number of researchers chose to extract their environmental samples prior to storage and to store the extracts. No evidence was found to suggest that sealed sample extracts, either for organics or trace metals, were not stable indefinitely. An investigation of the storage of sample extracts was not undertaken as a component of this study, but a few reports of extract storage are included.

4.5 Current Regulations for the Storage of Environmental Samples

Information on current storage techniques can be gained by looking at and evaluating current regulations governing sample storage. Some governing bodies provide detailed and rigid protocols for the storage of environmental samples. Analytical laboratories in these jurisdictions must abide by these regulations to produce legally defensible data. Other regulators do not specify storage times or maximum holding times for their environmental

samples. If storage studies were available to scientifically justify regulations governing holding times for specific analytes, laboratories would gain a great deal more flexibility in scheduling and carrying out analyses.

Specific storage conditions for priority pollutant analysis of environmental samples are not generally supported by references to experimental data, frequently refer to temperature of storage but not to holding time, and are often not included in the analytical protocol (Klemm 1990a and 1990b, Tetra Tech 1986, Gaskin 1980, McQuaker 1989, Gaskin 1991, Anon. 1983, Hart 1991, and Kalnins 1991). A look at some of the regulations in force in North America gives an overview of current analytical protocols.

4.5.1 USEPA Storage Protocols

The USEPA has developed many sets of protocols for storage of environmental samples for trace contaminant analysis. Recent discussions with a scientist at the USEPA Sample Control Center, indicate that USEPA storage specifications are not, in general, based on formal storage studies (Messing, pers. comm.). Instead they are based on logic and experience; samples containing volatile compounds must be analyzed quickly, and those containing more stable compounds are allowed longer storage periods. Because of the inflexibility of this system and the short period which laboratories have to extract environmental samples, there has been evidence in the US of falsification of data to meet storage protocols (Messing, pers. comm.). A summary of current storage protocols for some of the applicable EPA Methods can be found in Table 2 (Keith 1991).

EPA Trace Element Methods 200.7 (ICP for water and waste waters) and 6010 (AA for water, waste waters, soils and sediments) require samples to be stored at 4ºC and analyzed within 24 hours (Keith 1991). The 7000 series of methods (AA for water and waste waters) allow for storage periods of up to 6 months if the samples are acidified to pH<2 immediately upon sampling. Soil and sediment samples analyzed by 7000 series methods are required to be analyzed "... as soon as possible ..." (Keith 1991). Elements covered by these methods include aluminum, antimony, arsenic, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, selenium, silver, tin, vanadium, and zinc. Samples for mercury analysis must be acidified immediately and stored for no more than 28 days.

There is growing interest in streamlining analytical protocols in the US. In an overview document looking at the USEPA's current methods for water analysis and proposing method changes for the future, the authors recognize that "... there is a bewildering array of maximum storage times for the samples ..." and that "... these differences have proven to be troublesome for analytical laboratories ..." (Hites 1991). They go on to propose that "... Sampling needs to

be addressed in a module ... [and a] minimum set of sample preservation specifications (including holding times) should be given...".

Table 2. US EPA protocols for maximum holding times for environmental samples and sample extracts containing trace organic contaminants.

Compound/ Class	EPA Method Number	Temp (°C)	Maximum Holding Time (MHT) [in days]	
			Water/WW Sample Extract	Soil/Sed. Sample Extract
Semivolatile Organics	1625	4	7 40	14 40
Polycyclic Aromatic Hydrocarbons	8100 8310	4	7 40	14 40
Base Neutral and Acid Extractables	625	4	7 40	14 40
Phthalate Esters	8060	4	7 40	14 40
Chlorinated Hydrocarbons	8120	4	7 40	14 40
Chlorophenols	8040	4	7 40	14 40
Organochlorine Pesticides and PCBs	8080	4	7 40	14 40
Chlorinated Herbicides	8150	4	7 40	14 40
PCDD/PCDFs	8280	4	30 45	30 45

4.5.2 British Columbia Sample Handling and Preservation Requirements

The most recent edition of the British Columbia Environmental Laboratory Manual (1994) includes information on sample preservation and storage, assembled by a panel of experts including staff from the BC Ministry of Environment, Lands and Parks and representatives of analytical laboratories. Permitted dischargers must have their environmental samples analyzed by laboratories which are registered with the Ministry and which can demonstrate that the methods outlined in the Laboratory Manual are followed.

While the Manual cautions analysts to consider sample stability, little information is provided on maximum holding times for samples stored for specific analyses. In general, storage times reflect the current accepted limits specified by the USEPA, and in some cases Canadian government guidelines. The following statement illustrates the informal approach to sample storage: "... As a general rule, samples should be prepared and analyzed as soon as practical after submission to the laboratory ...".

Most of the methods covered by the Manual refer to the collection and analysis of water samples. These water samples can be stored for metal analysis for up to 6 months at 4ºC once they are acidified to pH $\leq$ 2, while biota samples must be stored frozen. Sediment samples can be stored indefinitely if frozen, or up to 6 months at 4ºC without preservation. No freezer temperature is specified.

Storage of samples for semivolatile organic compounds follows the USEPA guidelines shown in Table 2 with the exception that water samples containing organochlorine pesticides and PCBs are allowed to be held for 14 days at 4ºC prior to extraction, and the extracts held for up to 30 days prior to analysis. Acidification is recommended for chlorophenols and acid extractable herbicides. Soils, sediments, and biota must be stored frozen.

4.6 Specimen Banks

The primary goal of most environmental specimen banks is to provide storage facilities to preserve the integrity of environmental samples for long periods of time. Much can be learned from the specimen banking experience which can be applied to the selection of medium term sample storage conditions important to analytical laboratories.

While specimen banking is a relatively new concept, specimen banks have proposed that storage at -150ºC will allow samples to be stored without degradation for 50 or 60 years (MAS 1993). Time will be the ultimate judge of this estimate. A recent survey of individuals and institutions holding arctic specimens revealed that 71% keep samples indefinitely, with 52% of those storing samples in a frozen state (Wakeford 1993). Of this group, 28% keep samples at

<-80ºC, 30% at <-40ºC, and 42% from 0ºC to -40ºC. Most respondents (73%) felt that their method of storage was adequate.

In a mission statement on specimen banking in the Nordic countries, the writers make an unreferenced assessment of current storage technology (Giege 1993): "The project Group recognises that storage of specimens at -80ºC or lower is the only method which allows samples to be used for most kinds of environmental studies ... all material collected for joint international use should be stored at the lowest temperature possible, -80ºC or lower...".

In a feasibility study for a proposed Great Lakes Specimen Bank, prepared by the Michigan Audubon Society Consultant, UMA Engineering Ltd., (MAS 1993), it was agreed that no single standard storage method could be recommended for all type of chemicals and environmental sample matrices. "...there is an emerging consensus that the best general method is deep freezing at temperatures of -80 to -196ºC...a temperature of -80ºC seems necessary to minimize enzymatic activity and surface absorption in an archived sample...". The study investigates all aspects of specimen banking and makes recommendations for the storage of specimens. The stability of tissue samples under different storage conditions is also discussed. The limitation of this study is that it refers mainly to tissue preservation over very long periods of time. It does not address other sample matrices nor does it look at short term storage conditions, and as such, many of the needs of the typical analytical laboratory are not investigated.

Liquid nitrogen storage is the preservation method recommended by the most of the major specimen banks (MAS 1993). While some samples may not require such low temperature storage, liquid nitrogen freezers offer the advantage of being relatively maintenance-free, low cost, and unaffected by power failures. Liquid nitrogen shipping containers are also available for transporting relatively small samples from the field to the specimen bank.

One of the mandates of most specimen banks is to monitor storage stability of contaminants in environmental samples over time. Because of the potentially large number of samples available in long term storage facilities, specimen banks provide an excellent opportunity for such experiments to be undertaken. However, for storage studies to be carried out, consideration must be given to a number of parameters when samples are first targeted for collection: sample size, number of specimens, sub-sampling technique, storage temperatures, available space, sampling handling and treatment, etc.

Environmental specimen banks have also taken on a role in provision of the Standard Reference Materials (SRMs) used by analytical laboratories to standardize techniques. There appears to be a general move towards the provision of "...fresh, homogeneous, unperturbed, and well characterized materials..." as SRMs (Wise 1993, Backhaus 1993). These "third generation reference materials" will gradually replace the more common freeze dried, doped

SRMs because they more closely represent real environmental samples. Data on the storage of SRMs has been made available by NBSB and is included in this report (Sections 4.2.6.1 and 4.2.6.2).

Analytical methodology plays an important role in the analysis of samples stored over long periods of time. A CWS study involving re-analysis of gannet eggs in 1984, 15 years after the original analyses took place, showed consistently higher levels of DDE and PCBs than had been originally detected (Turle 1988). The authors note that, in retrospect, PCB data from the 1960s and 70s must be interpreted with caution. A similar study involving recent reanalysis of Lake Ontario trout also showed higher levels of p,p'-DDE than was detected in the same samples in the 1970s (Huestis 1995). Method changes should spawn statistical comparisons between old and new methods, and overlap in method use would help to ensure that results can be compared without bias.

Some storage stability assumptions appear to be circumstantial. Several reports on the analysis of samples after extended storage periods have questioned the possibility of chemical changes occurring during storage. In many cases, losses are assumed not to have occurred only because earlier samples show higher contamination than current samples. The following is an example which comes from the GESB: "Because of extreme low temperature, it is granted that the samples are not subject to chemical changes during long-term storage... The storage and preparation of samples sufficiently recognize the demands of organic and inorganic analysis because it can be shown that samples are not altered during these procedures. This fact can be clearly confirmed with the results of the presented time series, which could not show a decreasing trend of contamination of the environment with ongoing time, taking into account that most of the studies about sample stability yield up in a decreasing amount of compound during long-term storage. Thus the scientific and decisive relevance of the results is guaranteed by the storage concept of the GESB..." (Oxynos 1994). This approach highlights and reinforces the need for more rigorous storage studies.

Out of these observations comes one of the most exciting roles of specimen banks. Stored samples can be recovered in years to come when as yet unknown analytes become targets for evaluation. Studies are already being undertaken to look retrospectively at lake trout samples archived over the past 15 years (Huestis 1995) for PCB congeners which analytical technology did not allow to be detected during much of that time period. It is clear that samples being archived today will provide an excellent opportunity for the application of new analytical methods, and the establishment of trends in environmental contamination of which we are not aware at this time. It is indeed crucial that specimen banks use the best and the most comprehensive preservation techniques available at the time of storage, and that sample stability is closely monitored.

Detailed information on a number of environmental specimen banks around the world can be found in Appendix II.

5. DISCUSSION AND CONCLUSIONS

5.1 Summary of Sample Storage Information

While no conclusions can be drawn for analyte/matrix combinations where no data was found, a summary of the information gathered in Section 4 of the report is given below.

5.1.1 Chlorinated Phenols and Anisoles

A single storage study on the stability of chlorophenols in wastewater samples indicated stability for up to 5 months at -15ºC. Drinking and surface waters were stable for up to 15 weeks when chemically preserved and stored at 4ºC. Further study is indicated for all matrices except water. No data was found for chlorinated anisoles.

5.1.2 Chlorinated Paraffins

No data were found. Full study recommended.

5.1.3 Polychlorinated Dibenzodioxins and Dibenzofurans

The persistence of PCDD/Fs in the environment provides an indication of their inherent stability in stored samples. It is clear that storage condition for PCDD/Fs need be less rigorous than conditions required for other semivolatile organic compounds. Since no storage studies specific to environmental samples were located, a recommendation for full studies would normally be expected. However, because of the current level of understanding of dioxin stability, long term confirming studies may be all that are needed to confirm suspected dioxin stability in stored environmental samples.

5.1.4 Polychlorinated Biphenyls

There has been a major focus on measuring PCB concentrations in the environment world-wide since their sale was banned in the late 1970's. Data on PCB concentrations has been

influenced significantly in recent years by changes in analytical methodology, and PCBs are now often reported as specific congeners. Several important studies on the stability of PCBs in environmental samples have shown that sediment samples stored either frozen at -20ºC or freeze dried and stored at room temperature, are stable for up to 6 years. A number of studies document the stability of PCBs in tissue samples frozen at -20 to -25ºC for at least one year, and other studies show some loss of PCBs in the second year of storage at this temperature. There may be a slight advantage to storage at -40ºC if the samples must be kept for more than one year. Water samples may be kept at 4ºC for at least 15 weeks when preserved with chloroform. While a wealth of information is available to document the stability of PCBs in frozen tissue and water samples, studies to confirm current understanding of PCB stability are indicated for sediments and vegetation. Further studies will be necessary for all media if congener specific information is required.

5.1.5 Chlorinated Pesticides

Because of the nature and diversity of compounds falling into the category of "chlorinated pesticides", determination of storage stability is more complex for this group than for other categories of compounds.

Limited data indicate that sediment samples stored at -20ºC are subject to significant decreases in the concentration of DDT metabolites over the first two months of storage, and slower analyte loss in subsequent months. The most abundant data on storage of environmental samples has been generated for chlorinated pesticides in tissues, particularly fish tissues and liver samples. Results of several studies carried out at -20ºC show that most chlorinated pesticides are stable for up to one year. Only one study indicated a significant loss of Mirex and p,p'-DDD on storage of fish homogenates for 1 year at -20ºC. Results for storage at lower temperatures make it unclear as to whether decreased storage temperatures result in increased analyte stability in tissue samples. Freeze drying was evaluated as a sample preparation technique but is not recommended due to the volatility of many of the pesticides. Insufficient data were gathered for this report on the stability of chlorinated pesticides in water, however, Neaves and Fong found a large volume of data and recommended that no study is required.

Based on the available data, confirming studies will be needed for sediments along with a full study for vegetation. Although the storage of tissue samples for organochlorine pesticides appears to have been well documented, there are a few analytes, such as mirex and DDD, which may require additional storage stability studies.

5.1.6 Polycyclic Aromatic Hydrocarbons (PAHs)

Based on SRMs and unpublished data, PAHs appear to be stable in sediments and tissues stored at -20ºC, but there is insufficient data to confirm this deduction. It is not clear that preservation of water samples by acidification to pH < 2 or with $Na_2S_2O_3$ precluded loss of some PAHs on storage. For atmospheric particulate samples stored at -20ºC, the 12% decrease in benzo[a]pyrene concentration observed over 4 years occurred primarily in the first 6 months. Little work has been done on the storage of PAHs in environmental samples and full studies are recommended for all matrices.

5.1.7 Halogenated Diphenyl Ethers

No data found. Full study recommended.

5.1.8 Phthalate Esters

A single report on storage of tissues for phthalate esters indicated no loss of concentration over 2 years. A full study is recommended for all matrices.

5.1.9 Organotin Compounds

A number of studies indicate that butyltin compounds are stable in sediments stored at -20ºC for up to 6 months, and one study extends this conclusion to tissues storage. Stability of butyltins in water samples, was achieved by reducing pH or by frozen storage. TBT, MBT, DBT and TTBT concentrations were stable for approximately 6 months in samples stored at -20ºC. Acidification of water samples allowed storage at 4ºC for at least 3 months. The presence of suspended particulates in water samples appears to reduce recoveries of butyltin compounds. Sufficient storage stability investigation appears to have been carried out on sediments and water, while more information will be necessary to develop guidelines for the storage of biota samples.

5.1.10 Metals

Storage procedures for environmental samples to be analyzed for trace metals are normally different from procedures required to safeguard the stability of trace organic contaminants. There is ample evidence that samples of sediment and tissues stored at -20ºC are protected indefinitely against trace metal loss. Most samples can be freeze-dried at room temperature or

elevated temperatures prior to storage and analysis. However, an exception must be made for the metals such as mercury and arsenic where samples must be protected against loss by volatilization. Water samples are typically filtered, acidified, and stored at 4ºC to maintain the stability of trace metal concentrations. Little study is indicated for metals in environmental samples, with the possible exception of metal speciation.

5.2 General Discussion and Conclusions

The availability of information on the stability of contaminants in environmental samples during storage varies widely amongst sample matrices and target analytes. The greatest volume and depth of information appears to be related to the storage of tissues for organochlorine pesticides and PCBs.

The currently accepted method of storing most environmental samples, other than water, for the analytes in question, is freezing at -20ºC. Based on the information currently available for medium term storage of environmental samples, freezing to -20ºC appears to provide adequate protection for most of the target analytes examined in this study. Because this is the temperature of standard commercial freezers, it is a very convenient temperature for storage of environmental samples in the laboratory.

At present, all laboratories store environmental samples for some period of time prior to analysis. However, since many jurisdictions do not have regulations governing storage duration, the storage periods vary greatly from laboratory to laboratory. In conjunction with storage temperature, storage studies will need to thoroughly investigate storage duration for at least one, and preferably two, years. While most samples are initially analyzed before two years has elapsed, there is often call for reanalysis of stored samples. Storage studies will give concrete data by which to confidently evaluate results of analyses performed after prolonged storage.

The major drawbacks to storage at this temperature appear to be loss of water from the sample, and microbiological degradation of the sample, although little evidence was found to show that either of these problems influenced concentrations of analytes in environmental matrices covered in this study. For example, tissue samples stored at -25ºC retained stable levels of trace elements, selected pesticides and PCB congeners, although samples did not retain their colour and consistency (Zeisler 1992). The more important issue appears to be the length of time during which the concentration of each analyte is stable in a given matrix at -20ºC.

Most reports of environmental analyses do not provide details of the time interval between sampling and initiation of freezer storage. It has been shown that for some very fragile tissue samples, such as zooplankton, a rapid decrease in analyte concentration, related to lipid loss,

is observed during the first 24 hours after sampling. While this behaviour is not typical of most tissue samples, it does point out the need to carefully investigate the behaviour of contaminants once environmental samples have left their sampling location.

In designing a sample storage study, consideration must be given to the timing of the initial analysis of samples. In the past, some studies have compared results of analyses performed after samples had been frozen or preserved. For a more accurate look at analyte concentration changes with time, samples should be analyzed as soon as possible after receipt at the laboratory and preferably while still fresh (Kiriluk 1995). If freezer storage is available at the sampling site, then a comparison between fresh and frozen samples may be appropriate to determine the effect of freezing on initial contaminant concentrations.

For long-term storage, temperatures of -80°C or less are recommended, and most specimen banks maintain samples in liquid nitrogen freezers at between -120 and -196ºC (MAS 1993, Wise 1984, Wise 1988). No problems have been reported with freezing at -80°C, and one group reported better stability for some samples at this temperature than at -196ºC (Hyatt 1986).

The scarcity of literature reporting investigations of sample storage in analytical laboratories suggests that current protocols have been adopted for historical reasons, and there has been little motivation to question these protocols. Several scientists indicated that formal studies of sample stability were not usually carried out by analytical laboratories because of the time and expense involved. Reproducibility of analytical results and inter-laboratory calibrations have been the primary tests of the adequacy of a laboratory's storage protocols. Unfortunately, analytical reproducibility of samples stored under the same conditions does not adequately reflect sample integrity over the period of time between sampling in the field and analysis in the laboratory.

The question of whether to store samples as whole specimens, specimen sub-samples, or as homogenates must be carefully considered prior to sample storage. Whole samples, while advantageous for retrospective analytical work, may be difficult to accommodate. They may also necessitate thawing and re-freezing to obtain sub-samples, yielding a potential for contaminant degradation or volatilization, and compromising the potential for reanalysis. Cryogenic homogenization, developed at NIST, provides excellent homogeneity and allows sub-sampling to take place without sample thawing (Wise 1984). However, this technique may not be practical for analytical laboratories because of financial and time constraints. The problem of water evaporating from a sample and condensing as ice within the storage container suggests that there may be a benefit in sub-sampling whole specimens prior to storage. Each sub-sample could then be treated as a complete sample and the results reported on a wet weight basis.

In designing storage studies for multiple analytes, particularly the group of chlorinated pesticides, consideration will need to be given to the selection of analytes. It may be more preferable to select representative compounds from each major group than to try to evaluate the stability of all known contaminants in each class of compounds.

While this study did not document the concentrations of contaminants selected for study in each storage experiment, it is clearly important to consider this issue in designing further storage studies. There appears to be a trend toward preparing SRMs using unspiked environmental samples (Wise 1993, Backhaus 1993) and this approach may work well in cases where suitable samples can be obtained. Since contaminant concentrations in environmental samples vary widely between locations, additional useful information may be gathered by incorporating samples known to have different levels of the same contaminant into each storage study (Quevauviller 1991). A comparison could then be made between the behaviour of an analyte at or near the method detection limits, and its behaviour at much higher contaminant concentrations.

Another consideration in the storage of environmental samples is the choice of sample containers. This issue was touched on only briefly in this report, however, selection of appropriate sample containers and sampling tools which do not adsorb the target analytes was addressed in a report on tributyltin adsorption (Carter 1989). Other authors referred to the degradation of aluminum packaging over long periods of time (Wise, pers. comm.), and to the fragility of sample vials under cryogenic conditions. Sample containers must also prevent contamination from occurring by diffusion into the container. It has been proposed that packing in dry ice may allow CO_2 to enter the sample container and possibly alter the chemistry of the sample (MAS 1993).

At the time of sampling in the field, consideration must also be given to the ultimate use of the sample itself. There may be several goals to be met for a single sample or composite. If the sample is to be analyzed immediately, then storage may not be a critical consideration. If there is the possibility that the sample will need to be held for a moderate period of time prior to analysis, then consideration must be given to appropriate shipping conditions, sampling containers, sub-sampling procedures, homogenization, extraction, etc. prior to storage. If long term storage of part or all of a sample is also important, then the amount of sample collected must be adequate for this purpose, and suitable sample storage containers and facilities chosen prior to storage. Since samples taken for organic and trace metal analyses may require different sample handling procedures, consideration must be given to the type of sampling handling equipment and storage containers where there is a possibility that samples may need to be analyzed for both organics and metals.

Sample storage studies can be influenced greatly by changes in analytical methodology with time. This is particularly important for long term studies in which analytical methods, analysts,

and methods of data interpretation may change over the duration of the study. Preliminary findings in a recent study carried out at the Great Lake Fisheries Specimen Bank (see Section 4.2.5.2) suggest that a change in analyst during the study may have compromised some of the data (Kiriluk 1995). Final reporting of this multi-year storage study of organochlorine pesticides in fish tissue will not be possible until the analytical techniques of the two primary analysts have been investigated and their methods justified. In developing storage studies, analytical methodology and QA/QC procedures will need to be carefully considered and plans made to deal with possible changes in techniques over the duration of the study.

A contribution towards understanding sample storage stability has been made by laboratories preparing standard reference materials (SRMs). Specimen banks, tissue archives, and laboratories which develop SRMs have often generated systematic publishable or published studies of sample stability. Extrapolating the results of these studies to environmental samples must be done with care since SRMs are often prepared and stored in a manner different from environmental samples (eg. freeze drying, ampouling, shipping, etc.). In addition, the original concentration of analytes in SRMs is not usually critical. Instead, the critical concentrations in SRMs are those found when sub-samples are analyzed and concentrations confirmed following the preparation of the SRMs.

Several sources reported loss of frozen samples because of accidental warming (Hyatt 1986, Ambe 1993, Wakeford, pers. comm.). Considering the substantial investment which goes into collection and storage of environmental specimens, the possibility of freezer failure must not be underestimated. Alarm systems must be installed and backup storage facilities located to prevent catastrophic losses of samples. Good freezer control and adequate refrigeration capacity are also essential to avoid fluctuations in storage temperatures. One reference suggests that freezers should be powerful enough to ensure samples reach their storage temperature in a maximum of 6 hours (Sprenger 1981).

In conclusion, the time period over which each individual analyte is stable in each matrix must be thoroughly investigated. Standard "maximum holdings times" would be the natural result of these investigations and analytical laboratories would gain vital QA/QC information to ensure the accuracy of their results along with allowing more flexible analytical schedules. Sample storage temperatures must also be investigated for many analyte/matrix combinations either to confirm -20ºC as an adequate temperature for sample storage or to determine a more suitable storage temperature.

6. RECOMMENDATIONS

It is clear that there is not enough information available on the effects of storage of environmental samples on target analyte concentrations, to ensure that analytical results generated in the laboratory reflect the true concentrations of these analytes in the environment. The need for additional storage studies has been voiced clearly in reference to water samples, but the same perspective can be taken on other sample matrices: "... Only a few studies have been conducted to determine sample storage times and conditions, and of possible sample changes during storage. Recommended sampling and sample storage conditions for most analytes are then extrapolated from these few investigations ..." (Koester 1993).

Table 3. Recommendations for storage of environmental samples in analytical laboratory facilities (less than 2 years).

Compound Class	Matrix			
	Sediment	Vegetation	Tissue	Water
Chlorophenols	F	F	F	N
Chlorinated Paraffins	F	F	F	F
PCDD/Fs	C	F	C	C
PCBs	C	F	N	C
Chlorinated Pesticides	C	F	N (C)	N
PAHs	F	F	F	F
Halogenated Diphenylethers	F	F	F	F
Phthalate Esters	F	F	C/F	F
Organotins	N	F	C	N
Metals	N	ID	N	C

F = Insufficient data, full storage study recommended.
C = Additional study required to confirm storage temperature and/or duration.
N = No further study required.
ID = Insufficient data gathered to make a recommendation.

In planning future storage stability studies, investigations into the effects of storage temperature and storage duration are of primary importance. Of secondary importance are the questions of suitable sample packaging and subsampling methods to allow for long term

storage or sample archiving. Based on the information gathered in this report, it is recommended that studies into the stability of environmental samples be carried out at -20ºC for periods of up to two years. The recommended areas of study are indicated in Table 3. If studies are to be directly applicable to typical analytical service laboratories, they must take into consideration the range of storage facilities available in such laboratories.

It is anticipated that well documented storage studies will offer a trade-off between storage temperature and maximum holding times. This will provide a more scientific approach to sample storage conditions for analytical laboratories than would either arbitrary requirements imposed by regulators, or subjective decisions made by laboratory staff.

It is hoped that the results of the major sample storage study recommended here will one day be incorporated into sampling and analytical protocols, and that they will benefit both analytical laboratories and their clients and, ultimately, our fragile environment.

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APPENDIX II

ENVIRONMENTAL SPECIMEN BANKS

1. German Environmental Specimen Bank (GESB)

(Backhaus 1993, Schladot 1993, Kemper 1993)

T = <-150ºC
Air, biota (all trophic levels), sediment, soil
40,000 samples
Analyze for chlorinated hydrocarbons, PCBs, PAHs, PCDD/F, organometallics, and trace elements.

Specimen banking in Germany began as a pilot project in 1975 and successfully became a permanent institution in 1985. Samples are characterized, prepared, and stored at the Research Centre, KFA-Julich which is operated by the Institute of Applied Physical Chemistry. Its three main aims are retrospective identification and quantification of old and new environmental chemicals, retrospective replicate analysis of stored samples, and retrospective analysis of environmental protection legislation.

Extended sampling campaigns are performed for a number of species, and specimens are normally collected every two years. Investigations now include information on atmospheric input of contaminants, speciation of compounds, and mass balance studies. Single 5 kg samples are used to generate 500 standardized subsamples which are then archived at -150ºC.

Another component of the GESB, the Environmental Specimen Bank for Human Tissue, is an archive for human tissue samples (Kemper 1993). An initial pilot phase (1978-83) was used to investigate reliable storage temperatures and container materials. Up to 1991, 300,000 samples had been collected and stored at -85ºC. The collection contains 31 types of tissues obtained from live and deceased specimens and samples have been analyzed for trace metals and chlorinated organic compounds.

2. National Biomonitoring Specimen Bank in the USA (NBSB)

(Zeisler 1992, Wise 1989, Wise 1984)

T = <-150ºC
Human soft tissue (liver), a marine accumulator (mussels Mytilus edulis), a food accumulator, and an air pollutant accumulator
Analyze for organochlorine pesticides and trace elements

In 1979, the US National Institute of Standards and Technology (NIST), in conjunction with the USEPA, established a pilot Environmental Specimen Bank. The NBSB now consists of 6 projects, 4 of which archive environmental specimens:

Specimen Bank	Authority	Matrix	Contaminant
Environmental Specimen Bank	EPA	tissues	PCBs pesticides* metals
National Status and Trends Program	NOAA	sediment tissues	metals pesticides PCBs/PAHs
AMMTAP	NOAA/MMS	tissue	metals pesticides PCBs
NMMTB	NOAA/OPR	tissue	metals pesticides PCBs

*organochlorine pesticides only

NBSB provides a wealth of experience and detailed protocols for the collection, processing, long-term storage and analysis of different specimen types including human liver, sediment, bivalves, fish, marine mammals, and total human diet. Two 150 g samples are collected in Teflon bags, frozen in liquid nitrogen as soon as is feasible, and shipped to NIST where they are stored at -150ºC. Cryogenic homogenization using Teflon disk mills was developed at NIST to address the need to produce identical sample aliquots to be used in comparison of analytical techniques and for evaluation of sample storage stability. Sample handling at cryogenic temperatures reduces the risk of losses of analytes due to volatilization, and to analyte degradation which could occur on thawing and re-freezing.

One of the main mandates of NBSB is to evaluate the stability of contaminants in specimens during long term storage. Detailed results of the analysis of stored samples for trace elements and organic contaminants (chlorinated organics and PCBs) are described in sections 10.2, 4.2, and 5.2 of this report.

3. Alaska Marine Mammals Tissue Archival Project (AMMTAP)

(Zeisler 1993, Schantz 1993, Becker 1993)

T = -150ºC
Marine mammals
Analyze for chlorinated pesticides, PCBs, methyl mercury and trace elements

Initiated in 1987 by Mineral Management Service (MMS) of the US Department of the Interior, and NOAA as part of the National Biomonitoring Specimen Bank (NBSB) program of NIST. Samples of blubber, liver, kidney, and muscle from northern fur seal, ringed seal and beluga whale from several

locations have been archived at -150ºC for future analysis. Blubber samples were analyzed for 15 PCB congeners and 12 chlorinated pesticides for 10 different animals (Schantz 1993). Liver, kidney and muscle tissues from 12 animals were analyzed for 36 trace elements and methyl mercury (Zeisler 1993). As a result of extremely low contaminant levels, collection of muscle tissue has been discontinued.

4. National Marine Mammal Tissue Bank (NMMTB)
(Lillestolen 1993)

T = -150ºC
Marine mammals
Analyze for chlorinated pesticides, PCBs, and trace elements

The NMMTB was initiated in 1989 as part of the NBSB. Its role is similar to that of the AMMTAP but applies to mammals from US locations other than Alaska. Samples of blubber and liver from marine mammals (harbour porpoises, pilot whales and others in the future) found stranded or caught incidentally by fishermen, are collected in Teflon jars and stored in liquid nitrogen freezers at -150ºC. Subsamples of tissue homogenates are (a) analyzed by NIST for chlorinated pesticides, PCBs and trace elements, (b) archived indefinitely, (c) supplied to researchers on request, and (d) used in evaluating the stability of specimens during long term storage. (See NBSB section)

5. Great Lakes Fisheries Specimen Bank (GLFSB)
(Hyatt 1993, MAS 1993, Huestis 1995, Kiriluk 1995)

T = -20 to -30ºC, -80ºC
Fish, benthic invertebrates, and plankton
>12,000 samples
Analyze for PCBs, organochlorine pesticides, dioxins and furans, PAHs, chlorinated diphenyl ethers, organotins and heavy metals.

Canada's Department of Fisheries and Oceans (DFO) initiated specimen banking activities in 1977 and has since archived more than 12,000 specimens of fish and benthic invertebrates that have been analyzed for chlorinated organics and trace metals. Housed at the Great Lakes Laboratory for Fisheries and Aquatic Sciences (GLLFAS) in Burlington, ON, the GLFSB's mandate is concerned with contaminant levels in whole fish as they reflect water quality in the international waters of the Great Lakes. Recent acquisitions of specimens from the whole of Canada, including marine species, have changed the focus of the specimen bank from regional to national.

Samples are analyzed shortly after collection to establish real-time contaminants levels, and replicate samples are stored for historical study. Studies are currently underway to define appropriate storage conditions and times for chlorinated hydrocarbons in a variety of biological tissues. Section 4.2 contains the description of a major study carried out at NFSB investigating the effects of storage conditions, ranging from freeze drying at +60ºC to freezing at -196ºC, on the stability of chlorinated organics in coho

salmon and zooplankter.
Samples are frozen whole then homogenized and subsampled for analysis and archiving when necessary. For storage studies, five replicate samples are used for each storage condition/time period to be examined. A detailed Contaminants Surveillance Program ensures accurate and timely data handling. Ongoing studies will document changes in contaminant concentrations with repeated thawing and re-freezing.

Details of two research projects on the storage of fish tissue homogenates for PCBs and organochlorine pesticides can be found in Sections 4.2 and 5.2 of this report (Huestis 1995, Kiriluk 1995).

6. Great Lakes Sediment Bank
(Bourbonniere 1986, Mudroch 1991)

T = RT
Sediment

In 1986, a study was undertaken to evaluate the feasibility of establishing a Great Lakes Sediment Bank at the National Water Research Institute in Canada. Bottom sediments were collected from Lake Ontario and processed and stored at NWRI. Details of a storage study on the stability of trace organic contaminants in the sediments under three different sets of storage conditions can be found in Sections 4.2 and 5.2.

7. National Specimen Bank of the Canadian Wildlife Service (CWS)
(Elliot 1987)

T = -20ºC, -40ºC, -80ºC, -150ºC
>30,000 specimens
Birds and their eggs, mammals, bivalves, fish, reptiles, amphibians

The CWS National Specimen Bank is a collection of wildlife samples preserved by deep freezing for future analysis. The CWS maintains a comprehensive collection of birds, particularly the herring gull and its eggs. It also houses samples of many types of mammals originating in temperate and arctic regions. The CWS, as do other specimen banks, has very specific requirements for collection data and labelling procedures, and protocols for field handling, shipping, sample preparation, and storage. Storage facilities have recently been updated with the purchase of a liquid nitrogen freezer.

The CWS maintains a computerized National Registry of Toxic Chemical Residues, a repository for information on wildlife specimens analyzed for toxic chemicals or specimens deposited in the CWS National Specimen Bank. The registry was started in 1964 and a Scientific Information Retrieval data base management system added in 1980.

8. Proposed US-Canada Great Lakes Regional Specimen Bank (GLRSB)

(Kerry 1993, MAS 1993)

A feasibility study conducted recently by UMA Engineering for the Michigan Audubon Society draws together information from current US/Canadian environmental sample collections and other major specimen banks (CWS, NBSB, GESB, etc.), and makes recommendations for a regional specimen bank to house biological samples from the Great Lakes region of North America. Collection, handling, shipping, storage, and QA/QC protocols for archiving samples of fish, birds eggs, mammal liver (including human), and bivalves are discussed in this report.

The report's recommendations in the area of sample handling and storage include:

(i) development of contaminant-free sampling equipment,
(ii) rigorous data collection records including chain-of-custody information,
(iii) shipment of samples in liquid nitrogen shippers or dry ice coolers,
(iv) splitting of specimens into duplicate samples or duplicate sample pools (approx. 150 g each), one for long-term storage and one for analytical investigation,
(v) homogenization of samples under cryogenic conditions and only as needed,
(vi) storage of specimens in either -80ºC or liquid nitrogen (-120 to -196ºC) freezers upon receipt at GLRSB,
(vii) use of Teflon containers, and
(viii) restriction of access, installation of a 24 hour alarm system, and provision for a backup cooling system or alternate freezer facilities to ensure the safety of stored samples.

In a section dealing with storage protocols, the report states that "...LN_2 is the best available method to preserve the samples. Under no circumstances should specimens be banked at a temperature higher than -80ºC...". This conclusion is based on recommendations by two of the pioneers in specimen banking, the US EPA NBSB and the GESB. At -80ºC and lower, enzyme activity and surface absorption are minimized, and the risk of degradation of target contaminants is thus lessened. The report does not give details of storage studies supporting the -80ºC temperature recommendation, but lists a number of key references. It then goes on to quote the NBSB study (section 4.2) comparing storage of human liver samples at -25 and -150ºC (Zeisler 1992, Wise 1989) in which no degradation of PCBs or 4,4'-DDE was detected during a 7 year study.

9. Canadian Human Specimen Bank

(Subramanian 1993)

Plans for establishing a Canadian human specimen bank as part of the Great Lakes Health Effects Division (GLHED) program were initiated by Health and Welfare Canada in 1990 as a commitment to the IJC under the Great Lakes Surveillance Plan. A report providing a comprehensive review of international specimen banking, and mapping out a strategy for the new Canadian Human Specimen Bank was prepared by GLHED in 1991 (Iyengar 1991).

10. Biological Specimen Bank for Smelter Workers
(Gerhardsson 1993)

T = -20ºC
Human organs
Analyze for trace metals

In 1975, a specimen bank was initiated by the Departments of Medicine and Environmental Medicine at the University of Umea, Sweden to collect information on trace elements in organs from workers at a non-ferrous copper smelter. A large variety of tissue samples are taken from deceased smelter workers and control individuals from four different locations. Information from trace metal analyses is used to determine the influence of occupational exposure. No stability studies were mentioned.

11. Japan's National Institute for Environmental Studies (NIES)
(Ambe 1993, Ambe 1992)

T = 20, -20, -85, and -115ºC
Atmospheric, water, sediment and soil, biological (tissue and vegetation) samples and SRMs.
3000 samples

Environmental specimen banking activities in Japan began in 1980 at NIES. Research activities are now focusing on the study of long term preservability in stored samples, and the problems associated with operation of a full-scale specimen bank. Two sample storage experiments have been carried out to date. One looked at the stability of benzo[a]pyrene in atmospheric particulate matter (section 6.5), and the second evaluated the stability of organochlorines spiked artificially into homogenized mussel tissue (section 4.2). This small-scale specimen bank is planned to continue as a demonstration project.

12. Others

Co-ordination of specimen banking activities is underway in the Nordic countries: Sweden, Norway, Finland and Denmark. Under the authority of the Nordic Council of Ministers and their Working Group for Environmental Monitoring, a Project group recommends storage of biological specimens (plant and animal) at -80ºC or lower for samples which will be put to international use (Giege 1993).

The Greenland Environmental Research Institute and several Danish universities house a collection of more than 20,000 arctic specimens (Poulsen 1993). Proposed co-operation between the two countries is expected to resolve current differences in storage conditions.

Specimen banking in the new Russian Republic dates back a number of years. Banked samples of air, soil, and vegetation from the area of a capacitor plant have been used to correlate PCB contamination with abnormally high concentrations of PCBs in breast milk of local women (Bobovnikova 1993).

Scientists in Slovakia have banked extracts of environmental samples for 5 to 10 years without any changes in concentration being noted (Trnovec 1993). A summary of this study describing the analysis of trace metals in a variety of matrices can be found in Section 10.2.

New specimen banking activities are in the planning stages or have recently been established in Venezuela, Thailand, and Sri Lanka among other countries.