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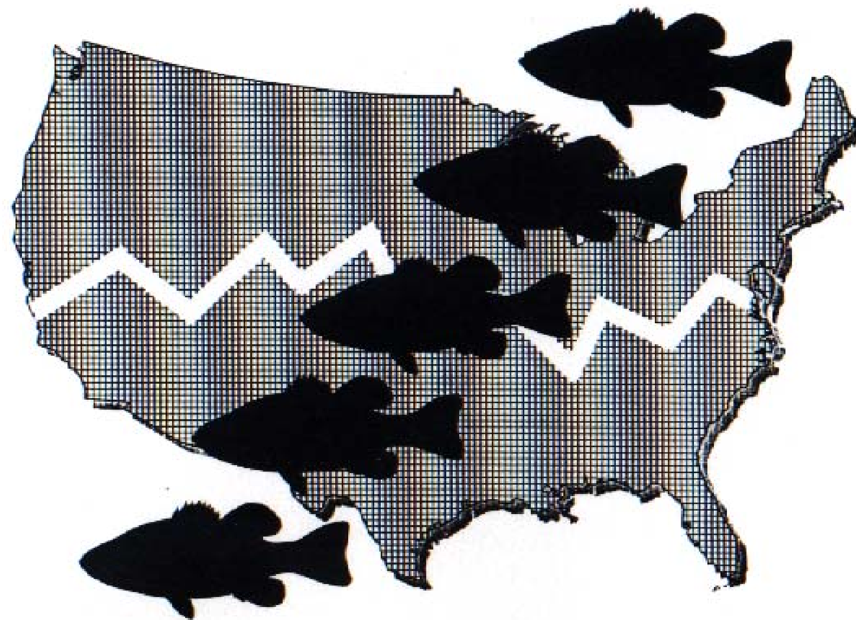
Quality Assurance Report for the National Study of Chemical Residues in Lake Fish Tissue: Analytical Data for Years 1 through 4



Prepared for:

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology

Prepared by:

CSC Environmental Programs Group

Prepared under:

MOBIS Contract No. GS-23F-9820, Task 68-C-00-137

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TABLE OF CONTENTS

Page

Chapter 1 Introduction . . . 1
- Section 1.1 Description of Study Objectives and Study Design . . . 1
- Section 1.2 Study Participants . . . 3
- Section 1.3 References . . . 3

Chapter 2 QA Program . . . 5
- Section 2.1 Collaborative Study Design . . . 5
- Section 2.2 Implementation of Approved Quality Assurance Project Plans for Sampling and Analysis Activities . . . 5
- Section 2.3 Implementation of a Field Orientation/Training Program . . . 6
- Section 2.4 Development of Study-Specific Sample Documentation and Sampling Kits . . . 6
- Section 2.5 Daily Monitoring of Sampling and Laboratory Activities . . . 6
- Section 2.6 Weekly to Monthly Reporting of Project Status as Appropriate . . . 7
- Section 2.7 Monthly Project Meetings among EPA Headquarters Staff and Contractors Responsible for Coordination of Activities . . . 7
- Section 2.8 Implementation of Thoroughly Documented Methods That Included All QC Elements Needed to Support Quality Objectives Established for the Study . . . 7
- Section 2.9 Three Levels of Data Quality Assessment and Application of Standardized Data Qualifiers . . . 8
- Section 2.10 Implementation of Standardized Data Format to Allow All Results to be Reported Consistently and Accurately to Data Users . . . 8

Chapter 3 Analytical Methods Employed . . . 9
- Section 3.1 Sample Prep Lab Procedures . . . 9
- Section 3.2 Dioxins/Furans . . . 10
- Section 3.3 Polychlorinated Biphenyls (PCBs) . . . 11
- Section 3.4 Total Mercury . . . 11
- Section 3.5 Arsenic Species . . . 11
- Section 3.6 Organochlorine Pesticides . . . 12
- Section 3.7 Organophosphorus Pesticides . . . 12
- Section 3.8 Semivolatile Organics . . . 12

Chapter 4 Data Quality Assessment . . . 15
- Section 4.1 Pre-Qualification Review . . . 15
- Section 4.2 Individual Data Package Review . . . 19
- Section 4.3 Overall Data Quality Assessment . . . 30

LIST OF EXHIBITS

Exhibit 1-1 Study Design Highlights 2
Exhibit 1-2 Overview of Study Participants 3
Exhibit 3-1 National Lake Fish Tissue Study Target Analytes and Corresponding Analysis Methods 13
Exhibit 4-1A Summary (Target Analytes) of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study 34
Exhibit 4-1B Summary (Non-Target Analytes) of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study 48

Chapter 1
Introduction

This report documents the quality of data gathered during the *National Study of Chemical Residues in Lake Fish Tissue* (hereafter referred to as either the "National Lake Fish Tissue Study" or, more simply, "the Study." Please note that the abbreviated name for the study changed from "National Fish Tissue Study" during 2004.). For reference purposes, this report provides a brief overview of the study and outlines the primary participants and the analytical parameters measured. Additional details concerning the design and implementation of this study can be found in the references listed at the end of this chapter.

SECTION 1.1 DESCRIPTION OF STUDY OBJECTIVES AND STUDY DESIGN

The National Lake Fish Tissue Study is a screening-level study designed to estimate the national distribution of selected persistent, bioaccumulative, and toxic (PBT) chemical concentrations in fish tissue from lakes and reservoirs of the contiguous United States. The study involves the collection of predator and bottom-dwelling fish from 500 randomly selected lakes and reservoirs in the lower 48 states (excluding the Great Lakes) over a period of 4 years (~125 lakes per year). The study began during the fall of 1999; however, full implementation did not commence until 2000. For this reason, tissue samples collected during the 1999 mobilization and 2000 implementation periods cumulatively represent Year 1 of the Study.

The study design resulted from a comprehensive planning effort that included a national workshop involving more than 50 scientists from state, federal, and tribal agencies to obtain technical input on sampling design, target analytes, sampling methods and data management. The final study design is described in Reference 1 of this chapter and highlighted in Exhibit 1-1.

Implementation of the study is a collaborative effort being led by the Environmental Protection Agency's (EPA) Office of Science and Technology (OST), within the Office of Water (OW), with extensive support from participating states and tribes, EPA Regions, and the Office of Research and Development's (ORD) Environmental Monitoring and Assessment Program (EMAP). States, Tribes, and EPA Regional staff collected most of the fish for the study. Contractor support is being provided by Tetra Tech (for study design, orientation/training, workshops, field sampling activities, data compilation, and project reporting), CSC Environmental Programs Group ([formerly DynCorp Environmental] for sampling kits, sample coordination and tracking, data review and reporting, and database development), and the following laboratories: Axys Analytical Services in Sydney, British Columbia, Canada; Battelle Ocean Sciences in Duxbury, MA; Battelle Marine Sciences in Sequim, WA; and Pacific Analytical Inc., in Carlsbad, CA (see Exhibit 1-2, Overview of Study Participants.)

The 500 lakes sampled for the study were statistically selected to meet study objectives. To allow study objectives to be met in the event that all four years of sampling could not be completed, the lakes were statistically sub-sampled and classified as Year 1, Year 2, Year 3, or Year 4 lakes. These statistical designations did not always conform to resources available at the state level in the assigned years. Therefore, some states requested and received permission to collect samples from a lake in a year other than the year that the lake was designated to be sampled. Consequently, it is important to note that the Year 1, Year 2, Year 3, and Year 4 data

sets described in this report do not correspond to the annual statistical subsets of lakes. To distinguish the annual statistical subsets from the lakes actually sampled during the first through the fourth years of the study, they are now being referred to as Panel 1, Panel 2, Panel 3, and Panel 4 lakes. The 500 lakes sampled during the four years of the study consisted of 120 Panel 1 lakes, 109 Panel 2 lakes, 105 Panel 3 lakes, 109 Panel 4 lakes, and 57 lakes from a set of statistically drawn "reserve" lakes.

Exhibit 1-1 Study Design Highlights

Objective

Estimate the national distribution of selected persistent, bioaccumulative, and toxic (PBT) chemical concentrations in fish tissue from lakes and reservoirs of the contiguous U.S.

Sample Sources

500 randomly selected lakes and reservoirs (each defined as a permanent body of water having at least one hectare in surface area with a minimum of 1,000 m^2 of open (unvegetated) water, a minimum depth of one meter, and a permanent fish population)

Sample Types

- Edible tissue (i.e., skin-on fillet) composites of targeted predator species
- Total body tissue (i.e., whole fish) composites of targeted bottom-dwelling species

Composite Definition

Five individual fish of the same species that:
- Satisfy legal requirements of harvestable size or weight (or are of consumable size if no legal harvest requirements are in effect)
- Are of similar size so that the smallest individual within the composite is no less than 75% of the total length of the largest individual
- Are collected at the "same" time (i.e., as close to the same time as possible but no more than 1 week apart)
- Are of adequate size to allow analysis of target study analytes

Target Species

Selected in accordance with EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis, Third Edition* (U.S. EPA 2000), with preferences as cited in References 1, 2, and 3 of this chapter.

Field QC

- Development and implementation of the Sample Collection Activities QAPP (Reference 2), Field Sampling Plan (Reference 3), and SOPs (References 2 and 3)
- Collection of replicate fish samples from 10% of the lakes to estimate sampling variability
- Use of experienced fisheries biologists to ensure use of proper procedures
- Distribution of standardized sampling kits to control contamination and ensure proper documentation
- Daily tracking and coordination of sample shipments through a centralized source
- Regional orientation/training workshops to ensure all field personnel understood objectives and design of study and to ensure consistent application of required sample collection, handling, and shipping procedures
- Field audits to ensure consistent sample collection, handling, and shipping procedures

Exhibit 1-1 Study Design Highlights

Laboratory QC

- Development and implementation of the Analytical Control and Assessment Activities QAPP (Reference 4)
- Use of centralized Sample Prep Laboratory to minimize variability during sample grinding, homogenizing, and compositing
- Identification of quantifiable measurement quality objectives (MQOs)
- Implementation of standardized sample tracking, lab analysis, data reporting, and data review procedures
- Use of pure and traceable reference standards
- Demonstration of instrument calibration and system performance
- Periodic calibration verification
- Verification that each laboratory could achieve the required detection and quantitation levels
- Analysis of initial and ongoing QC samples to demonstrate each laboratory's ability to achieve precise and accurate results with the method
- Analysis of blanks to demonstrate the absence of contamination

SECTION 1.2 STUDY PARTICIPANTS

A detailed list of participants referenced in this report is provided in Reference 3 of this chapter. Exhibit 1-2 below identifies the parties cited in this report.

Exhibit 1-2 Overview of Study Participants

Study Participants

EPA Office of Science and Technology:
Leanne Stahl, National Study Manager, Field Support Manager
Cynthia Simbanin, Analytical Project Manager

Sample Control Contractor (SCC): CSC Environmental Programs Group (formerly DynCorp Environmental)

Study Design and Field Support Contractor: Tetra Tech, Inc.

Sampling Teams: State, Tribal, and Regional contacts listed in Reference 3

Sample Prep Laboratory: Axys Analytical Services

Analysis Laboratories: Axys Analytical Services (PCBs, Dioxins/Furans)
Battelle Ocean Sciences (Semivolatile Organics)
Battelle Marine Sciences (Mercury and Arsenic)
Pacific Analytical Inc. (Pesticides)

SECTION 1.3 REFERENCES

Additional information regarding the design and implementation of this study can be found in the following references:

(1) U.S. Environmental Protection Agency. 1999. *National Study of Chemical Residues in Lake Fish Tissue: Study Design*. Prepared by Tetra Tech, Inc. for USEPA, OW/OST, Washington, DC.

(2) U.S. Environmental Protection Agency. 2000. *Quality Assurance Project Plan for Sample Collection Activities for a National Study of Chemical Residues in Lake Fish Tissue.* Prepared by Tetra Tech, Inc., for USEPA, OW/OST, Washington, DC.

(3) U.S. Environmental Protection Agency. 2000. *Field Sampling Plan for the National Study of Chemical Residues in Lake Fish Tissue*. Prepared by Tetra Tech, Inc. for USEPA, OW/OST, Washington, DC.

(4) U.S. Environmental Protection Agency. 2000. *Quality Assurance Project Plan for Analytical Control and Assessment Activities in the National Study of Chemical Residues in Lake Fish Tissue*. Prepared by CSC Environmental Programs Group (formerly DynCorp Environmental) for USEPA, OW/OST, Washington, DC.

The first three documents are available from Leanne Stahl, National Study Manager, and the final document is available from Cynthia Simbanin, Analytical Project Manager, at the following addresses:

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Chapter 2
QA Program

At the outset of the National Lake Fish Tissue Study, EPA managers recognized that data gathered from the study would be used extensively by individuals responsible for making environmental, economic, and policy decisions. Environmental measurements always contain some level of uncertainty. Decision makers, therefore, must recognize the uncertainty associated with the data on which their decisions are based. In recognition of this, the study managers established a quality assurance (QA) program intended to ensure that data produced under the National Lake Fish Tissue Study would meet defined standards of quality within a specified level of confidence (see Chapter 1, References 2 and 4).

> Implementation of the QA Program ensured that all Measurement Quality Objectives (MQOs) were met in Year 1, Year 2, Year 3, and Year 4 of the Study and that not a single sample was lost or misidentified.

The study QA program prescribes minimum requirements to which all organizations that gather data are required to adhere. All of these elements were followed during each year of the study, and data quality was defined, controlled, and assessed through these QA program activities. The remainder of this chapter highlights the QA program employed during the study.

SECTION 2.1 COLLABORATIVE STUDY DESIGN

Development of the study design was a collaborative effort among EPA's Office of Water (OW), Office of Research and Development (ORD), and Office of Prevention, Pesticides, and Toxic Substances (OPPTS), with significant involvement by biologists, chemists, and statisticians in OW's Office of Science and Technology (OST), statisticians in ORD's Environmental Monitoring Assessment Program (EMAP), and chemists in OPPTS. The draft design was reviewed by experts throughout federal, state, and tribal organizations (including EPA, the National Oceanic and Atmospheric Administration (NOAA), U.S. Geological Survey (USGS), and state and tribal environmental, wildlife, and fisheries management agencies) via a two-day workshop. Input obtained during this workshop was incorporated into the final study design.

SECTION 2.2 IMPLEMENTATION OF APPROVED QUALITY ASSURANCE PROJECT PLANS FOR SAMPLING AND ANALYSIS ACTIVITIES

Two Quality Assurance Project Plans (QAPPs) were developed and approved by EPA to support this study. The *Quality Assurance Project Plan for Sample Collection Activities for a National Study of Chemical Residues in Lake Fish Tissue* (May 2000) establishes data quality goals for all sample collection and handling activities and describes the QA/Quality Control (QC) techniques employed by field teams and the field support contractor to support those goals. The *Quality Assurance Project Plan for Analytical Control and Assessment Activities in the National Study of Chemical Residues in Lake Fish Tissue* (September 2000) establishes MQOs for laboratory data generated during the study and describes QA/QC techniques employed by laboratory and sample control contractor (SCC) staff to ensure these goals are met.

SECTION 2.3 IMPLEMENTATION OF A FIELD ORIENTATION/TRAINING PROGRAM

Because the study design relied on a large number of state, tribal, and federal sampling teams, EPA established a field orientation and training program to ensure that personnel responsible for sampling activities within each organization understood the study objectives, were familiar with paperwork developed specifically to document sample collection activities under the study, and were prepared to collect, document, and ship samples in accordance with the standard operating procedures (SOPs) and the sample collection QAPP.

SECTION 2.4 DEVELOPMENT OF STUDY-SPECIFIC SAMPLE DOCUMENTATION AND SAMPLING KITS

The study design calls for collection of fish samples by multiple teams from participating states, tribes, and EPA Regions. To ensure samples were consistently documented by such a large and diverse group, several documentation materials were custom-designed for the study. These forms include a:

- *Field Record Form* to document information about each lake sampled and individual specimens collected from the lake,
- *Sample Identification Label* to accompany and identify each fish specimen,
- *Chain-of-Custody Form* to provide constant tracking information for all samples, and
- *Chain-of-Custody Label* to seal each shipping container.

These forms were being provided annually in *custom-made sampling kits* prepared by the SCC. The kits also contained contaminant-free materials needed to store each specimen (i.e., solvent-rinsed aluminum foil and food grade polyethylene tubing), a reference instruction sheet with contact phone numbers, and pre-completed forms needed to ship the specimens to the Sample Prep Laboratory for homogenization and compositing. In addition, sample *Traffic Reports* were created for use by the Sample Prep Laboratory to document each homogenized composite aliquot that was sent to either an Analysis Lab or to the Sample Repository for long-term storage. Implementation of these tools in all four sampling years of the study (2000-2003) was highly successful.

SECTION 2.5 DAILY MONITORING OF SAMPLING AND LABORATORY ACTIVITIES

To ensure effective communication among all organizations involved in the study, the field support and SCC contractors were tasked with establishing and implementing a series of procedures for the following activities: preparing and distributing sampling kits; coordinating and tracking sample shipments; identifying corrective actions in the event of lost shipments; reviewing Field Record Forms to identify and notify EPA of fish samples that deviated from the sample criteria and recommending corrective actions; obtaining laboratory analyses; reviewing laboratory data; and generating a STORET-compatible database of study results. These activities were highly successful in controlling the quality of data during all four years of the study. Not a single sample was lost during the study, and all potential deviations from the study design were mitigated by these early identification techniques.

Section 2.6 Weekly to Monthly Reporting of Project Status as Appropriate

Each of the contractors routinely reported the status of project activities to EPA so that the National Study Manager could monitor study progress (e.g., samples collected, samples analyzed, etc.), notify senior EPA management of potential problems and success stories, and communicate project status to other organizations supporting the study. Such communications provided a real time means through which the study manager could notify study participants of important issues (e.g., the need to halt sample shipments until air traffic returned to normal following the 2001 terrorist attacks; clarifications concerning the amount of dry ice needed when shipping coolers; and the need for alternate documentation procedures to streamline the shipment of samples from the field through Customs to the Sample Prep Laboratory).

Section 2.7 Monthly Project Meetings among EPA Headquarters Staff and Contractors Responsible for Coordination of Activities

During Year 1 of the study, the National Study Manager held monthly meetings with EAD staff responsible for managing laboratory and data review activities and with each team of contractors responsible for daily tracking of activities. With a full year of study experience behind the teams, the frequency of these meetings was reduced to an as-needed basis in the remaining years of the study. The purpose of each meeting was to review study progress, discuss upcoming schedules, and identify and resolve issues. Depending on project activities, additional staff were brought into these meetings to facilitate planning and resolve issues. Notably, OW representatives responsible for developing and managing STORET were invited to meetings that included discussions of database designs, STORET data upload, and data distribution plans. Likewise, EAD statisticians responsible for interpreting study results were invited to participate in discussions of the procedures used to review, qualify, and report laboratory results.

Section 2.8 Implementation of Thoroughly Documented Methods That Included All QC Elements Needed to Support Quality Objectives Established for the Study

A suite of EPA 1600-series methods was employed to support the study. Each participating laboratory, including the Sample Prep Laboratory, was required to demonstrate their ability to practice these methods before preparing or analyzing samples collected in the study. Chapter 3 describes these methods in detail.

SECTION 2.9 THREE LEVELS OF DATA QUALITY ASSESSMENT AND APPLICATION OF STANDARDIZED DATA QUALIFIERS

All analytical data generated during the study were subjected to three levels of review:

- A pre-qualification review was performed on data submitted by each laboratory to demonstrate that the labs were qualified to prepare and/or analyze tissue samples collected during the study.

- Each submission of tissue sample results was carefully scrutinized to verify that the samples were analyzed as directed and that supporting QC results demonstrated the quality of results generated. In evaluating these submissions, data reviewers employed a suite of standardized data qualifiers and abbreviated qualifier codes to consistently and accurately document the quality of all data generated so that both the primary data users (statisticians) at EPA Headquarters and secondary data users within states, tribes, and other organizations could make informed decisions regarding their use.

- A third level of data review was performed at the conclusion of each of the four annual data review processes to determine if overall data quality supported study objectives. These end-of-year evaluations indicated that all MQOs were met for all four years of the study. Chapter 4 describes the data quality assessment procedures employed in the study.

SECTION 2.10 IMPLEMENTATION OF STANDARDIZED DATA FORMAT TO ALLOW ALL RESULTS TO BE REPORTED CONSISTENTLY AND ACCURATELY TO DATA USERS

All data generated during the study are being compiled in a centralized, custom-developed database designed for the following: eventual upload of results to the national STORET database system, statistical manipulation of results, export of results to user-friendly formats such as Excel spreadsheets, and consistency in data format and nomenclature across laboratories and over time.

Chapter 3
Analytical Methods Employed

To control variability among tissue sample results, all samples collected during the study were analyzed by a single set of methods, and all analyses performed with a given method were performed by only one laboratory. Further control of variability was ensured by utilizing a single laboratory to prepare, composite, homogenize, and aliquot samples in a strictly controlled, contaminant-free environment. The methods employed by the Sample Prep Laboratory and by each Analysis Laboratory are described below. A complete list of the chemicals measured by these laboratories is provided at the end of this chapter in Exhibit 3-1.

SECTION 3.1 SAMPLE PREP LAB PROCEDURES

Each composite tissue sample prepared by the Sample Prep Lab consisted of five individual fish (where available) of a single species. Bottom-dwelling species were prepared as whole fish composites (i.e., the entire specimen, including the head, skin, internal organs, muscle, and bones were thoroughly homogenized). Predator/gamefish were prepared as skin-on (scales removed) fillet composites. All tissue sample preparation, filleting, and homogenization activities were performed in accordance with EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis, Third Edition*, November 2000 (the "Fish Advisory Guidance") in a strictly controlled, contaminant-free environment. Equipment rinsates were collected during each day of sample preparation activities and analyzed to document the absence of contamination. Upon receipt of samples from the field, the Sample Prep Lab:

- Checked that each shipping container arrived undamaged and verified that samples were still frozen and in good condition upon receipt. All samples collected and shipped throughout the four years were received frozen. Fish composite samples that did not conform to established study criteria (i.e., "nonroutine" composite samples) were frozen pending a determination by EPA concerning procedures for analysis. EPA documented all processing and analysis decisions for nonroutine fish composite samples (e.g., accepting composites with fewer or greater than 5 specimens), and these decisions were entered into the study database.

- Forwarded all associated paperwork to the field support contractor for full verification of completeness and accuracy. All QA problems were successfully resolved and reported back to the SCC for entry of field data into the database.

- Verified that all specimens listed on the paperwork for each composite were included in the shipment and were properly wrapped and labeled. Deviations were rare, and they were immediately reported to EPA (via SCC) for resolution and noted in the study database.

- Signed the chain-of-custody form and forwarded it to the Field Support Contractor with a copy to the SCC.

- Documented information about each specimen in a laboratory notebook.

All grinding and homogenization activities were performed in accordance with Section 7.2.2.9 of the Fish Advisory Guidance (for fillet composites) and Appendix G of the guidance (for whole fish composites) with the following exceptions:

- The laboratory was required to use equipment known to be free from contamination of all target analytes at the concentrations of interest.
- For predator fillets, the Sample Prep Lab used the *entire* fillet from both sides of each fish (skin-on, scales removed, with belly flap attached) instead of measuring and homogenizing equal weights of each fillet.
- For bottom feeders, each entire fish was homogenized and included in the composite instead of compositing equal weights of individually homogenized fish.

Once homogenized and composited, the Sample Prep Lab aliquoted samples for distribution to each of the Analysis Labs. If volume allowed, the Sample Prep Lab also prepared "extra volume" aliquots for shipment to a sample repository for archiving. Aliquots intended for organics analyses were placed into 125-mL trace-organics clean amber jars with fluoropolymer (FEP)-lined lids. Metals aliquots were stored in 125-mL I-Chem™ Level III trace metals clean (or equivalent) glass jars, also equipped with FEP-lined lids. Each aliquot was further stored inside two food-grade plastic bags to avoid sample loss in the event of breakage. To avoid breakage, the jars were filled to no more than 80% capacity. All aliquots were frozen (-20°C) pending distribution to the Analysis Labs and the Sample Repository.

The Sample Prep Lab determined the lipid content of each sample. This lipid determination was performed using the procedure described in EPA Methods 1613B and 1668A, and is the same procedure used in EPA's National Dioxin Study.

The Sample Prep Lab assigned a unique five-digit EPA sample number to each composite tissue sample and documented the sample number, and corresponding percent lipids result, on a Traffic Report that accompanied each aliquot to the designated Analysis Lab. The Sample Prep Lab also prepared a series of "blind composite duplicates" on 5% of the samples. These blind composite duplicates were also assigned five-digit EPA sample numbers and sent to the Analysis Labs in exactly the same manner as were true field tissue sample aliquots. The blind sample aliquots were used by EPA to verify that Sample Prep Lab procedures were yielding homogeneous aliquots and to characterize variability arising from the entire sample preparation, re-distribution, and analysis processes.

SECTION 3.2 DIOXINS/FURANS

The presence and concentration of seventeen 2,3,7,8-substituted chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDDs and CDFs) in each sample was determined by a slightly modified version of EPA Method 1613, Revision B (*Tetra- through Octa- Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS*, EPA-821-B-94-005).

Modifications were made to the procedures specified in Method 1613B in order to allow for determination of dioxins and furans at levels ten times lower than those specified in the method. Specifically, the method was modified to increase the tissue sample size used for analysis and to

add a sixth calibration solution that contained all the method-specified analytes at levels lower than the levels specified in the method to verify linearity at the lower concentrations targeted.

SECTION 3.3 POLYCHLORINATED BIPHENYLS (PCBS)

EPA Method 1668, Revision A (*Chlorinated Biphenyls Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS*, EPA-821-R-00-002) was used to determine PCB congener concentrations in tissue samples collected during the study. There are 209 possible congeners, 12 of which have toxicological significance (i.e., the "toxic" PCBs identified by the World Health Organization). Method 1668A can unambiguously determine 126 of the 209 congeners as separate chromatographic peaks. The remaining 83 congeners do not appear as separate peaks, but elute from the gas chromatograph in groups of 2 to 6 congeners that cannot be completely resolved by the instrumentation. Ten of the 12 "toxic" congeners are resolved, and the remaining two congeners (PCB 156 and PCB 157) elute as a congener pair. Because PCB 156 and 157 have identical toxicity equivalency factors, however, it is possible to accurately calculate PCB toxic equivalence based on the 12 toxic congeners.

For reporting purposes, each tissue sample is associated with 126 results that represent the 126 single PCB congeners, and another 33 results that represent co-eluting congener groups for the remaining 83 congeners, for a total of 159 PCB congener "results." In addition, each sample is associated with 10 values corresponding to the 10 possible levels of chlorination for the parent biphenyl. Each of these 10 values represents the sum of the concentrations of all of the congeners in a given level of chlorination (i.e., a total of the monochlorinated PCBs, a total of the total dichlorinated PCBs, etc). Finally, each sample is associated with a total PCB value, which represents the sum of the 126 congener results plus the 33 values for the co-eluting congeners. All told, states and other study partners receive 170 unique PCB records for each sample (126 + 33 + 10 + 1), and 11 of these records represent totals drawn from the first 159 records (126 + 33).

SECTION 3.4 TOTAL MERCURY

Total mercury (Hg) concentrations were determined by EPA Method 1631, Revision B (*Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*) and its Appendix *(Digestion Procedures for the Determination of Total Mercury in Tissue, Sludge, Sediment, and Soil).*

SECTION 3.5 ARSENIC SPECIES

Total inorganic arsenic, arsenic (III), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) were directly determined by Method 1632, Revision A (*Chemical Speciation of Arsenic in Water and Tissue using Hydride Generation Quartz Furnace Atomic Absorption Spectrometry*). Arsenic (V) was determined by mathematically subtracting the measured concentration of arsenic (III) from the measured concentration of total inorganic arsenic. Strictly speaking, the techniques provided in Method 1632A allow for determination of the valence states of arsenic (III) and arsenic (V) rather than the species of inorganic arsenic. The actual species of inorganic arsenic are assumed to be those predicted by a geochemical equilibrium model. Total arsenic (which includes organic forms such as arsenobetaine) was not measured. Although it is commonly found in fish, arsenobetaine was not targeted in the study because of its low toxicity to fish and humans.

SECTION 3.6 ORGANOCHLORINE PESTICIDES

Organochlorine pesticides and total Aroclors were determined by Method 1656, Revision A (*Organo-Halide Pesticides in Wastewater, Soil, Sludge, Sediment, and Tissue by GC/HSD*), except that tissue sample extracts were concentrated by a factor of five beyond method-specified levels before instrumental analysis. This modification ensured that all target pesticides could be quantified at levels equal to or lower than the screening values published in EPA's Fish Advisory Guidance.

SECTION 3.7 ORGANOPHOSPHORUS PESTICIDES

EPA Method 1657, Revision A (*Organophosphorus Pesticides in Water, Soil, and Tissue by GC/FPD*) was used to determine the presence and concentration of organophosphorus pesticides listed in Exhibit 3-1.

SECTION 3.8 SEMIVOLATILE ORGANICS

The remainder of the target organic analytes were analyzed by a modified version of EPA Method 1625, Revision C (*Semivolatile Organic Compounds by Isotope Dilution GC/MS*). The modifications involved fractionating the samples by gel permeation chromatography (GPC) to yield a fraction containing the phthalates and some of the lower molecular weight lipids and a lipid-free fraction containing the polar target compounds. The phthalate/lipid fraction was further cleaned using Alumina and then recombined with the lipid-free fraction so that all target analytes could be determined in a single run. Please note that 3,3'-dichlorobenzidine was originally included as a target analyte in this study. However, the nature of this compound (and benzidines in general) often results in poor recoveries of this compound and its labeled analog, 3,3'-dichlorobenzidine-d6, from tissue samples and Ongoing Precision and Recovery (OPR) samples. Historically, the recovery problems have led to exclusion of large numbers of analytical results for 3,3'-dichlorobenzidine. Therefore, rather than exclude a large percentage of these results and include other results for this compound with recoveries that are very disparate from the other target analytes, EPA decided not to report results for 3,3'-dichlorobenzidine.

During Year 4 of the study only, the laboratory employed a Florisil cleanup instead of an Alumina cleanup in order to mitigate lipid interferences that were resulting in an excessive number of reextractions and reanalyses in the Year 3 samples. The use of Florisil did reduce the number of reanalyses required, suggesting that this approach should be considered in any further studies.

Exhibit 3-1 **National Lake Fish Tissue Study Target Analytes and Corresponding Analysis Methods**		
Analysis Method	**Target Analyte**	
Dioxins and Furans by Isotope Dilution High-resolution Gas Chromatography (GC)/Mass Spectrometry (MS) *(Method 1613, Revision B)*	2,3,7,8-TCDD 2,3,7,8-TCDF 1,2,3,7,8-PeCDD 1,2,3,7,8-PeCDF 2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDD 1,2,3,6,7,8-HxCDD 1,2,3,7,8,9-HxCDD 1,2,3,4,7,8-HxCDF	1,2,3,6,7,8-HxCDF 1,2,3,7,8,9-HxCDF 2,3,4,6,7,8-HxCDF 1,2,3,4,6,7,8-HpCDD 1,2,3,4,6,7,8-HpCDF 1,2,3,4,7,8,9-HpCDF OCDD OCDF
Total Mercury by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry *(Method 1631, Revision B with Appendix A - Digestion procedures for Total Mercury in Tissue, Sludge, Sediment, and Soil)*	Mercury	
Arsenic Speciation by Arsine Generation, Chromatography, and Atomic Absorption Spectrometry *(Method 1632, Revision A)*	Arsenic (III) Arsenic (V) Dimethylarsinic acid (DMA)	Monomethylarsonic acid (MMA) Total inorganic arsenic
Polychlorinated Biphenyls by Isotope Dilution High-resolution GC/Mass Spectrometry *(Method 1668, Revision A)*	209 congeners, including the following 12 "dioxin-like" congeners: 3,3',4,4'-TeCB 3,4,4',5-TeCB 2,3,3',4,4'-PeCB 2,3,4,4',5-PeCB 2,3',4,4',5-PeCB 2',3,4,4',5-PeCB	 3,3',4,4',5-PeCB 2,3,3',4,4',5-HxCB 2,3,3',4,4',5'-HxCB 2,3',4,4',5,5'-HxCB 3,3',4,4',5,5'-HxCB 2,3,3',4,4',5,5'-HpCB
Organochlorine Pesticides by GC/HSD *(Method 1656, Revision A)*	2,4'-DDD (TDE) ‡ 2,4'-DDE ‡ 2,4'-DDT ‡ 4,4'-DDD (TDE) 4,4'-DDE 4,4'-DDT Aldrin *cis*- and *trans*-Nonachlor Dicofol Dieldrin Endosulfan sulfate Endosulfan I Endosulfan II Endrin Ethalfluralin (Sonalan) Heptachlor Heptachlor epoxide Isodrin Kepone (Chlordecone) Methoxychlor Mirex Octachlorostyrene ‡ Analytes were added to the target analyte list after Year 1 of the study.	Oxychlordane Aroclor-1016 Aroclor-1221 Aroclor-1232 Aroclor-1242 Aroclor-1248 Aroclor-1254 Aroclor-1260 Pendamethalin (Prowl) Pentachloronitrobenzene (PCNB) *cis*-Permethrin *trans*-Permethrin Toxaphene Trifluralin α -BHC α -Chlordane (*cis*-Chlordane) β -BHC γ -BHC (Lindane) γ -Chlordane (*trans*-Chlordane) δ -BHC Pentachloroanisole
Organophosphorus Pesticides by GC/FPD *(Method 1657, Revision A)*	Chlorpyrifos Diazinon Disulfoton Ethion Paraoxon	Parathion (ethyl) Terbufos Terbufos sulfoxide Terbufos sulfone

Exhibit 3-1
National Lake Fish Tissue Study Target Analytes and Corresponding Analysis Methods

Analysis Method	Target Analyte	
Semivolatile Organic Compounds by Isotope Dilution GC/MS *(Method 1625, Revision C with modifications for tissue)* [See Section 3.8 for note concerning the analyte 3,3'-dichlorobenzidine.]	1,2,4,5-Tetrachlorobenzene	2,4-Dimethylphenol*
	1,2,4-Trichlorobenzene	2,4-Dinitrophenol*
	1,2-Dichlorobenzene	2,4-Dinitrotoluene*
	1,3-Dichlorobenzene	2,6-Dinitrotoluene*
	1,4-Dichlorobenzene	2-Chloronaphthalene*
	2,4,5-Trichlorophenol	2-Chlorophenol*
	4,4'-Methylenebis (2-chloroaniline)	2-Nitrophenol*
	4-Bromophenyl phenyl ether	2-Picoline*
	4-Nonylphenol	4-Chloro-3-methylphenol*
	Acenaphthene	4-Chlorophenylphenyl ether*
	Acenaphthylene	4-Nitrophenol*
	Anthracene	alpha-Terpineol*
	Benzo[a]anthracene	Biphenyl*
	Benzo[a]pyrene	Bis(2-chloroethoxy) methane*
	Benzo[b]fluoranthene	Bis(2-chloroethyl) ether*
	Benzo[g,h,i]perylene	Bis(2-Chloroisopropyl) ether*
	Benzo[j]fluoranthene	Carbazole*
	Benzo[k]fluoranthene	Di-*n*-octyl phthalate*
	Bis(2-ethylhexyl) phthalate	Di-*n*-propylnitrosamine*
	Butyl benzyl phthalate	Dibenzofuran*
	Chrysene	Dibenzothiophene*
	Dibenzo[a,h]anthrancene	Diethyl phthalate*
	Di-*n*-butyl phthalate	Dimethyl phthalate*
	Diethylstilbestrol (DES)	Diphenyl ether*
	Fluoranthene	Diphenylamine*
	Fluorene	Hexachlorocyclopentadiene*
	Hexachlorobenzene	Hexachloroethane*
	Hexachlorobutadiene	Isophorone*
	Indeno[1,2,3-cd]pyrene	*n*-Decane*
	Naphthalene	*n*-Docosane*
	Nitrobenzene	*n*-Dodecane*
	Pentachlorobenzene	*n*-Eicosane*
	Pentachlorophenol	*n*-Hexacosane*
	Perylene	*n*-Hexadecane*
	Phenanthrene	*n*-Nitrosodimethylamine*
	Phenol	*n*-Nitrosodiphenylamine*
	Phenol, 2,4,6-tris(1,1-dimethylethyl)-	*n*-Octacosane*
	Pyrene	*n*-Octadecane*
	Tetrabromobisphenol A	*n*-Tetracosane*
	1,2,3-Trichlorobenzene*	*n*-Tetradecane*
	1,2-Diphenylhydrazine*	*n*-Triacontane*
	2,3,6-Trichlorophenol*	*p*-Cymene*
	2,4,6-Trichlorophenol*	2-Methyl-4,6-Dinitrophenol*
	2,4-Dichlorophenol*	Styrene*

*This analyte was not considered a target analyte for study purposes, but since it was listed as a target analyte in Method 1625C, it was determined as a means of obtaining additional useful data for the study.

Chapter 4
Data Quality Assessment

Three levels of review were applied to all data generated in the National Lake Fish Tissue Study. First, a pre-qualification review was performed prior to analysis of tissue samples to verify that each laboratory was qualified to analyze the tissue samples in accordance with the prescribed methods. Second, ongoing reviews were performed to verify that the results of each data submission were, in fact, generated in accordance with all method and study requirements. Finally, overall data quality was evaluated at the end of Year 1, Year 2, Year 3, and Year 4 to verify that data as a whole were meeting established MQOs. The procedures employed for each of these three data review levels are described in Sections 4.1 through 4.3. Section 4.1 describes the procedures employed for reviewing laboratory prequalification data submitted prior to analysis of tissue samples during the study. Section 4.2 describes our process for reviewing individual data packages as they were submitted throughout the study, and describes our assessment of overall data quality in terms of the items reviewed. Section 4.3 discusses initial MQOs established for the study, and summarizes how well the overall study data set measured up against those MQOs. Where applicable, quantitative measures (e.g., percent of data that met Criterion X) of data quality are presented; these measures were calculated using results from study target analytes. Specific data qualifiers applied to the target analytes are presented in Exhibit 4-1A, and specific data qualifiers applied to non-target analytes measured during the study are presented in Exhibit 4-1B.

SECTION 4.1 PRE-QUALIFICATION REVIEW

Prior to preparing or analyzing tissue samples collected during the study, each laboratory was required to submit data demonstrating their ability to achieve the *sensitivity*, *precision*, and *accuracy* goals established for the study.

> Labs did not analyze fish tissue samples until they submitted pre-qualification data demonstrating they could achieve the sensitivity, precision, and accuracy goals defined for the study.

4.1.1 Sensitivity

4.1.1.1 Sensitivity Goals

Analytical sensitivity reflects the minimum concentration of an analyte above which a data user can be reasonably confident that the analyte was reliably detected and quantified. For this study, the *method detection limit (MDL)* and the *minimum level (ML)* of quantitation were used to define the sensitivity of each measurement process.

The MDL is defined as the measured concentration at which there is 99% confidence that a given analyte is present in a given sample matrix. Prior to analyzing tissue samples collected in Year 1 of the study, all laboratories were required to perform MDL studies in accordance with the procedures specified by EPA at 40 CFR 136, Appendix B.

Quantitative sensitivity in this study was established by the ML. The ML is defined as the lowest concentration at which the entire analytical system gives a recognizable signal and acceptable calibration for an analyte. The ML is equivalent to the lowest calibration standard analyzed by a specific analytical procedure, assuming that all the method-specified sample weights, volumes, and processing steps have been employed. The EPA 1600-series methods described in Chapter 3 specify MLs for tissue and/or aqueous samples. Generally speaking, MLs are approximately three times greater than the MDL and are comparable to the American Chemical Society's Limit of Quantitation.

In accordance with study objectives, each laboratory was required to demonstrate it could achieve MLs that were equal to or lower than those listed in the analytical method they would be using in the study. The only exceptions were as follows:

- The laboratory tasked with analyzing tissue samples for dioxins/furans was required to achieve MLs that were ten times lower than those specified in EPA Method 1613B. This was accomplished by increasing the tissue sample size used for analysis (to increase measurement sensitivity) and by adding a sixth calibration solution that contained all the method-specified analytes at levels lower than the levels specified in the method (to verify linearity at the lower target concentrations).

- The laboratory tasked with analyzing the organochlorine pesticide tissue samples was instructed to further concentrate its sample extracts in order to quantify all the target pesticides at levels that were equal to or lower than the screening values published by EPA in the Fish Advisory Guidance.

- The laboratory tasked with determining total mercury was permitted to target a ML of 2 ng/g instead of the 1 ng/g figure cited in the tissue appendix to Method 1631B. The allowed ML of 2 ng/g was considered to be acceptable because it is well below the EPA's recommended screening value for mercury.

4.1.1.2 Sensitivity Assessments

The Sample Prep Lab was responsible for receiving, filleting (where appropriate), homogenizing, aliquoting, and distributing samples to the analysis laboratories. Because these processes could theoretically affect the results generated by all other laboratories, it was critical to demonstrate that the Sample Prep Lab processes would not introduce contamination of any target analytes at the levels of interest in this study. To do so, the Sample Prep Lab was required to analyze equipment blanks (rinsates) before preparing any samples collected in the study. In conjunction with this, the Sample Prep Lab was required to perform MDL studies to demonstrate the lab's ability to measure blanks at the levels of interest in the study. These Sample Prep Lab MDL studies were performed in reagent water. Reagent water was used as a reference matrix for the equipment rinsates generated to demonstrate the absence of contamination.

The Analysis Labs were required to perform their MDL studies in a reference tissue matrix, using the same analytical methods they would be using in the study (see Exhibit 1-2). When possible, actual fish tissue was used for the MDL studies. However, it was often not possible to locate fish that were free of the analyte of interest at the low detection limits targeted in this study. In such cases, an alternative matrix, such as chicken breast or corn oil, was used.

Each MDL submission was reviewed to verify that:

- The MDL procedures specified at 40 CFR 136, Appendix B were followed correctly, with respect to the number of replicates, the spiking levels, and the statistics applied in determining the MDL;

- The laboratory used the analytical method(s) they would be employing in the study to analyze actual tissue (or, for the Sample Prep Lab, equipment rinsate) samples when performing their MDL studies;

- The laboratory performed their MDL studies on a calibrated instrument;

- The laboratory used an appropriate reference matrix when performing their MDL studies; and

- The MDLs determined by the laboratory supported the ML that would be targeted in the study (i.e., the MDLs were at least three times lower than the target MLs).

After evaluating the MDL results and, where necessary, obtaining clarification or missing information from the laboratories, the MDL data submitted by each laboratory for metals, dioxins, PCB congeners, and pesticides were deemed to meet method and study requirements. For the semivolatile organic analytes, all of the MDL study results were below the method-derived MLs for tissue samples; however, a few instances occurred in which the measured MDL was above this objective. These cases included the following target analytes: 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, di-*n*-butyl phthalate, and bis(2-ethylhexyl) phthalate. This was not considered to be an issue because the instrument was calibrated at the ML and non-detects were reported at the ML rather than the MDL. To further clarify, any result above the calculated MDL that met method-specified criteria would be reported as a "hit"; however, any result detected below the calculated MDL would be reported as a non-detect at the ML (see Section 4.2.2). Also, the measured MDLs for some of the analytes, such as the phthalates, were considered to be elevated by analyte contributions from the reference matrix used, and it was evident that other analytes, such as the dichlorobenzenes, could be detected at much lower levels (the measured MDLs were elevated by variability among the replicate measurements).

4.1.1.3 Reporting Thresholds for Sensitivity

As noted above, all of the 1600-series methods used for this study list MLs for aqueous and/or tissue samples. The appendices to Methods 1631B (mercury) and 1632A (arsenic) give tissue-based MDLs and MLs. These method-specified thresholds were used as reporting thresholds in the study for arsenic, but, as noted above, the laboratory-determined mercury ML of 2 ng/g was approved for use in this study. For consistency, the laboratory-determined mercury MDL also was used as the reporting threshold for detection limit sensitivity.

Method 1613B (dioxins/furans) provides MLs applicable to solids and tissues but, with the exception of 2,3,7,8-TCDD, does not provide corresponding MDLs. Because the method was modified as described in Section 3.2 to quantify dioxins/furans at levels 10 times lower than specified in the method, the quantitation limit thresholds reported in the database reflect the method-specified MLs divided by a factor of 10. Laboratory-determined MDLs were used as the

reporting threshold for detection limit sensitivity. As noted above, these laboratory-determined MDLs were at least a factor of three lower than the target MLs.

Method 1668A (PCB congeners) provides estimated MDLs and MLs that were derived based on the standard deviation of single lab blank measurements. Because these levels have not been finalized, laboratory generated MDLs and MLs were used as the detection and quantitation limit thresholds, respectively. As noted above, the laboratory's calibration curve encompassed their ML, and the laboratory-determined MDLs were at least a factor of three lower than the target MLs.

Methods 1656A (organochlorine pesticides) and 1657A (organophosphorus pesticides) provide recommended MLs for tissue samples and list recommended calibration standards intended to support the ML. These method-specified MLs were used as reporting thresholds for Method 1657A. For Method 1656A, the method MLs were divided by a factor of 5 to account for the five-fold extract concentration step described in Section 3.6 of this report and in the sensitivity goals discussion above. The laboratory MDLs, determined in fish tissue, were used as the threshold for reporting detection limit sensitivity. As noted above, these MDLs supported the MLs targeted in the study.

Method 1625C (semivolatile organics) does not specify MLs in tissue, nor does the method provide MDLs in either tissue or aqueous samples. Therefore, target tissue-based MDLs were mathematically derived from the method-specified aqueous MLs by converting aqueous units to solid units, accounting for the sample mass used in the tissue measurements, and dividing the resulting tissue MLs by 3. These method-derived MDLs and MLs were used as reporting limits for the semivolatile analytes.

4.1.2 Initial Demonstration of Precision and Accuracy

Prior to analyzing tissue samples collected during all four years of the study, each laboratory was required to demonstrate its ability to achieve precise and accurate results with the required analytical method. To do so, laboratories were required to prepare and analyze Initial Precision and Recovery (IPR) samples as described in each method.

IPR samples consisted of four aliquots of a reference matrix spiked with a known level of the target analytes. The reference matrix was chosen to serve as an indicator of method performance that could be expected for the tissue samples collected in the study. Accuracy was measured by determining the average recovery in the replicate IPR samples; precision was measured by calculating the relative standard deviation (i.e., RSD = standard deviation/mean) of the measured levels in the IPR samples.

Each laboratory's IPR submission was reviewed to verify the following:

- An appropriate reference matrix and spiking levels were used to prepare the four replicate samples.
- The designated 1600-series method was used to analyze the samples.
- The samples were generated on a properly calibrated instrument.
- Calculations of analyte recovery and precision were performed correctly.

After evaluating these factors and, where necessary, obtaining clarification or missing information from the laboratories, the IPR data submitted by each laboratory was deemed to meet method and study requirements and the laboratories were considered to be pre-qualified to analyze samples collected in Year 1 through Year 4 of the study.

SECTION 4.2 INDIVIDUAL DATA PACKAGE REVIEW

Upon completion of tissue sample preparation activities, each sample was assigned to a "sample delivery group" (SDG) for analysis purposes. Each SDG consisted of an arbitrarily assigned group of 20 samples assembled to simplify sample distribution and minimize the number of Quality Control (QC) samples that had to be prepared by each analytical laboratory. (Most methods specify that QC samples be prepared at a frequency of one per 20 tissue samples or one per analytical batch, whichever is smaller.) Laboratories were also asked to report their data by SDG to standardize the size of each data package and expedite data review processes.

> All Year 1, Year 2, Year 3, and Year 4 field results and supporting QC data were carefully reviewed to determine if the method had been properly followed and if all systems were in control during sample preparation and analysis. QC deviations were assessed to determine if they had an impact on the data, and these deviations and assessments were coded directly into the study database with flags and descriptive comments.

When submitting results for each SDG, the laboratories were required to submit all results associated with analysis of the samples in the SDG. This included results of the fish tissue samples analyzed, as well as results associated with any supporting QC measurements (e.g., instrument calibration, blank, and spike data). Results were to include both summary level data (the final measurement) and raw data (spectra, chromatograms, bench worksheets, etc.).

Each data package was thoroughly reviewed to ensure the following:

- All samples listed on the corresponding field and Sample Prep Lab documentation were analyzed and that results were provided.
- When possible, each analyte was uniquely identified with a Chemical Abstract Registry Service Number (CAS Number) to eliminate any ambiguity in naming conventions.
- All required QC samples were analyzed and these QC samples met specified acceptance criteria.
- Results were provided for each sample analyzed, including any dilutions and reanalyses, and for all associated QC samples.
- Required data reporting forms and/or electronic formats were provided for each of the field tissue sample and/or associated QC analyses.
- Raw data associated with each field tissue sample and QC sample was provided with each data package, and the instrument output (emission intensity, peak height, area, or other signal intensity) was traceable from the raw data to the final result reported.
- Any problems encountered and corrective actions taken were clearly documented.

If anomalies were found, the laboratory was contacted and asked to provide the missing data, clarifications, and/or explanations so that a comprehensive data review could be performed to verify the quality of their results. Results of these data reviews were documented directly in the database through the application of standardized data qualifier flags and descriptive comments concerning the reliability of the flagged results. Exhibit 4-1A (Target Analytes) at the end of this report summarizes flags and comments applied to the data for target analytes as a result of the review process described in subsections 4.2.1 - 4.2.7. Exhibit 4-1B (Additional or Non-Target Analytes) summarizes the flags applied to the 49 "non-target," semivolatile organic analytes measured as part of this study.

It is important to note that, because several of the methods used in the National Lake Fish Tissue Study contain a large number of analytes that are being tested simultaneously, there is always a small statistical probability that QC results for some of these compounds will occasionally fail to meet method specifications. Likewise, the large number of samples collected and the complex matrices being analyzed suggest some probability of occasional QC failures. In other words, the presence of QC failures and data qualifiers in the Year 1, Year 2, Year 3, and Year 4 data sets does not automatically suggest poor data quality in this study. To the contrary, EPA believes that the overall quality of data generated in this study was high, as evidenced by the limited number of failures described in Sections 4.2.1 through 4.2.7, and by the fact that the overall data set met all MQOs established for the study, as described in Section 4.3.

4.2.1 Assessment of Instrument Calibration

All of the methods used in the study required the laboratory to calibrate their instruments using a series of standards that covered a range of target analyte concentrations. This initial calibration provides a quantitative determination of instrument response and generates qualitative criteria for analyte identification. Methods 1631B (mercury), 1632A (arsenic), 1656A (organochlorine pesticides), and 1657A (organophosphorus pesticides), require a three-point calibration (i.e., the use of three calibration standards that contain the target analytes at low, medium, and high concentrations). Methods 1625C (semivolatile organics) and 1668A (PCBs) specify the use of five standards to calibrate the instrument. Method 1613B (dioxins/furans) requires the use of five standards, but as noted above in Section 4.1.1, a sixth calibration standard was used in this study to achieve lower quantitation limits.

The relationship between the response of an analytical instrument to the concentrations or amounts of an analyte introduced into the instrument is referred to as the "calibration curve." The 1600-series methods used in the study contain specific criteria for determining the linearity of calibration curves. When the applicable criterion is met, the calibration curve is considered to be sufficiently linear to permit the laboratory to use an average response factor or calibration factor, and it is assumed that the calibration curve is a straight line that passes through the zero/zero calibration point. If the calibration curve is not linear, an alternative approach must be used to quantify sample results. This means that a regression line or other mathematical function must be employed to relate instrument response to the concentration.

Each data submission was reviewed to verify that the appropriate number of calibration standards were used and that the resulting calibration curve either met the linearity specifications in the method or that the calibration curve was used to quantify samples. *All Year 1, Year 2,*

Year 3, and Year 4 data were generated on instruments that met the linear calibration requirements specified in the referenced method.

Initial calibration data submitted with each data package also were examined to verify that the calibration curve encompassed the MLs targeted in this study. The use of the ML as a point on the calibration curve is the principal means by which to assure that measurements made at the quantitation level are reliable. *All Year 1, Year 2, Year 3, and Year 4 data were generated on instruments that were properly calibrated at or below the MLs in this study.* In addition, all but two of the results reported in the study were analyzed within the instrument calibration range. These semivolatile organic results were coded with "REXC" to indicate that the result exceeded the calibration range and were further qualified as an "Estimated Value."

Because analytical instruments are subject to drift over time, analytical methods typically require periodic analysis of standards to verify the instrument remains calibrated throughout the duration of analysis. The 1600-series methods used in the National Lake Fish Tissue Study specify that the calibration verification be performed by analyzing a mid-point standard. The concentration of each analyte in this standard is determined using the initial calibration data and compared to the specifications in the method. If the results are within the method specifications, the laboratory is allowed to proceed with analysis without recalibrating and to continue using the initial calibration data to quantify sample results. If calibration verification results fall outside the required limits, the laboratory is required to recalibrate their instrument before proceeding with sample analysis. The frequency of this calibration verification varies according to the instrumentation (more frequent verification is required for instruments that are highly prone to drift) and is specified in the method. Verifying calibration at the method-specified frequency allows for early identification of problems and minimizes the need to reanalyze samples that might otherwise have been analyzed on an uncalibrated instrument.

Each Year 1, Year 2, Year 3, and Year 4 data package received was reviewed to verify that:

- Calibration verification was performed at the required frequency using appropriate calibration standards, and
- Results of the calibration verification met method-specified acceptance criteria, or
- If results did not meet method specified criteria, the laboratory re-calibrated the instrument before proceeding with sample analysis.

If the calibration verification requirements were not met, the reviewer evaluated the data package to verify that the laboratory followed the corrective action dictated by the method and that results were not affected. Although 284,973 field tissue sample results were generated during the first, second, third, and fourth years of the study, only 107 (6 samples in Year 1; 63 samples in Year 2; 0 samples in Year 3; and 38 samples in Year 4) of these results were flagged with calibration verification (CalVer) qualifiers. In Year 1 of the study, the six results flagged were for organochlorine pesticides, an outcome that is not surprising given the large number of pesticides targeted by the method and the fact that each pesticide is determined on two separate columns. When the analyte was found using both columns, the lowest of the measured results was reported since that value could be supported by both measurements. Reported results that failed to meet the recovery specifications for Method 1656A were qualified with either "LVER" to indicate a low recovery of the calibration standard or "HVER" to indicate a higher recovery of the standard. Failure to recover the analyte from the CalVer sample at all resulted in the application of a "NVER" flag. In Year 2 of the study, 63 of the arsenic speciation results (Method 1632A)

were qualified with "LVER" indicating a low recovery of the calibration standard. In Year 4 of the study, all of the 38 "LVER" and "NVER" codes were also associated with other data qualifiers.

> 99.96% of the 284,973 field tissue sample results generated in the four years of the study met all instrument calibration requirements.

4.2.2 Reporting Thresholds

Each laboratory was instructed to report all positive results that met all method-specified criteria (i.e., "hits") down to the MDL. The labs were further instructed to apply a "J" flag to any results reported above the MDL but below the ML.[1] The purpose of the "J" flag was to indicate that, although the analyte was detected, it was detected at a value below the quantitation limit. In other words, the presence of a "J" flag suggests the reported value is qualitative (the analyte is definitely present) but not quantitative (the reported value is an estimate of the true concentration).

Note: The PCB lab was instructed to include these "J" flagged results when reporting congener totals for the PCBs. Because nearly all samples had at least a few "J" flagged PCB congeners, nearly all the samples (964 samples out of a total of 1,003 samples analyzed for PCBs) also have a "J" flag on total PCB congener values.

The MDL is designed to provide a 99% level of confidence that when an analyte is reported as being present, it really is present. The converse is not true, however. If an analyte is reported as not being present at the MDL level, a 50% possibility exists that the result is a false negative. To ensure that results reported as non-detects are reliable indicators of the true concentration at which the analyte could not be detected, the reporting threshold for non-detects was set at the ML. (For further clarification, see final paragraph of Section 4.1.1.2)

> Positive results were reported to the MDL and flagged with a "J" if the results were below the ML. J-flagged results were further annotated as "Estimated Values" to caution users that the results are qualitative rather than quantitative.

All field tissue sample results submitted during Year 1, Year 2, Year 3, and Year 4 of the National Lake Fish Tissue Study were carefully reviewed to verify that the laboratory adhered to these reporting conventions.

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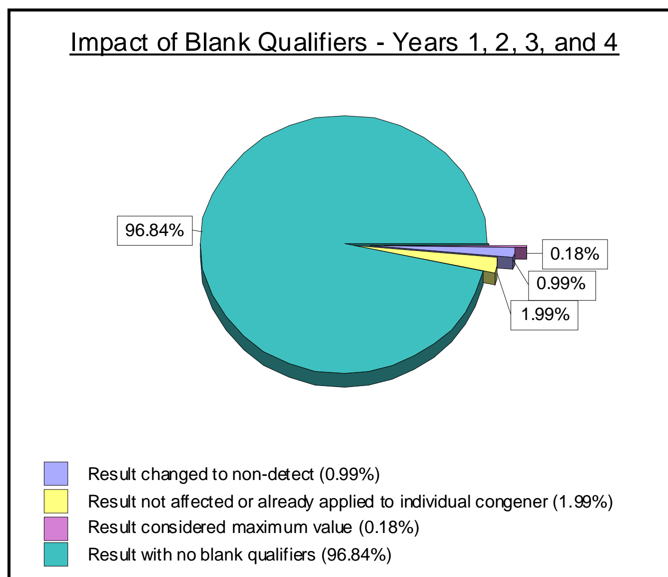
[1] MDLs reported in the study database reflect the study MDLs approved for the study, as discussed in Section 4.1.1. Because MDLs are determined in a single laboratory, measured MDL results are subject to slight changes over time and between laboratories. For analytes, such as hexachlorobenzene and terbufos, where the recommended screening value was below the "study MDL," but above a lab-determined MDL, any results reported between the study MDL and the lab-determined MDLs were reported in the database with a "LJS" (e.g., "J flagged result was between the lab and study MDLs").

4.2.3 Analysis of Blanks

Blanks are used to verify the absence of contamination that may occur at any point in the measurement process. In the National Lake Fish Tissue Study, many analytes were targeted at extremely low concentrations comparable to or lower than those typically found in the ambient environment. Therefore, frequent analysis and assessment of blanks was critical to determine if measured sample concentrations were biased by the presence of contamination during sample collection, handling, or analysis. In this study, the following blanks were used:



- *Equipment blanks* were used by the Sample Prep Lab to verify that the procedures and equipment used to prepare, fillet, homogenize, and or aliquot the samples were not introducing contamination. These blanks consisted of reagent water (i.e., water known to be free of the target analytes) that was run through all equipment used to process the samples in the facility where sample processing occurred. Each year, blanks were prepared and analyzed before any samples were prepared to verify the equipment and procedures to be used in the study were clean. The laboratory was required to reduce contamination to a level below all target MLs, and requested to reduce contamination below the target MDLs to the maximum extent possible. (It was understood that complete elimination of some ubiquitous contaminants, such as mercury and certain PCB congeners, at the low levels targeted in this study would be extremely difficult, if not impossible.) After lab cleanliness was verified, the lab continued to prepare and analyze an equipment blank on each day of sample preparation/homogenization activity.

- *Calibration blanks* were used during metals analysis to verify that contamination was not being introduced by the analytical system. These calibration blanks were to be analyzed immediately after calibration verification.

- *Method blanks* were used by each Analysis Lab to verify that contamination was not being introduced by the analytical process (i.e., the combination of the sample digestion or extraction procedures and the analytical system).

All equipment blank results submitted by the Sample Prep Lab were evaluated to verify that the equipment blanks were free of contamination below the MLs targeted in this study, and that any contamination reported below the ML but above the MDL was considered when evaluating corresponding field tissue sample results. To evaluate the potential contribution of equipment contamination to corresponding field tissue samples, the mass of contaminant detected in the equipment blank was determined by multiplying the concentration of the contaminant reported in the equipment blank by the volume of rinse water used to generate the blank. The worst-case concentration of the contaminant in each corresponding tissue composite was calculated by

dividing the mass of the contaminant in the equipment blank by the total tissue mass of each composite. This calculation assumes that any equipment-related contamination in the composite sample would be equally distributed through the sample by the compositing process. The effects of equipment blank contamination were then assessed as follows:

- If the analyte was detected in the equipment blank but was not detected in the associated field tissue samples, the sample data were considered to be acceptable.

- If the analyte was detected in the associated field tissue samples at levels far greater (i.e., at least ten times more) than the levels detected in the equipment blank, the effect of the blank was considered to be negligible and the field tissue sample data were considered to be acceptable. Such data were qualified with "B" and "RNAF" to indicate that equipment blank contamination was present but the sample result was not affected by it.

- If the analyte was detected in the field tissue samples at levels close to (i.e., within 5 times) the level detected in the equipment blank, there are no means by which to ascertain if the tissue result reported was due to contamination. In such cases, the result reported by the lab was changed to a non-detect at the ML and coded with "B" and "RNON" qualifiers to indicate the change.

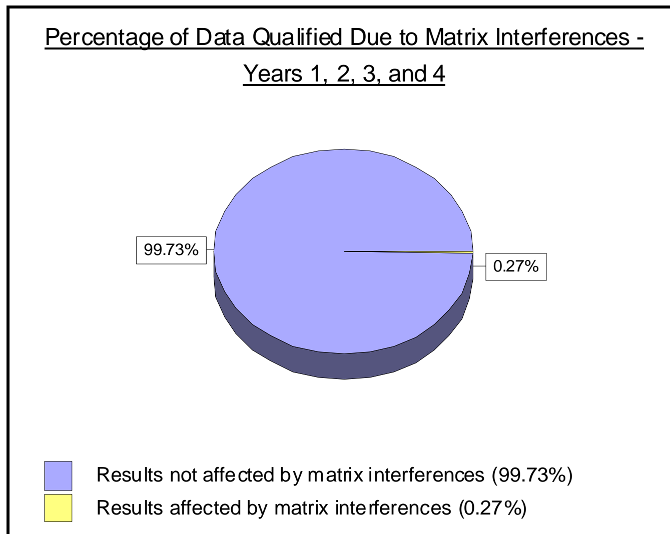
- If the analyte detected in the field tissue sample was more than 5 times but less than 10 times the concentration detected in the equipment blank, the field tissue sample result was coded with "B" and "RMAX" qualifiers to indicate a possible high bias from contamination.

Blanks submitted by each Analysis Lab were reviewed and flagged according to the same approach. In applying these rules, data reviewers were careful to consider the impact of dilution on the field tissue sample results (i.e., the concentration of a diluted sample was compared to the blank result multiplied by the dilution factor that was applied to the sample). For example, if 12 ppb of contaminant was found in the blank, and the associated sample extract was diluted by a factor of 6 relative to the extract from the blank, then the sample result would have to be greater than 12 x 6 x 10, or 720 ppb to be considered acceptable. (The result times the dilution factor times the 10 times rule described above.) If the sample result was reported to be between 360 ppb and 720 ppb, it would be flagged with "B" and "RMAX" qualifiers to indicate a possible high bias resulting from contamination as dictated by the between 5 times and 10 times rule described above.

In all, 9,094 of the 284,973 field tissue sample results generated during Year 1 through Year 4 of the study were qualified due to blank contamination, and more than 6,000 of these qualifiers were applied to PCB congeners (other than the 12 dioxin-like congeners), which are ubiquitous in the environment and very difficult to eliminate at the low levels targeted in this study. In addition, most of the qualifiers were used to indicate that the results were determined to be not affected by the blank contamination (i.e., flagged with "B, RNAF") or were duplicatively applied as a result of PCB congener summation (i.e., if an individual congener received a blank qualifier flag, the qualifier also was applied to the corresponding congener total). It is important to note that no blank subtraction was performed to eliminate the effects of blank contamination detected

in the equipment, calibration, or method blank.[2] Instead, data associated with contaminated blanks were qualified so that data users could make decisions regarding data usability.

4.2.4 Spiked Sample Recoveries



All laboratories were required to spike field tissue samples to estimate the recovery of target analytes from the field tissue samples analyzed in this study. The GC/MS methods used to analyze dioxins/furans, PCB congeners, and semivolatile organics required that isotopically labeled analogs of the target analytes be spiked into each and every sample, including QC samples. This technique, known as isotope dilution, provides an extremely accurate means of quantifying a large number of analytes in the presence of matrix interferences, and each method specifies acceptable recovery windows for the labeled compounds. Because the isotope dilution technique incorporates recovery-correction into calculations of target analyte concentration, any results that fail to meet the method-specified recovery windows are considered to be estimated values. The gas chromatography (GC), atomic absorption (AA), and atomic fluorescence (AF) methods used to analyze pesticides, Aroclors, arsenic species, and mercury require that a matrix spike (MS) and a matrix spike duplicate (MSD) pair be prepared and analyzed with each batch of 20 field tissue samples.

QC samples are not subjected to the matrix spiking requirement. The methods provide precision and accuracy criteria that should be met for each analyte. Precision criteria are expressed as the relative percent difference (RPD) between the MS and MSD results, and accuracy criteria are expressed as acceptable recovery of each analyte. RPD is determined as:

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \times 100$$

where, C_1 is the concentration of the first spiked aliquot of the sample and C_2 is the concentration of the second spiked aliquot of the sample.

Unlike isotope dilution GC/MS techniques, the calculations involved in measuring analytes by GC, AA, and AF techniques do not include a recovery-correction component. Therefore, the direction of the MS recovery failure is used to estimate the directional bias of any associated sample results.

[2] Mercury results were corrected for bubbler blank contamination as per sample calculation procedures described in Method 1631B. As with results from other analyses, however, none of the mercury results were corrected for contamination observed in the equipment, calibration, or method blanks.

All labeled compound and MS/MSD results reported in Year 1, Year 2, Year 3, and Year 4 of the National Lake Fish Tissue Study were carefully reviewed to evaluate the quality of data. In reviewing these results, reviewers verified that appropriate spiking compounds and spiking levels were used, that samples were spiked at the correct frequency, and that spiked sample results met method-specified criteria. In evaluating spiked sample results, data reviewers applied the following rules:

- If the isotopically labeled analog of a target compound was recovered in a sample above method-specified criteria, the associated native compound was coded with "HLBL" to indicate the presence of a high labeled compound recovery. The result also was qualified with an "Estimated Value" descriptor to indicate that, although there was no question as to the identify of the analyte, there is some doubt as to the reliability of the measured concentration. In Year 1 through Year 4, only two results received this qualifier.

- If the isotopically labeled analog of a target compound was recovered below the method-specified criteria, the associated native compound in that sample was coded with "LLBL" to indicate the presence of a low labeled compound recovery. The result also was qualified with an "Estimated Value" descriptor. In Year 1 through Year 4, eighteen (18) results received this qualifier.

- In rare cases, a labeled analog is not recovered from the sample at all. Due to the extreme nature of this QC failure, any associated native (i.e., target) compound result is considered unreliable and should, therefore, be excluded from the database. In Year 1 through Year 4, this situation occurred only for semivolatile organic and PCB congener analytes in 39 samples (a total of 49 results). The 49 affected Year 1, Year 2, Year 3, and Year 4 results were excluded from the database, and the associated sample records were coded as "NLBL" and further qualified with "Exclude" to explain the absence of these results.

- If an analyte was recovered from an MS or MSD sample above method-specified criteria, all associated samples with positive results were coded with "HMSR" to indicate the high MS recovery. The associated samples also were qualified to indicate a "Potential High Bias" unless other flags (such as a "J") applied to the same result suggested that the sample should be qualified as an "Estimated Value." In Year 1, Year 2, Year 3, and Year 4, only 25 results were qualified with the "HMSR" flag (the analytes affected were arsenic (III); δ-BHC; cis-nonachlor; 4,4'-DDT; and dicofol). Ten of these results were qualified as having "Potential High Bias;" the remaining 15 results were reported below the quantitation limit or associated with "RPDX" and/or "RNF2" flags and were qualified as "Estimated Values." Non-detects in samples associated with the high MS/MSD recovery were not flagged because it was clear that the potential high bias indicated by the MS or MSD had no adverse impact on the sample result.

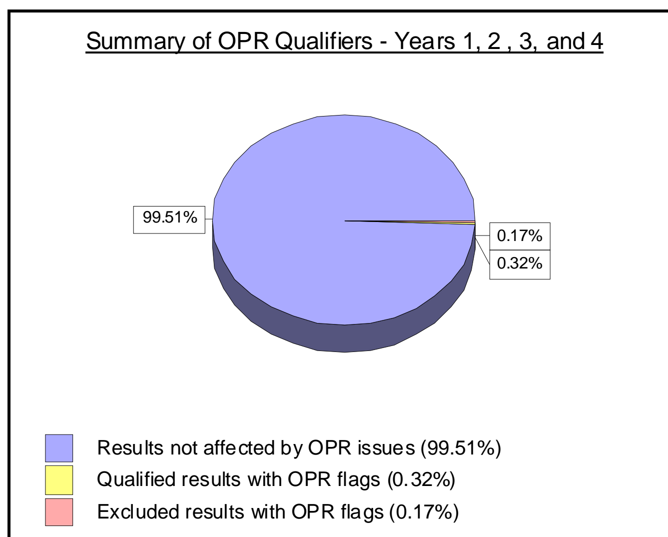
- If an analyte was recovered from an MS or MSD sample below method-specified criteria, all associated samples were coded with "LMSR" to indicate the low MS recovery. The associated samples also were qualified to indicate a "Potential Low Bias." All of the "LMSR" codes applied during Year 1, Year 2, Year 3, and Year 4 of the study were applied to those analytes determined by Methods 1632A and 1656A, with the majority applied to MMA (during Year 1 of the study). The number of MMA qualifiers in Year 1 of the study suggested that method improvements were needed to yield optimal results when determining

this form of arsenic and certain pesticides in tissue matrices. Improvements were implemented during the analysis of Year 2, Year 3, and Year 4 samples, and zero (0) MS failures were reported for MMA during Year 2, Year 3, and Year 4 of the study.

- Disulfoton was not recovered at all in the first MS/MSD pair analyzed in Year 1 of the study and in another pair analyzed in Year 4; also, for the Year 1 samples, there were low recoveries of the same compound in the ongoing precision and recovery sample (OPR, see section 4.2.5) analyzed with the batch. Disulfoton was not reported in any of the samples nor could it be seen in a careful review of the sample chromatograms. Because it is impossible to determine if the non-detects reported in the samples are valid, results for all 28 samples were excluded from the database. These samples were flagged with "LOPR, NMSR" (Year 1 samples) to indicate that there was no MS recovery and low OPR recovery and "NMSR" (Year 4 samples) to indicate that there was no MS recovery, respectively; all samples were further qualified with "Exclude" to explain the absence of results. Data users should note, however, that this compound was not detected in any of the samples analyzed in Year 1, Year 2, Year 3, or Year 4, even when laboratory and method performance was in control.

4.2.5 Ongoing Precision and Recovery

All laboratories that participated in the National Lake Fish Tissue Study were required to prepare and analyze an ongoing precision and recovery, or OPR, standard with each sample set and to meet OPR acceptance criteria specified in the 1600-series method used to analyze the samples. The OPR standards are identical to those used in the IPR demonstrations discussed in Section 4.1.2.



A single OPR standard is analyzed with each sample batch to verify that laboratory performance is in control during the analysis of field tissue samples. Whereas the calibration verification (discussed in Section 4.2.1) allows verification that the instrument remains in control during analysis of each batch, the OPR allows verification that the entire analytical process, including the instrumentation, is in control. Likewise, the OPR differs from the MS/MSD or labeled analog spikes described in section 4.2.4 in that the OPR is performed in a reference matrix to verify that all laboratory systems are in control, whereas the MS/MSD and labeled analogs are spiked into actual samples to verify that the method is working as expected in the actual matrices analyzed.

Each Year 1, Year 2, Year 3, and Year 4 data package generated in the National Lake Fish Tissue Study was reviewed to verify that OPR samples were prepared and analyzed at the required frequency and that recoveries met acceptable performance criteria. All of the methods used in this study list OPR acceptance criteria that are applicable to fish tissue, except for Method 1625C. Therefore, OPR data generated for Method 1625C were assessed against the OPR criteria specified in the method for aqueous samples. In evaluating OPR results, data reviewers applied the following rules:

- If the OPR results were only marginally outside the method criteria (i.e., results were within 10% of the method-specified windows), the data were considered to be acceptable without qualification. This is because all OPR windows established for the 1600-series methods used in this study reflect a 95% confidence interval.

- If the OPR recovery was below method-specified criteria, all tissue data associated with that OPR were qualified with either "LOPR" to indicate that a low OPR recovery of the target analyte, "LLRO" to indicate a low labeled analog recovery from the OPR, or "LNRO" to indicate a low native compound recovery from the OPR. Results with low target analyte recoveries (i.e., "LOPR" results) were further qualified to indicate a "Potential Low Bias." In Year 1, Year 2, Year 3, and Year 4, "LOPR" results were observed for 1.61% of the pesticides measurements, several of which were associated with other data qualifiers (e.g., data also were reported below the ML). One semivolatile organic result was flagged with "LLRO" due to low labeled compound recovery in the OPR. This sample was also flagged with other data qualifiers ("B", "LLBL", and "RNAF"), as well. Results with low native compound recoveries in the OPR (i.e., "LNRO" results) were further qualified to indicate a "Potential Low Bias." In Year 1, Year 2, Year 3, and Year 4, "LNRO" results were observed for 0.12% of the semivolatile organics measurements.

- In rare cases, the native compound was not recovered from the OPR sample or there was no OPR spike recovery. For these cases, the associated results were excluded from the database. The associated sample records were coded as either "NNRO" or "NOPR," respectively, and further qualified with "Exclude" to explain the absence of any results. This situation occurred for 1.14% of the semivolatile organic measurements made by Method 1625C (the vast majority of which were associated with the analyte tetrabromobisphenol A) and 0.03% of the pesticide measurements made by Method 1656A and 1657A.

4.2.6 Holding Time Assessments

Each data submission was reviewed to verify that all samples were received and maintained in frozen condition until analysis. When samples were thawed, the time between thawing and sample digestion, extraction, or analysis was assessed to verify that the holding times specified in each method were met. Samples that failed to meet the required holding times were coded with "HTEX" to indicate the holding time exceedance. If no other QC failures occurred, the data were coded to indicate a "Potential Low Bias." One semivolatile organics result (in Year 1) was analyzed outside the holding time and was reported below the ML. This result was coded with both "HTEX" and "J," and was further qualified as "Estimated Value." Thirteen other results also were coded with an "HTEX" flag, but these results were excluded for other reasons previously cited in this report. Finally, one semivolatile organics result (in Year 4) was analyzed outside the holding time and was associated with a low native recovery in the OPR. This result was coded with both "HTEX" and "LNRO," and was further qualified as "Potential Low Bias."

> 99.99% of the 284,973 sample results reported during Year 1 through Year 4 were determined within analytical holding times.

4.2.7 Method-Specific Considerations

Pesticides

Methods 1656A and 1657A rely on the use of GC techniques coupled with selective detectors to determine organochlorine and organophosphorus pesticides, respectively. The advantage of these techniques is that they allow for measurement of pesticides at lower concentrations than is possible with GC/MS. The disadvantage is that compound identification with the selective detectors is not as reliable as compound identification via GC/MS. To overcome this disadvantage, the methods require that any analytes detected be verified by analysis on a second column and that the results from the second column closely agree with the primary column results (the results measured on the two columns must agree within a factor of two).

When such verification occurs, the laboratory reports the lower of the two results. In reviewing the data package for each pesticide analysis, reviewers evaluated the data to verify that all positive results were detected on two columns and that the results agreed within a factor of two. When the analyte was detected on both columns, but the results did not closely agree, the lower result was reported in the database and coded with "RNF2" to indicate that the results did not agree within a factor of two and further qualified as an "Estimated Value." When the analyte was detected on one column but could not be confirmed on a second column, the result was coded "NCNF" to indicate the lack of confirmation and further qualified as an "Estimated Value." This approach was taken to advise data users about the possibility that an analyte might be present so that informed decisions can be made about the value of gathering additional data.

For all pesticides, except kepone, unconfirmed results were reported as non-detects unless confirmation on the second column was not possible due to co-elution. Because kepone cannot be independently resolved on the second column, unconfirmed kepone results were reported as detects, but qualified as described above. Several possible kepone hits are reported and flagged as "NCNF" in the database. One Year 1 result for *trans*-nonachlor and one 2,4'-DDE result from Year 2 could not be confirmed because the compound co-eluted with another analyte on the second column. The co-eluted results also differed from the first column result by more than a factor of two. These results were flagged with "NCNF, RNF2" and further coded as an "Estimated Value."

In addition, for Year 2, Year 3, and Year 4 of the study, several dicofol results were coded with "NCNF." The Year 2 results were also associated with high and low MS recoveries ("HMSR" and "LMSR", respectively), and one of the sample results was further coded with a "J" flag. These two results were given the overall qualifier of "Estimated Value." Finally, several 2,4'-DDE, alpha-chlordane, *cis*-nonachlor, ethalfluralin, gamma-chlordane, trans-nonachlor, and trifluralin results in the Year 2, Year 3, and Year 4 data sets were coded with "NCNF" (and possibly other flags), which produced an overall qualification of "Estimated Value."

Note: EPA reminds all data users that the identification of any pesticide qualified with "NCNF" or "RNF2" was not confirmed. Appropriate caution should be exercised when using such results.

Also, in Year 1 of the study, seven pesticide results were coded "MTRX" to indicate that the sample chromatograms suggested the presence of possible matrix interferences. In three of these cases, the pesticide was detected on both columns, but the results did not agree within a factor of two, so the data were further coded with an "RNF2" flag. One of these three results also was

reported below the ML on one of the columns, so this result was further coded with a "J" flag. All six of these MTRX flagged results were qualified to indicate that they should be considered "Estimated Values." In Year 4 of the study, one pesticide result for dicofol was qualified with the "MTRX" code and should be considered an "Estimated Value." In Year 3 of the study, 25 PCB congener results were flagged with the "MTRX" code. These results were further coded with "LLBL" or "NLBL" flags (See Section 4.2.4 - Spiked Sample Recoveries), and were qualified as an "Estimated Value" or "Exclude" from the database, respectively. There were no "MTRX" codes used for the results obtained from Year 2 of the study.

Semivolatile Organics

During Year 4 of the study, the laboratory initially reported "hits" for 4-nonylphenol in several of the samples. Since only one sample had a detected result for 4-nonylphenol during the first three years of the study, the semivolatile organics laboratory investigated the situation and identified several possible explanations, including 1) the possibility of contamination, 2) the fact that the interpretation was performed by a different lab staff member than in previous years, and 3) the less likely possibility that the hits were actually present in the samples.

Based on re-evaluation of Year 4 data and comparison to results from previous years, it was concluded that, for all but 7 samples, the compound detected did not meet appropriate identification criteria and should not be reported as 4-nonylphenol. It is not possible to tell with certainty whether or not 4-nonylphenol was actually present in any of the samples. The laboratory reported that the remaining "hits" for 4-nonylphenol were probably due to interference and/or contamination. The laboratory performed an independent study in an attempt to isolate the source of the contamination, evaluating the following:

- the procedures used to clean and prepare the Soxhlets for extraction
- the soap used to clean the extraction glassware
- the Florisil/alumina clean-up columns used during the extraction process

The results of all of their tests proved to be inconclusive. The sample results in which 4-nonylphenol was detected were reported as "hits" based on best professional judgement. However, these samples with concentrations of 4-nonylphenol detected above the MDL were qualified with a "SLIC" code to signify the result was probably due to interference and/or contamination.

SECTION 4.3 OVERALL DATA QUALITY ASSESSMENT

Upon completion of all data review and database development activities for Year 1, Year 2, Year 3, and Year 4, the full data set was evaluated to determine if the results overall were falling within the MQOs established in the study QAPP [3]. Assessment of the data against these MQOs is described in Sections 4.3.1 through 4.3.5 below.

[3] *Quality Assurance Project Plan for Analytical Control and Assessment Activities*, September 2000, Prepared for the U.S. EPA OW by CSC Environmental Programs Group (formerly DynCorp Environmental).

4.3.1 Precision

Precision is the degree of agreement among replicate measurements of the same property, under prescribed similar conditions. It can be expressed either as a range, a standard deviation, or a percentage of the mean of the measurements.

Ideally, precision is measured by subdividing samples in the field, preserving and numbering each split separately, and sending the aliquots to the analysis laboratory as "blind" duplicates. In this study, however, samples have to be homogenized, composited, and subdivided in a strictly controlled, clean laboratory environment. Therefore, the Sample Preparation Laboratory was required to prepare "duplicate composite pairs" on 5% of the samples analyzed. These duplicate composite pairs were sent to each analysis laboratory as "blind duplicates" (e.g., labeled with separate EPA sample numbers) and used to assess variability arising from the sample homogenization, compositing, aliquoting, shipping, and laboratory analysis processes.

Because agreement between results was expected to be better at higher concentrations, two MQOs were established. For sample results that were close to (i.e., less than 5 times) the ML, the MQO was that 90% of results from the original sample and the blind composite duplicates should agree within ±100%. For sample results that were well above (i.e., more than 5 times higher than) the ML, the MQO was that at least 90% of the results from the original and blind composite duplicate samples agree within ±50%.

A total of 54 blind composite duplicates were prepared and analyzed for all of the target analytes during Year 1, Year 2, Year 3, and Year 4 of the study. These analyses yielded approximately 15,350 pairs of results; of these, more than 7,050 pairs of results were detected in both the field tissue sample and its blind composite duplicate. (Agreement between non-detects could not be quantitatively analyzed and therefore is not included in this discussion.)

> The study MQO for precision was exceeded during Year 1, Year 2, Year 3, and Year 4 of the study. Over 99% (99.8%) of the duplicate composite pairs agreed within ±50% when the measured results were more than 5 times the ML, and 99.7% of the duplicate composite pairs agreed within ±100% when measured results were less than 5 times the ML.

Approximately 4,240 of these paired results were detected at concentrations that were at least 5 times greater than the ML, and all but eight (99.8%) of these results met the 50% MQO established for agreement between analytes detected at this level. The remaining pairs (approximately 2,810 paired results) were detected within 5 times the ML and subjected to the ±100% objective described above. All but five of these paired results (99.8% of the results), three in Year 1, one in Year 2, and three in Year 4 of the study, met the MQO. All of these paired results met the MQO for Year 3 of the study.

4.3.2 Bias

Bias is the systematic distortion of a measurement process that causes errors in one direction.[4] In this study, bias from the analytical process was measured by preparing and analyzing field tissue samples spiked with 1) the analytes of interest (i.e., MS samples), 2) isotopically labeled analogs of the target analytes, or 3) surrogate compounds that are expected to behave in a manner similar to the target analytes. Assessment of these spiked sample results was described in Section 4.2.4. The MQO for overall analytical accuracy in this study was for 80% of these spiked field tissue sample results to fall within the acceptance criteria specified for each method.

This goal was easily exceeded in all four years of the study, suggesting that overall, the methods selected for use in the study were appropriate for the analytes and matrices targeted.

> The study MQO for bias was exceeded during Year 1, Year 2, Year 3, and Year 4 of the study; 99.7% of the spiked sample results fell within method-specified acceptance criteria.

Although the MQO was established for the entire data set, it is useful from a QA perspective to evaluate bias across individual methods to verify that each method is working as planned. On this basis, it is clear that the individual methods are working as intended. No MS failures were observed for mercury; minimal spike (MS and surrogate samples) failures (0.19%) were observed for organophosphorus pesticides, and 98.91% of the spiked (MS and surrogate samples) organochlorine pesticide results met method-specified criteria.

In Year 1 of the study, MS samples performed for arsenic species also showed that, although the method works well in tissue for total inorganic arsenic and most of the other species measured, additional improvements were needed to yield optimal results for MMA. Such improvements were implemented during the Year 2 analysis, and no (0) MS failures were reported for MMA during Year 2, Year 3, or Year 4 of the study. Finally, all of the methods that involve spiking isotopically labeled compounds into each sample yielded a success rate of 99.6%, with only 0.02%, 0.02%, and 0.15% of the labeled analog recoveries failing to meet method specified recoveries for PCBs, dioxins/furans, and semivolatile organics, respectively.

4.3.3 Accuracy

Accuracy is a measure of the closeness of an individual measurement or the average of a number of measurements to the true value. Accuracy includes a combination of random error (precision) and systematic error (bias) components that result from sampling and analytical operations. Accuracy is determined by analyzing a reference material of known analyte concentrations or by reanalyzing a sample spiked with a known amount of analyte. Study objectives dictated that certified reference materials (CRMs) be sent to each laboratory to assess bias when available, feasible, and warranted. No certified reference materials were sent during Year 1, Year 2, Year 3, or Year 4 of the study.

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[4] *EPA Guidance for Quality Assurance Project Plans, EPA QA/G-5*, EPA Office of Research and Development, Washington, DC, 20460. EPA/600/R-98/018.

4.3.4 Sensitivity

As described in Section 4.1.1, all laboratories demonstrated their ability to achieve the study MQOs established for sensitivity, and 100% of the results generated during Year 1, Year 2, Year 3, and Year 4 of the study were generated on instruments that were calibrated to encompass MLs. In addition, all results reported below the ML have been qualified in the database to alert data users that, although the presence of the analyte was confirmed, the concentration reported in the database is an estimate because it falls below the quantitation limit.

4.3.5 Completeness

Completeness is defined in terms of the percentage of data that are collected and deemed to be acceptable for use in the study. Three measures of completeness can be defined, as follows:

Sampling Completeness: The number of valid samples collected relative to the number of samples planned for collection;

Analytical Completeness: The number of valid sample measurements relative to the number of valid samples collected; and

Overall Completeness: The number of valid sample measurements relative to the number of samples planned for collection.

The analytical completeness goal in this study was that EPA obtain valid measurements from 90% of the valid samples collected. This goal was exceeded during Year 1, Year 2, Year 3, and Year 4 of the study. Only two of the 1003 valid samples collected during the four years of the study could not be completely analyzed because they did not provide sufficient tissue. This resulted in EPA obtaining valid measurements from 99.8% of the valid samples collected in Year 1 through Year 4 of the study.

EPA obtained valid measurements from 99.8% of the samples collected in Year 1, Year 2, Year 3, and Year 4 of the study, thereby exceeding the MQO of 90% for analytical completeness during the study.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
B	Blank Contamination	The target analyte was detected in one or more of the blanks associated with this sample.	Applied to fewer than 0.05% of Year 1, Year 2, Year 3, and Year 4 results and only occurs for PCB congener totals in which some of the individual totals were associated with B flags.
B, J	Blank Contamination, Estimated Value	One or more of the PCB congeners contributing to the total was associated with a contaminated blank *and* one or more of the congeners was reported above the MDL and below the ML. In most cases, the impact of the flag on the total is negligible due to the relatively minor impact of the individual congener(s) the flags represent.	Applied to 0.78% of Year 1, Year 2, Year 3, and Year 4 results, but only for PCB congener totals. This combination only occurs for PCB congener totals in which B-flagged and J-flagged results from individual congeners are mathematically summed with unflagged results.
B, J, RMAX	Blank Contamination, Estimated Value, Result is a Maximum Value	The target analyte was detected in one or more of the blanks associated with this sample; the result was reported above the MDL but below the ML and is, therefore, an estimated value; *and* blank contamination was observed and the target analyte was reported in the sample at a concentration between 5 and 10 times higher than the blank value. The result was considered to be of acceptable quality, but data users are cautioned that it may be a maximum value due to possible influence of contamination.	Applied to 0.43% of the dioxin results in Year 1 through Year 4 of the study. This qualification occurs only because the sample result was exactly five times or ten times the blank contamination and the dioxin MDLs were more than ten times lower than the MLs at which the instruments were calibrated.
B, J, RNAF	Blank Contamination, Estimated Value, Result Not Affected	The target analyte was detected in one or more of the blanks associated with this sample; the result was reported above the MDL but below the ML and is, therefore, an estimated value; *and* blank contamination was present but was not considered to adversely impact the sample result. The presence of the analyte in the blank is not considered to adversely affect the data in cases where the sample results are more than 10 times the associated blank results or where the analyte is not detected in associated samples.	Applied to one 1,2,3,4,6,7,8-HpCDD result and three 2,3,7,8-TCDD results in the Year 1 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
B, LLBL, RNAF	Blank Contamination, Estimated Value, Result Not Affected	Blank contamination was present but was not considered to adversely impact the sample result. The presence of the analyte in the blank is not considered to adversely affect the data in cases where the sample results are more than 10 times the associated blank results or where the analyte is not detected in associated samples. *However,* the labeled analog of the target analyte was recovered from the field tissue sample below method-specified criteria, resulting in an estimated value flag.	Applied to two OCDD results in the Year 2 data set.
B, REXC, RNAF	Blank Contamination, Estimated Value, Result Not Affected	The target analyte was detected in one or more of the blanks associated with this sample; the field tissue sample result exceeded the instrument calibration range; *and* the blank contamination that was present was not considered to adversely impact the sample result. The presence of the analyte in the blank is not considered to adversely affect the data in cases where the sample results are more than 10 times the associated blank results or where the analyte is not detected in associated samples.	Applied to two bis(2-ethylhexyl)phthalate results in the Year 2 data set.
B, RMAX	Blank Contamination, Result is a Maximum Value	Blank contamination was observed and the target analyte was reported in the sample at a concentration between 5 and 10 times higher than the blank value. The result was considered to be of acceptable quality, but data users are cautioned that it may be a maximum value due to possible influence of contamination.	Applied to 0.15% of Year 1, Year 2, Year 3, and Year 4 results, including PCB congener, dioxin/furan, pesticide, and target semivolatile organic results.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
B, RMAX, RNF2	Blank Contamination, Result is a Maximum Value, Estimated Value	Blank contamination was observed and the target analyte was reported in the sample at a concentration between 5 and 10 times higher than the blank value; the result was considered to be of acceptable quality, but data users are cautioned that it may be a maximum value due to possible influence of contamination; *and* although the analyte was found in the field tissue sample using two different columns, the results reported on the two columns differed by a factor of more than two.	Occurred with one beta-BHC result in the Year 4 data set.
B, RNAF	Blank Contamination, Result Not Affected	Blank contamination was present but was not considered to adversely impact the sample result. The presence of the analyte in the blank is not considered to adversely affect the data in cases where the sample results are more than 10 times the associated blank results or where the analyte is not detected in associated samples.	Applied to 1.19% of the Year 1, Year 2, Year 3, and Year 4 results.
B, RNON	Blank Contamination, Result Reported as a Non-detect	When the sample result is less than five times the blank result, there are no means by which to ascertain whether or not the presence of the analyte may be attributed to contamination. Therefore, SCC recommends that the data be reported in the database as a non-detect at the ML, adjusted for dilution.	Applied to 0.98% of Year 1, Year 2, Year 3, and Year 4 results, with most of the occurrences for PCB congeners other than the 12 dioxin-like congeners.
HLBL	Estimated Value	The labeled analog of the target compound was recovered above method-specified criteria, suggesting a possible matrix interference (High Labeled Compound Recovery).	Applied to two results in the Year 1 PCB data set.
HMSR	Potential High Bias	High analyte recovery was observed in one or more MS samples associated with this result.	Applied to two Arsenic (III) results and two δ -BHC results in the Year 1 data set; and applied to six *cis*-nonachlor results in the Year 2 data set.
HMSR, J	Estimated Value	A high recovery of this result was observed in an associated MS sample suggesting the J-flagged result may have a high bias *and* the sample result reported was above the MDL but below the ML.	Applied to three Arsenic (III) results and two δ -BHC results in the Year 1 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
HMSR, J, NCNF	Estimated Value	High analyte recovery was observed in one or more MS samples associated with this result; the sample result reported was above the MDL (detection limit) but below the ML (quantitation limit); *and* the presence of the reported analyte was not confirmed on a second column.	Applied to one dicofol result in the Year 2 data set.
HMSR, J, RNF2	Estimated Value	High analyte recovery was observed in one or more MS samples associated with this result; the sample result reported was above the MDL (detection limit) but below the ML (quantitation limit); *and* although the analyte was found in the field tissue sample using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to one *cis*-nonachlor result in the Year 2 data set.
HMSR, RNF2	Estimated Value	High analyte recovery was observed in one or more MS samples associated with this result *and* although the analyte was found in the field tissue sample using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to three *cis*-nonachlor results in the Year 2 data set.
HMSR, RNF2, RPDX	Estimated Value	High analyte recovery was observed in one or more MS samples associated with this result *and* although the analyte was found in the field tissue sample using two different columns, the results reported on the two columns differed by a factor of more than two. Also, the RPD between the MS and MSD exceeded criteria.	Applied to one 4,4'-DDT result in the Year 2 data set.
HMSR, RPDX	Estimated Value	High analyte recovery was observed in one or more MS samples associated with this result *and* the RPD between the MS and MSD exceeded criteria.	Applied to four 4,4'-DDT results in the Year 2 data set.
HSSR	Potential High Bias	A high surrogate spike recovery was observed, suggesting a possible high bias in the result reported.	Occurred for one 4,4'-DDE result in the Year 1 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
HSSR, J	Estimated Value	A high recovery of the surrogate spike compound was associated with the reported result *and* the sample result was reported above the MDL but below the ML on at least one of the two columns on which the pesticide was detected.	Applied to one α-chlordane result in the Year 1 data set.
HTEX, J	Estimated Value	The sample result reported was above the MDL (detection limit) but below the ML (quantitation limit) *and* the holding time was exceeded when analyzing the sample.	Associated with one bis(2-ethylhexyl) phthalate result in the Year 1data set.
HTEX, LNRO	Potential Low Bias	The holding time was exceeded when analyzing the sample *and* the native compound of the target analyte was recovered below method criteria in the OPR associated with the sample.	Occurred for one naphthalene result in the Year 4 data set.
HTEX, NLBL	Exclude	The holding time was exceeded when analyzing the sample *and* the labeled analog was not recovered from the sample, suggesting severe matrix interferences.	Occurred for one pentachlorophenol result in the Year 4 data set.
HTEX, NNRO	Exclude	The holding time was exceeded when analyzing the sample; *and* the target analyte (native compound) was not recovered in the OPR sample associated with this result.	Applied to seven tetrabrormobisphenol A results in the Year 4 data set.
HVER, J, RNF2	Estimated Value	The sample result was determined on both pesticide columns, but the lower of the two results reported was below the quantitation limit, *and* the results reported were not within a factor of two of each other (possibly due to the low level reported). In addition, the lower of the two values was associated with a high calibration verification standard recovery.	Occurred for one 4,4'-DDT result in the Year 1 data set.
HVER, RNF2	Estimated Value	The sample result was determined on both pesticide columns, but the results reported were not within a factor of two of each other *and* the lower of the two values was associated with a high calibration verification standard recovery.	Occurred for two methoxychlor results in the Year 1 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
J	Estimated Value	The sample result reported was above the MDL (detection limit) but below the ML (quantitation limit).	Occurred throughout the Year 1, Year 2, Year 3, and Year 4 data sets.
J, LLBL	Estimated Value	The target analyte was reported above the MDL but below the ML; *and* the labeled analog of the target was recovered below method-specified criteria suggesting the possible presence of matrix interferences.	Applied to one HxCDD result and one PCB congener (PCB-3) result in the Year 1 and Year 3 data sets, respectively.
J, LLBL, MTRX	Estimated Value	The target analyte was reported above the MDL but below the ML; the labeled analog of the target was recovered below method-specified criteria, suggesting the possible presence of matrix interferences; *and* the chromatogram suggested possible matrix interferences with the sample.	Applied to three PCB congener (two PCB-3 and one PCB-8) results in the Year 3 data set.
J, LMSR	Estimated Value	The target analyte was reported above the MDL but below the ML in the sample *and* low analyte recovery was observed with one or more MS samples associated with this result.	Applied to one oxychlordane result in the Year 2 data set and four trifluralin results in the Year 4 data set.
J, LMSR, LOPR	Estimated Value, Potential Low Bias	The target analyte was reported above the MDL but below the ML in the sample; low analyte recovery was observed with one or more MS samples associated with this result; *and* the target analyte was recovered below method-specified criteria in the OPR associated with the sample.	Occurred for one ethalfluralin result in the Year 4 data set.
J, LMSR, RNF2	Estimated Value	The target analyte was reported above the MDL but below the ML in the sample; low analyte recovery was observed with one or more MS samples associated with this result; *and* the sample result was determined on both pesticide columns, but the results reported were not within a factor of two of each other.	Applied to one oxychlordane and one 2,4'-DDT result in the Year 2 data set. Also, applied to six trifluralin results in the Year 4 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
J, LMSR, RNF2, RPDX	Estimated Value	The target analyte was reported above the MDL but below the ML in the sample; low analyte recovery was observed with one or more MS samples associated with this result; the sample result was determined on both pesticide columns, but the results reported were not within a factor of two of each other; *and* the RPD between the MS and MSD exceeded criteria.	Occurred for one methoxychlor result in the Year 3 data set and one trifluralin result in the Year 4 data set.
J, LMSR, RPDX	Estimated Value	The target analyte was reported above the MDL but below the ML in the sample; low analyte recovery was observed with one or more MS samples associated with this result; *and* the RPD between the MS and MSD exceeded criteria.	Applied to two 4,4'-DDT results in the Year 3 data set.
J, LOPR	Estimated Value, Potential Low Bias	The target analyte was reported above the MDL but below the ML *and* the target analyte was recovered below method-specified criteria in the OPR associated with the sample.	Applied to two 4,4'-DDT results in the Year 1 data set, one methoxychlor result in the Year 2 data set, and four ethalfluralin results in the Year 1, Year 3, and Year 4 data sets.
J, LOPR, NCNF	Estimated Value	The target analyte was reported above the MDL but below the ML; the target analyte was recovered below method-specified criteria in the OPR associated with the sample; *and* the sample result was not confirmed on a second column.	Applied to five kepone results in the Year 1 data set and one ethalfluralin results in the Year 3 data set.
J, LOPR, NCNF, RNF2	Estimated Value	The target analyte was reported above the MDL but below the ML; the target analyte was recovered below method-specified criteria in the OPR associated with the sample; the sample result was not confirmed on a second column; *and* although this analyte was found in the field tissue sample using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to one ethalfluralin result in the Year 3 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
J, LOPR, NCNF, RNF2, RPDX	Estimated Value	The target analyte was reported above the MDL but below the ML; the target analyte was recovered below method-specified criteria in the OPR associated with the sample; the sample result was not confirmed on a second column; although this analyte was found in the field tissue sample using two different columns, the results reported on the two columns differed by a factor of more than two; *and* the RPD between the MS and MSD exceeded criteria.	Applied to four ethalfluralin results in the Year 3 data set.
J, LOPR, NCNF, RPDX	Estimated Value	The target analyte was reported above the MDL but below the ML; the target analyte was recovered below method-specified criteria in the OPR associated with the sample; the sample result was not confirmed on a second column; *and* the RPD between the MS and MSD exceeded criteria.	Occurred for one ethalfluralin result in the Year 3 data set.
J, LOPR, RNF2	Estimated Value, Potential Low Bias	The target analyte was reported above the MDL but below the ML; the target analyte was recovered below method-specified criteria in the OPR associated with the sample; *and* although this analyte was found in the field tissue sample using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to two 4,4'-DDT results in the Year 1 data set and twenty-two ethalfluralin results in the Year 3 and Year 4 data sets.
J, LVER	Estimated Value, Potential Low Bias	The target analyte was reported above the MDL but below the ML *and* low analyte recovery was observed in a calibration verification associated with this sample, suggesting the possibility of a low bias in the result.	Applied to three MMA results in the Year 2 data set.
J, MTRX, RNF2	Estimated Value	The target analyte was reported above the MDL but below the ML; the chromatogram suggested possible matrix interferences with this sample; *and* although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to one *cis*-permethrin result in the Year 1 data set.
J, NCNF	Estimated Value	The target analyte was reported above the MDL but below the ML *and* the result was not confirmed on a second column.	Applied to a few pesticide results (0.23%) in Year 1, Year 2, Year 3, and Year 4.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
J, NCNF, RNF2	Estimated Value	The target analyte was reported above the MDL but below the ML; the result was not confirmed on a second column; *and* although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to one cis-nonachlor, six 2,4'-DDE results, and six trifluralin results in the Year 3 data set.
J, NCNF, RNF2, RPDX	Estimated Value	The target analyte was reported above the MDL but below the ML; the result was not confirmed on a second column; although the analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two; *and* the RPD between the MS and MSD exceeded criteria.	Applied to four trifluralin results in the Year 3 data set.
J, RNF2	Estimated Value	The target analyte was reported above the MDL but below the ML *and* although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to a few pesticides results (0.79%) in Year 1, Year 2, Year 3, and Year 4.
J, RNF2, RPDX	Estimated Value	The target analyte was reported above the MDL but below the ML in the sample; the sample results reported on the two columns differed by a factor of more than two; *and* the RPD between the MS and matrix spike duplicate exceeded criteria.	Applied to four 2,4'-DDE results, one heptachlor result, one pendimethalin result, eight ethalfluralin, and seven trifluralin results in the Year 2, Year 3, and Year 4 data sets.
J, RPDX	Estimated Value	The target analyte was reported above the MDL but below the ML in the sample *and* the RPD between the associated MS and MSD exceeded criteria.	Applied to two 2,4'-DDE results, one 4,4'-DDT result, two endosulfan sulfate results, three pendimethalin results, and two trifluralin results in the Year 2, Year 3, and Year 4 data sets.
LLBL	Estimated Value	The labeled analog of the target analyte was recovered below method-specified criteria.	Applied to two PCB congener results (PCB-4 and PCB-19) in the Year 3 data set.
LLBL, MTRX	Estimated Value	The labeled analog of the target analyte was recovered below method-specified criteria *and* the chromatogram suggested possible matrix interferences with the sample.	Applied to six PCB congener results in the Year 3 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
LMSR	Potential Low Bias	Low analyte recovery was observed with one or more MS samples associated with this result.	Applied primarily to MMA results and a small number of total inorganic arsenic results in the Year 1 data set; also applied to a few pesticide results (0.43%) in the Year 2, Year 3, and Year 4 data sets.
LMSR, LOPR	Potential Low Bias	Low analyte recovery was observed with one or more MS samples *and* with the OPR sample associated with this result.	Applied to a few pesticide results (0.28%) in the Year 1, Year 2, Year 3, and Year 4 data sets.
LMSR, LOPR, RNF2	Estimated Value	Low analyte recovery was observed with one or more MS samples and with the OPR sample associated with this result; also, the sample results reported on the two columns differed by a factor of more than two.	Occurred for two ethalfluralin results in the Year 4 data set.
LMSR, NCNF	Estimated Value	Low analyte recovery was observed with one or more MS samples *and* the result was not confirmed on a second column.	Applied to one dicofol result in the Year 2 data set and two kepone results in the Year 3 data set.
LMSR, RNF2	Estimated Value	Low analyte recovery was observed with one or more MS samples *and* the sample results reported on the two columns differed by a factor of more than two.	Occurred for five trifluralin results in the Year 4 data set.
LNRO	Potential Low Bias	The native compound of the target analyte was recovered below method-specified criteria in the OPR sample.	Applied to five indeno(1,2,3-cd)pyrene and twenty-one naphthalene results in the Year 4 data set.
LNRO, LVER	Potential Low Bias	The native compound of the target analyte was recovered below method-specified criteria in the OPR sample *and* low analyte recovery was observed in a calibration verification associated with this sample, suggesting the possibility of a low bias in the result.	Applied to nineteen phenol results in the Year 4 data set.
LOPR	Potential Low Bias	Low analyte recovery was observed with the OPR sample associated with this result.	Applied to several organochlorine and organophosphorus pesticide results (1.1%) in the Year 1, Year 2, Year 3, and Year 4 data sets.
LOPR, NCNF	Estimated Value	Low analyte recovery was observed with the OPR sample associated with this result *and* the presence of the reported analyte was not confirmed on a second column.	Applied to four kepone results (kepone cannot be confirmed on the method-specified second column) in the Year 1 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
LOPR, NCNF, RNF2	Estimated Value	Low analyte recovery was observed with the OPR sample associated with this result; the presence of the reported analyte was not confirmed on a second column; *and* the sample results reported on the two columns differed by a factor of more than two.	Applied to six ethalfluralin results in the Year 3 data set.
LOPR, NCNF, RNF2, RPDX	Estimated Value	Low analyte recovery was observed with the OPR sample associated with this result; the presence of the reported analyte was not confirmed on a second column; the sample results reported on the two columns differed by a factor of more than two; *and* the RPD between the MS and matrix spike duplicate exceeded criteria.	Applied to five ethalfluralin results in the Year 3 data set.
LOPR, NCNF, RPDX	Estimated Value	Low analyte recovery was observed with the OPR sample associated with this result; the presence of the reported analyte was not confirmed on a second column; *and* the RPD between the MS and matrix spike duplicate exceeded criteria.	Applied to one ethalfluralin result in the Year 3 data set.
LOPR, NMSR	Exclude	Low analyte recovery was observed with the OPR sample associated with this result *and* the analyte was not recovered at all in the MS sample associated with the result. The analyte was not detected in the sample, but the recovery problems observed in the OPR and the MS make it impossible to confirm the reliability of these non-detects.	Applied to eighteen disulfoton results in the Year 1 data set.
LOPR, RNF2	Estimated Value	The analyte was detected on both columns, but the results were not within a factor of two of each other *and* the lower of the results reported was associated with a low OPR standard recovery.	Applied to one ethalfluralin result in the Year 1 data set; eight methoxychlor and one endosulfan sulfate result in the Year 2 data set; fifteen ethalfluralin results in the Year 3 data set; and one ethalfluralin results in the Year 4 data set.
LOPR, RNF2, RPDX	Estimated Value	The analyte was detected on both columns, but the results were not within a factor of two of each other; the lower of the results reported was associated with a low OPR standard recovery; *and* the RPD between the associated MS and MSD exceeded criteria.	Applied to five 4,4'-DDT results in the Year 2 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
LOPR, RPDX	Estimated Value	The lower of the results reported was associated with a low OPR standard recovery *and* the RPD between the associated MS and MSD exceeded criteria.	Applied to six 4,4'-DDT results in the Year 2 data set.
LVER	Potential Low Bias	Low analyte recovery was observed in a calibration verification associated with this sample, suggesting the possibility of a low bias in the result.	Applied to two 4,4'-DDT results in the Year 1 data set and several (5.2%) arsenic species results in the Year 2 data set.
LVER, RNF2	Estimated Value	The analyte was detected on both columns, but the results on the two columns differed by more than a factor of two, *and* the lower of the two results reported was associated with a low recovery of calibration verification standard.	Applied to one 4,4'-DDT result in the Year 1 data set.
MTRX	Estimated Value	The chromatogram suggested possible matrix interferences with the sample.	Applied to one methoxychlor and two *trans*-permethrin results in the Year 1 data set; also applied to one dicofol result in the Year 4 data set.
MTRX, NLBL	Exclude	The chromatogram suggested possible matrix interferences with the sample; *and* the labeled analog was not recovered from the sample, suggesting severe matrix interferences. Because it is impossible to determine if the analyte is present or not present, the reported target analyte result was excluded from the database.	Applied to sixteen PCB congener results in the Year 3 data set.
MTRX, RNF2	Estimated Value	The chromatogram suggested possible matrix interferences *and* although this analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to one methoxychlor and one *cis*-permethrin result in the Year 1 data set.
NCNF	Estimated Value	The result was not confirmed (either no elution or co-elution on a second column).	Applied to several analytes in the Year 1, Year 2, Year 3, and Year 4 data sets. Occurred for 0.17% of all the pesticide results in the study. (Please note that kepone cannot be confirmed on the method-specified second column.)

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
NCNF, RNF2	Estimated Value	The result was found on one column but co-eluted with another compound on the second column *and* the co-eluted results differed from the other column results by a factor greater than two.	Applied to one *trans*-nonachlor result in the Year 1 data set; three 2,4'-DDE results in the Year 2 and Year 3 data sets; and one trifluralin result and one alpha-chlordane result in the Year 3 data set.
NCNF, RNF2, RPDX	Estimated Value	The result was found on one column but co-eluted with another compound on the second column; the co-eluted results differed from the other column results by a factor greater than two; *and* the RPD between the associated MS and MSD exceeded criteria.	Applied to five trifluralin results in the Year 3 data set.
NCNF, RPDX	Estimated Value	The result was found on one column but co-eluted with another compound on the second column *and* the RPD between the associated MS and MSD exceeded criteria.	Applied to one trifluralin result in the Year 3 data set.
NLBL	Exclude	The labeled analog was not recovered from the sample, suggesting severe matrix interferences. Because it is impossible to determine if the analyte is present or not present, the reported target analyte result was excluded from the database.	Applied to a few (0.08%) semivolatile organic results in the Year 1, Year 2, Year 3, and Year 4 data sets.
NLRO, NNRO	Exclude	Neither the target analyte (native compound) nor the labeled analog were recovered in the OPR sample associated with this result.	Applied to three phenol results in the Year 1 data set.
NMSR	Exclude	No analyte recovery was observed with one or more MS samples.	Occurred with ten disulfoton results in the Year 4 data set.
NNRO	Exclude	The native compound was not recovered in the OPR sample suggesting that the method may not be working for this analyte.	Applied to 1.06% of the semivolatile organic results in Year 1, Year 2, Year 3, and Year 4 data sets.
NNRO, NVER	Exclude	The native compound was not recovered in the OPR sample suggesting that the method may not be working for this analyte *and* there was no recovery of the analyte in the calibration verification sample associated with this result.	Applied to nineteen tetrabromobisphenol A results in the Year 4 data set.
NOPR	Exclude	There was no recovery of the analyte in the OPR sample associated with this result.	Applied to eighteen endosulfan sulfate results in the Year 2 data set.

Exhibit 4-1A (*Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
RNF2	Estimated Value	Although the analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two.	Applied to a few (0.68%) organochlorine pesticide results in the Year 1, Year 2, Year 3, and Year 4 data sets.
RNF2, RPDX	Estimated Value	Although the analyte was found using two different columns, the results reported on the two columns differed by a factor of more than two *and* the RPD between the MS and MSD exceeded criteria.	Applied to one 2,4'-DDE result in the Year 2 data set; three 2,4'-DDE results, one 4,4'-DDT result, two heptachlor, and two trifluralin results in the Year 3 data set; and one ethalfluralin result in the Year 4 data set.
RPDX	Estimated Value	The RPD between the MS and MSD exceeded criteria.	Applied to seven 2,4'-DDE results in the Year 2 data set and one 2,4'-DDE result, one 4,4'-DDT result, and two trifluralin results in the Year 3 data set.

Exhibit 4-1B (*Additional or Non-Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
B, HTEX, RNON	Blank Contamination, Result Reported as a Non-detect, Potential Low Bias	The analyte was detected in one or more of the blanks associated with this sample; the holding time was exceeded when analyzing the sample; *and* when the sample result is less than five times the blank result, there are no means by which to ascertain whether or not the presence of the analyte may be attributed to contamination. Therefore, SCC recommends that the data be reported in the database as a non-detect at the ML, adjusted for dilution.	Occurred for one *n*–octadecane and one *n*-tetradecane result in the Year 4 data set.
B, RMAX	Blank Contamination, Result is a Maximum Value	Blank contamination was observed and the analyte was reported in the sample at a concentration between 5 and 10 times higher than the blank value. The result was considered to be of acceptable quality, but data users are cautioned that it may be a maximum value due to possible influence of contamination.	Applied to six non-target analytes (one *n*–decane, two *n*–hexadecane, and three *n*–octadecane results) in the Year 3 and Year 4 data sets.
B, RNAF	Blank Contamination, Result Not Affected	Blank contamination was present but was not considered to adversely impact the sample result. The presence of the analyte in the blank is not considered to adversely affect the data in cases where the sample results are more than 10 times the associated blank results or where the analyte is not detected in associated samples.	Applied to 0.47% of the non-target, semivolatile analytes in the Year 2 and Year 4 data sets.
B, RNON	Blank Contamination, Result Reported as a Non-detect	When the sample result is less than five times the blank result, there are no means by which to ascertain whether or not the presence of the analyte may be attributed to contamination. Therefore, SCC recommends that the data be reported in the database as a non-detect at the ML, adjusted for dilution.	Applied to 0.82% of the non-target, semivolatile analytes in the Year 2, Year 3, and Year 4 data sets.
HNRO	Potential High Bias	High native compound recovery in the OPR associated with the sample.	Applied to one *n*–octadecane result in the Year 4 data set.

Exhibit 4-1B (*Additional or Non-Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
HNRO, HTEX, LLRO	Exclude	A combination of QC failures that suggest the reliability of the result is questionable. In this case, the native compound was recovered above method-specified criteria and the labeled analog was recovered below method-specified criteria in the OPR associated with the sample (HNRO and LLRO). In addition, the holding time was exceeded (HTEX) and, although the compound was reported in a sample dilution, it was not found in the neat (undiluted) analysis.	Applied to one 2,6-dinitrotoluene result in the Year 1 data set.
HNRO, J	Estimated Value	High native compound recovery in the OPR associated with the sample; the sample result reported was above the MDL (detection limit) but below the ML (quantitation limit).	Applied to seven *n*–octadecane results in the Year 4 data set.
HTEX	Potential Low Bias	The holding time was exceeded when analyzing the sample.	Associated with one di-*n*-octyl phthalate, two *n*-eicosane, seven *n*-hexadecane, and six *n*-octadecane results in the Year 1 and Year 3 data sets. Also, associated with several non-target analytes for seven samples in the Year 4 data set.
HTEX, J	Estimated Value	The sample result reported was above the MDL (detection limit) but below the ML (quantitation limit) *and* the holding time was exceeded when analyzing the sample.	Associated with five *n*–decane , one *n*–eicosane, fourteen *n*-hexadacane, seven *n*–octadecane, and six *n*–tetradecane results in the Year 1, Year 3, and Year 4 data sets.
HTEX, NLBL	Exclude	The holding time was exceeded when analyzing the sample *and* the labeled analog was not recovered from the sample, suggesting severe matrix interferences.	Applied to four 2-methyl-4,6-dintrophenol results in the Year 4 data set.
HTEX, NLRO, NNRO	Exclude	The holding time was exceeded when analyzing the sample *and* neither the analyte (native compound) nor the labeled analog were recovered in the OPR sample associated with this result.	Applied to seven 4-nitrophenol results in the Year 4 data set.
J	Estimated Value	The sample result reported was above the MDL (detection limit) but below the ML (quantitation limit).	Occurred throughout the Year 1, Year 2, Year 3, and Year 4 data sets.

Exhibit 4-1B (*Additional or Non-Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
J, LLBL	Estimated Value	The analyte was reported above the MDL but below the ML *and* the labeled analog of the target was recovered below method-specified criteria, suggesting the possible presence of matrix interferences.	Applied to one *n*–tetracosane result in the Year 1 data set.
J, LLRO	Estimated Value	The analyte was reported above the MDL but below the ML in the sample *and* the labeled analog of the analyte was recovered below method criteria in the OPR associated with the sample.	Applied to one 2,4-dimethylphenol result in Year 1.
LLBL	Estimated Value	The labeled analog of the analyte was recovered below method-specified criteria.	Applied to one *n*-hexadecane result in the Year 1 data set.
LLRO	Result Not Affected	The labeled analog of the analyte was recovered below method-specified criteria in the OPR sample.	Associated with nineteen carbazole, nineteen *n*–nitrosodiphenylamine, and nine *n*-triacontane results in the Year 4 data set.
LNRO	Potential Low Bias	The native compound of the analyte was recovered below method-specified criteria in the OPR sample.	Applied to twelve 2,3,6-trichlorophenol, nineteen hexachlorocyclopentdiene, three *n*-docosane, three *n*-hexacosane, and fourteen *n*-octacosane results in the Year 4 data set.
NLBL	Exclude	The labeled analog was not recovered from the sample, suggesting severe matrix interferences. Because it is impossible to determine if the analyte is present or not present, the reported analyte result was excluded from the database.	Applied to a few (0.24%) non-target organic results in the Year 2, Year 3, and Year 4 data sets.
NLBL, NLRO, NNRO	Exclude	The labeled analog was not recovered from either the native sample nor from the Ongoing Precision and Recovery (OPR) sample associated with this result, suggesting severe matrix interferences; also, the analyte was not recovered from the OPR. The failures suggest poor method performance for this analyte.	Applied to six 2,4-dinitrophenol results in the Year 1 data set; and sixteen 4-nitrophenol results in the Year 3 data set.
NLRO	Result Not Affected	The labeled analog was not recovered in the OPR sample associated with this result.	Applied to two 4-nitrophenol results in the Year 3 data set.

Exhibit 4-1B (*Additional or Non-Target Analytes*) - Summary of Data Review Qualifiers Used for Year 1 through Year 4 of the National Lake Fish Tissue Study			
Qualifiers Applied (SCC Code)	**Full Length Description or Recommendation (Comment)**	**Explanation of Code (Reason)**	**Notes on When This Occurs**
NLRO, NNRO	Exclude	Neither the analyte (native compound) nor the labeled analog were recovered in the OPR sample associated with this result.	Applied to a few non-target organic results (0.18%) in the Year 1, Year 3, and Year 4 data sets.
NNRO	Exclude	The native compound was not recovered in the OPR sample, suggesting that the method may not be working for this analyte.	Applied to 0.45% of the non-target organic results in Year 1, Year 2, Year 3, and Year 4 data sets.
REXC	Estimated Value	The result exceeded the instrument calibration range.	Applied to one di-*n*-octyl phthalate result in the Year 1 data set.