

**QUALITY ASSURANCE PROJECT PLAN**  
**AND**  
**SAMPLING ANALYSIS AND ASSESSMENT PLAN**  
**FOR FISH TISSUE SURVEYS**  
**FOR THE STATE OF COLORADO**

Water Quality Control Division  
Department of Public Health and Environment  
State of Colorado

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## ***A. Project Management***

This document describes the quality assurance and quality control procedures that are used while investigating potential contaminants in fish tissue from lakes, reservoirs and streams for the contaminants in fish monitoring program.

This Quality Assurance Project Plan was prepared according to the document *EPA Requirements for Quality Assurance Project Plans*, EPA QA/R-5 (USEPA 2001) and *EPA Guidance for Quality Assurance Project Plans*, EPA QA/G-5 (USEPA 2002). The collection methods, procedures and protocols follow the guidelines and recommendations of *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1: Fish Sampling and Analysis*, Third Edition (USEPA 2000).

### 1.0 Project Organization

The State of Colorado's project manager is Lucia Machado, staff personnel of the Monitoring Unit, Water Quality Control Division, Colorado Department of Public Health and Environment. Roles and responsibilities include the development of this project plan, the applicable standard operating procedures and other pertinent documents, project design, site selection, coordination of data gathering efforts and mechanisms for data exchange with the Colorado Division of Wildlife and other potential partners such as the U.S. Geological Survey, the U.S. Fish and Wildlife Service, etc., field work (with partners), preparation and submittal of samples to the laboratories, revision of results for data quality, statistical analysis, preparation of reports to management and recommendations on the issuance or rescindance of fish consumption advisories.

Robert Griffith, staff personnel of the Monitoring Unit, is the project quality assurance officer. Roles and responsibilities include review of the project plan and standard operating procedures, and collaboration with laboratory-related activities, equipment and supplies, and scheduling of field work.

Robert McConnell, Manager of the Monitoring Unit, approves the project plan and subsequent project-related activities, supervises project implementation and analysis, reviews data results and reports and decides on the appropriateness of issuing or rescinding fish consumption advisories.

Monitoring unit personnel provide field and laboratory support and data entry.

Division of Wildlife regional fisheries biologists partner with the project manager and other Water Quality Control Division personnel on conducting sampling activities. They review and provide comments on this project plan, provide site-specific information on the waterbodies to be studied, such as fish species mostly found at the location and patterns of harvesting and consumption of the fish from the location. They also collect the fish to be analyzed and coordinate delivery with Monitoring Unit personnel.

Other agencies or entities that might be involved in this study are County and City Health Departments, Parks and Recreation Divisions, University personnel, and others.

## 2.0 Problem Definition and Background

In the past, the Water Quality Control Division, the Colorado Division of Wildlife, US EPA, US Fish and Wildlife, Bureau of Reclamation, Denver Health Department and others have conducted several fish tissue studies, in several lakes and reservoirs across Colorado. Results from these studies indicate that certain contaminants were present in fish tissue at many of the sampling sites.

Based on these historical data, the Water Quality Control Division is initiating further fish tissue studies, with the objective of investigating selected waterbodies throughout the state of Colorado for the presence of certain contaminants of concern. The information gathered from these studies is used to assess the potential risk that consumption of these fish may pose to the population. Based on the assessments, the department can decide whether to take further action, including conduct targeted studies, issue fish consumption advisories or rescind existing fish consumption advisories. The assessments may also help evaluate the potential risk that these contaminants may pose to wildlife that consume these fish.

Colorado is comprised of over 100,000 miles of rivers. Many of Colorado's rivers originate in the pristine high alpine environment of the Rocky Mountains and flow downstream through the high desert or high plains environment. Several major rivers have their headwaters in Colorado and flow downstream through multiple states. There are seven major river basins in Colorado, consisting of the Arkansas, Rio Grande, San Juan, Colorado, Green, Platte and Republican rivers. The estimated number of lakes, reservoirs and ponds greater than ten acres in Colorado is 1,533. The estimated acreage of lakes, reservoirs and ponds is 164,029. The majority of lakes in Colorado is man-made, resulting from the damming of rivers for the construction of reservoirs.

Because fish spend their entire life in a waterbody, they can be important indicators of water quality. Some toxic pollutants can be present in the water column or in the sediments at concentrations below analytical detection limits, but still be found in fish due to physiological and feeding mechanisms. The investigation of certain contaminants in fish tissue is an important tool to evaluate aquatic systems and to evaluate the health of game and sport fish consumed by anglers.

## 3.0 Project Description

The overall objective of this project is to investigate whether ***the concentrations of certain contaminants in fish tissue are above the screening level for those contaminants.*** Corollary objectives are: 1) Obtain accurate and representative data on the concentrations of the contaminants of concern; 2) Conduct an investigation that encompasses as many desirable fisheries in the state as possible; 3) Respond to local concerns as readily as

possible, as they become known to the Monitoring Unit; 4) Work collaboratively with the Division of Wildlife; 5) Share the data with the public as readily as possible; 6) Provide recommendations to management as they become available.

The Monitoring Unit designed a statistically based sample plan that generates enough data to answer this question with a known degree of confidence. Details of the sample plan are found in Section B. 7.0. The study involves the collection of fish from several lakes, reservoirs and streams in the State of Colorado, within a five-year period. After the first five years, the cycle can be repeated, depending on available resources.

Sampling activities are conducted by the Water Quality Control Division and the Division of Wildlife staff personnel, although other partners might be involved in the field work as well. Fish tissue samples are routinely analyzed by the Department of Public Health and Environment, Laboratory Services Division although other laboratories, such as U.S. EPA Region 8 Laboratory may also be involved at times.

Field teams consist of one experienced fisheries biologist, one field technician and a quality control specialist. Division of Wildlife fisheries biologists provide the expertise on fish sampling equipment, identification of fish species and are responsible for the actual collection of the fish. Water Quality Control Division personnel provide assistance with field work, assume responsibility for site quality control and documentation, and prepare and submit the samples to the laboratory.

The study targets fish that are most likely to be caught and consumed by the public. The target fish vary among the waterbodies, depending on the prevalent fish in each lake, reservoir, stream or river. The decision is made by the fisheries biologist at the time of sampling, based on his or her knowledge on what species to sample.

The goal is to collect two target species, with a total of 120 fish specimens from each waterbody, 60 specimens per species, divided in two size classes (30 larger and 30 smaller fish). Each group of 30 fish is composited in five composite samples of six fish per sample, so that each composited sample consists of a composite of six fish of the same target species and of the same relative size. The design of the study incorporates as much flexibility as possible, to allow for each waterbody's intrinsic characteristics.

Sampling time is restricted to biological, physical and meteorological conditions, and often coincides with Division of Wildlife's sampling associated with fisheries management and population surveys. The fisheries biologist and the project manager coordinate the best times for the sampling events. Field activities began in the spring of 2004, and are planned to continue for 4 more years. Although this document is being developed for a five year period, it can be reviewed and updated as needed to allow for pertinent feed-back, but not more often than once a year and after the sampling season, so that the field work can be accomplished consistently each year.

Currently, the Department is investigating the presence of mercury, arsenic and selenium in fish tissue, in the State of Colorado. In the future, depending on available resources, the Department may expand the investigation to other metals and to organic compounds.

The Department is currently using U.S.EPA recommendation of 6 to 12 months as appropriate holding time for archived fish material, while still maintaining integrity of the sample material. The Department is conducting a holding time study to validate this recommendation.

#### 4.0 Quality Objectives and Criteria for Measurement Data

##### 4.1 Project Quality Objectives

The results from the Colorado Fish Tissue Study will allow the Water Quality Control Division to evaluate the extent to which certain contaminants are present in fish, in selected waterbodies. The results are reported as an “x” number of composite samples having contaminant levels above the screening level. Major sources of uncertainty inherent to the study are due to the following: 1) sampling only a fraction of the fish species from each lake; 2) unknown variability in pollutants concentration in fish population; 3) collecting a small number of fish relative to the lake population; 4) compositing the fish samples; and 5) variability in the laboratory analysis process.

The quality objectives of this project are related to the fish tissue collection method and to the laboratory procedures. Methods and procedures for the collection of fish tissue described in this document are intended to reduce the magnitude of the sources of uncertainty (and their frequency of occurrence) by applying the following approaches:

- use of standardized sample collection and handling procedures, and
- use of trained scientists to perform the sample collection and handling activities

The following approaches are intended to measure the data quality objectives as they relate to laboratory procedures:

- a laboratory blank for each 10 composite samples (10%)
- matrix spike and matrix spike duplicate for 10% of the samples collected
- a quality control spike sample of known quality and concentration for laboratory comparison

##### 4.2 Measurement Performance Criteria

Measurement performance criteria are quantitative statistics that are used to interpret the degree of acceptability or utility of the data to the user. The following defines the criteria for this study:

### *Precision*

Precision is a measure of internal method consistency or variability in sample results. It is generally attributed to sampling activities and/or laboratory analysis. It can be expressed either as a range, a standard deviation or percentage of the mean of the measurements (relative range or relative standard deviation).

In order to control for field-related variability, sampling activities will be standardized by the strict adherence to the procedures and methods described in this sampling plan, and field sampling will be conducted by trained professionals (this will also help prevent *bias*).

For this study, because samples must be composited and subdivided in a strictly controlled, clean laboratory environment, the project manager prepares duplicate composite samples on 10% of the samples to be analyzed. These duplicates are labeled with unique separate numbers and sent to the laboratory to be analyzed with the routine samples. The results from these duplicate samples are used to assess variability arising from sample compositing, aliquoting, shipping and laboratory analysis processes. The study measurement quality objectives requirements for analytical precision are that results from 90% of these duplicate composite samples agree within  $\pm 50\%$  for values greater than 5x the minimum level of quantification and that 90% of these duplicate composite samples agree within  $\pm 100\%$  for values less than 5x the minimum level.

In the case that this percentage is exceeded, additional material is extracted from the frozen fish tissue and the analyses are repeated. If the high percent difference persists after the second round of analyses, an effort is attempted to collect fish at the questionable site, depending on available resources and logistics.

In addition to the duplicate composite samples, the laboratory also employs a suite of laboratory quality control measures (initial precision and recovery samples, matrix spike and matrix spike duplicate samples) that provide information about the precision associated with various components of the analytical process. These quality control elements and associated requirements are described in more detail in the laboratory's Quality Assurance Project Plan and are not part of this project plan. The results are provided to the Water Quality Control Division as part of the Laboratory Information Management System reports.

Some major criteria for laboratory data are:

- 5-point calibration (in fish tissue) must have a correlation coefficient of 0.995
- Laboratory fortified blank, one per analytical batch, recovery must be 85-115%
- Duplicate, run at 10% frequency, percent difference must be 0-10%
- Spike, run at 10% frequency, recover must be 70-130 %
- Reference, one per analytical batch, recover must be 90-110%

- Continuing calibration verification, run after every 10th sample, recovery must be 90-110%
- Blank, run at 10% frequency, must be non-detect for mercury

### *Bias*

Bias is the systematic and consistent distortion of a measurement process that causes errors in one direction. Bias within the sampling and processing is controlled by training of field procedures and of the sample preparation procedures in the laboratory and by strict adherence to protocols. Bias within the analytical process is measured by preparing and analyzing field samples spiked with analytes of interest (matrix spike samples).

### *Accuracy*

Accuracy is the measure of the combination of bias and precision of an analytical procedure. It reflects the closeness of a measured, observed value to a true value. Accuracy is inferred from recovery data determined by sample spiking and/or analyses of reference standards. Spiked samples are run at a 10% frequency or one per set of samples, whichever is greater. The criterion for spike recovery is 60% to 140%.

Percent recovery is calculated using the following equation:

$$A = \{(x_{ss} - x_s) / T\} \times 100\%, \text{ where:}$$

A = recovery for the added spike;  
 $x_{ss}$  = result for the spiked sample;  
 $x_s$  = result for the sample;  
T = true value of the added spike

### *Analytical Sensitivity*

Analytical sensitivity is included in the laboratory's Quality Assurance Project Plan and is reported to the Water Quality Control Division in terms of the method detection limits and the minimum levels that are used to define the sensitivity of each measurement process. Measurement quality objectives requirements for detectability are that the laboratory has a demonstrated capability to achieve the method detection limits and the minimum levels for 100% of the samples to be analyzed.

### *Representativeness*

Representativeness expresses the degree to which data accurately and precisely represent a characteristic of a population, parameter, variations at a sampling point, a process condition or an environmental condition. In order to achieve this, this study was statistically designed so that the target population of fish in each waterbody is represented by a statistically valid sub-sample. This item is discussed further in Section B. 7.0. The preservation of the representativeness of the collected samples is assured by adhering to the

sample handling protocols for storage, preservation and transportation, as described in this document. Proper documentation records that the protocols have been followed and sample identification and integrity have been assured.

### *Comparability*

The objective of this parameter is to assure that data developed during this investigation are either directly comparable, or comparable with defined limitations, to literature data or other applicable criteria. Comparability is dependent on the proper design of the sampling plan and on adherence to accepted sampling techniques, standard operating procedures and quality assurance guidelines.

In order to fulfill the objectives of this study, all samples are collected and prepared according to the procedures described in this project plan and associated standard operating procedures. These procedures are consistent with the recommendations of U.S.EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis*, Third Edition (USEPA 2000). The laboratory conducts a Quality Assurance/Quality Control spike analysis for the parameter of concern; the result should be within 60% to 140% of the known or reported concentration.

The procedures for this study are also consistent with the National Study of Chemical Residues in Lake Fish Tissue, conducted by the U.S.EPA Office of Water, Office of Science and Technology and Engineering and Analysis Division. The national study is a screening level study designed to estimate the national distribution of the mean concentrations of selected persistent, bio-accumulative and toxic chemicals in fish from lakes and reservoirs. It involves the collection of predator and bottom-dwelling fish from 500 randomly selected lakes and reservoirs on the continental United States, over a 4-year period; it started in 2000. Lakes and reservoirs in Colorado are also being sampled as part of this study, and the data results from both studies will be gathered to evaluate potential contamination in fish tissue in Colorado.

All field personnel involved with sampling have adequate training, appropriate experience and use one standardized protocol for sample collection.

### *Completeness*

This is a measure of the amount of valid data collected and deemed to be acceptable for use in the study, as compared to the amount of data expected to be obtained. Three measures of completeness are defined:

- 1) Sampling completeness, defined as the number of valid samples collected relative to the number of samples planned for collection
- 2) Analytical completeness, defined as the number of valid sample measurements relative to the number of valid samples collected, and

- 3) Overall completeness, defined as the number of valid sample measurements relative to the number of samples planned for collection.

Sampling and analytical completeness goal in this study is to obtain valid measurements from 90% of the valid samples collected. In case this percentage is lower than 90%, the effects on the study conclusions and recommendations are re-evaluated during data analysis. Fish specimen archives are kept frozen, in labeled vials, for a year, at the state laboratory.

## 5.0 Special Training Requirements

The field sampling team consists of one experienced fisheries biologist, one field technician and a quality control specialist, all of whom are trained on all field procedures detailed in this protocol. The fisheries biologist, in the majority of the cases, is staff personnel from the Division of Wildlife, and the field technician and quality control specialist (who may be the same person) consists of staff personnel from the Water Quality Control Division.

This protocol and associated standard operating procedures are distributed to all personnel involved in the field activities. Project orientation sessions are coordinated by the project manager, who also provides instructions on all the field sampling and sample handling activities.

Minimum skills required of the laboratory analysts performing work for this study are described in the laboratory's Quality Assurance Project Plan.

## 6.0 Documentation and Records

Thorough documentation of all field sample collection and handling activities is necessary for proper processing in the laboratory, for ensuring data integrity and, ultimately, for the interpretation of study results. Field sample collection and handling are documented in writing (for each sampling site) using the following forms and labels:

- A Field Record Form that contains information about each individual fish specimen and lake site (can be found in the SOPs)
- A Sample Identification Label that accompanies and identifies each sample or labeled vials
- A Chain of Custody Form that provides tracking information for all samples (can be found in the SOPs)
- Sample Preparation Record Form for each composite sample (can be found in the SOPs)

The field record form documents the sampling date, time, sampling crew names, sampling site location/description and sample description, common name of fish species collected

(scientific names are compiled later), length of each specimen, and the method of fish collection. The field record form also contains a unique tracking code for tracking each fish specimen. The ten-character code follows the format:

- The initial for Colorado (CO)
- Year of collection (YY)
- Waterbody identification code (first four letter in the waterbody's name)
- Species identification code (consult Appendix B)
- Numbering order of fish specimens (001, 002, etc.)

Field record forms are completed by the quality control personnel in the field. All entries are made in ink, with no erasures. If an incorrect entry is made, the information is crossed out with a single strike mark and initialed and dated by the recorder. Two copies are made of this form, one for the Division of Wildlife and one for the Water Quality Control Division. The originals are kept in a project-dedicated binder.

All fish samples (fillets or portion of a fillet) are kept in 50 ml Nalgene vials, with screw-on caps. The vials are labeled with indelible ink (i.e. Sharpie) and the information on the vials coincides with specimen and sample information on the field record form. In the occasions when fish are filleted in the field, a sample identification label is used and placed inside the aluminum foil package with the accompanying fillet.

Chain of custody forms accompany each container of samples and document sample identity (coincide with information on the field record), sampler relinquishment name, date and time and project manager receipt date and time. The field personnel responsible for quality control are also responsible for the delivery of the samples to the laboratory.

Sample preparation record form is completed at the laboratory, for each lake, and it includes information on every replicate composite sample. It includes the name of the persons filleting, coring and preparing the replicate composite samples; information about each individual fish included in each composite sample; composite sample number, as generated by Water Quality Control Division personnel; the weight of each replicate composite sample; any general comments or remarks. The table describing the compositing scheme, i.e., which fish make up each replicate composite sample, is attached to the sample preparation record, and also kept in the project-dedicated binder, in the Monitoring Unit.

If any changes are necessary during the sample collection and handling activities, a note is made in the field record form, and the project manager is notified as soon as practically possible, preferably prior to the change actually occurring. Every effort is made for the project manager to be accessible, either by being on site or by cellular telephone.

NOTE that only minor changes can be acceptable and that major modifications can jeopardize data integrity and statistical power and hence, the efforts related to the whole project.

## 6.1 Analytical Lab Records

The state laboratory is required to submit summary reports of all analytical results, in electronic format and hard copy. They are also required to submit copies of the raw data and any other information that would allow an independent reviewer to verify the calculations performed and trace the final results to the raw data. The reports should include a description of any problems encountered and comments on the performance of any part of a method. The results should be reported consistently in regard to reporting units.

### ***B Data Acquisition***

## 7.0 Sampling Process Design

The objective of the Colorado Fish Tissue Study is to investigate whether, for certain fish of specific size classes, the fish tissue concentrations of certain target analytes are above the screening level for those analytes.

### *7.1 Rationale for Selection of Waterbodies for Sampling*

This study follows the Tier 1 – Screening Studies objectives, as outlined in U.S.EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis*, Third Edition (USEPA 2000). The primary aim of this study is to investigate frequently fished sites where commonly consumed fish species can be potentially contaminated and may pose a risk to human health. Ideally, screening studies should include all waterbodies where commercial, recreational or subsistence fishing is practiced. Since this is not practical and given the existing resources available for the implementation of this study, the Monitoring Unit has made the decision to use a targeted or tiered method to select lakes for the study. In other words, the site selection process does not follow a random process. Initially, only lakes and reservoirs were ranked and slated for sampling. In future years, streams or rivers may also be included. Waterbodies were chosen based on the following criteria:

- 1) Whether there are historical data on contaminants in fish tissue above the screening levels.
- 2) Whether there is a high incidence of population harvesting fish from a waterbody. Urban lakes are assumed to be in this category. Division of Wildlife, County and City Health Departments, etc, are consulted on this item.
- 3) The need to update current fish consumption advisories
- 4) Whether there are any on-going collaborative studies with other entities such as the U.S. Geological Survey, U.S. Fish and Wildlife Service, Universities, etc.

Once a list was derived applying these criteria, the waterbodies were apportioned to each year, in a five-year plan to complete the study. For a complete list of waterbodies to be investigated, consult Appendix A.

Another consideration associated with sampling fish is whether the waterbody contains threatened or endangered species. In order to address this, fish collection will only occur in the presence of an experienced fisheries biologist, who will identify the fish species prior to field collection and ascertain that no threatened and endangered species are harmed.

## 7.2 *Rationale for Selection of Parameters*

Mercury – it is a naturally occurring element that is neither created nor destroyed. It enters the environment as a result of natural events such as volcanoes, fires and surface emissions and from anthropogenic sources such as combustion and commercial products. Mercury and its compounds are persistent, bioaccumulative and toxic and they pose risks to humans and to ecosystems. Since the 19<sup>th</sup> century, the total amount of mercury in the environment has increased by a factor of two to five above the pre-industrial levels. As the quantity of available mercury in the environment has increased, so have the risks of neurological and reproductive problems for humans and wildlife. The exposure route for mercury begins when it is emitted in the air and deposits on land and water. It is then captured by biological activity, transformed in methylmercury, taken up by the food web and eventually reaches larger fish, and wildlife and humans who consume the fish. Mercury adversely impacts wildlife and humans, especially children and women of childbearing age. It is also the leading cause of impairment in the Nation's estuaries and lakes. It is cited in nearly 80% of Fish Consumption Advisories reported by the states in the National Listing of Fish and Wildlife Advisories, in 2000.

Arsenic - it can be found naturally on earth in small concentrations. It occurs in soil and minerals and it may enter air, water and land through wind-blown dust and water run-off. Arsenic generally bio-accumulates in fish in the less harmful organic form. Human exposure may occur by ingesting contaminated water, soil, or air at contaminated sites. High levels of inorganic arsenic in food or water can be fatal. Arsenic damages many tissues including nerves, stomach and intestines, and skin. Breathing high levels can cause sore throat and irritated lungs. Low-level exposure may lead to nausea, vomiting, diarrhea, decreased production of red and white blood cells, abnormal heart rhythm, blood vessel damage, and "pins and needles" sensations in the hands and feet. The U.S. Department of Health and Human Services (DHHS) has determined that arsenic is a known carcinogen. Breathing inorganic arsenic increases the risk of lung cancer. Ingesting inorganic arsenic increases the risk of skin cancer and tumors of the bladder, kidney, liver, and lung (Agency for Toxic Substances and Disease Registry).

Selenium – Selenium is a naturally occurring element that is widely but unevenly distributed in the earth's crust. It is also an essential dietary element that prevents damage to tissues by oxygen. However, when consumed in amounts higher than the recommended

daily allowance (RDA), selenium is toxic to humans and animals. Although selenium bioaccumulates, that is, accumulates in tissues of aquatic organisms, it is not significantly biomagnified. Unlike mercury or polychlorinated byphenyls (PCBs), concentrations of selenium do not increase significantly in animals at each level of the food chain going from prey to predator.

The Department is currently using an action level for mercury that is equal to 0.5 µg/g. For Arsenic and Selenium, the department is using U.S.EPA screening values that are taken from *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis*, Third Edition, (USEPA 2000), page 5-11. For Arsenic that value is 1.2 µg/g; and for Selenium it is 20 µg/g; the non-carcinogenic values were used. Calculating action level involves toxicological studies, risk assessment, data and resources; it is an on-going effort undertaken by the Department. Screening levels for other contaminants become available, the Department will adopt those. In the meantime, the Department will continue to consult with U.S. EPA for the recommended values. The Department may also expand the fish tissue study to address other contaminants of concern, including organic compounds, as resources allow it.

### 7.3 Rationale for Determining Sample Size

In order to be able to evaluate concentrations of contaminants in fish tissue, one strategy can be to verify the concentration of the contaminants of concern in every specimen of fish in all waterbodies (a census approach). This alternative is not very practical or realistic. The other strategy is to select a statistically representative sample such that an answer for all fish of the same species can be generated using a sub-set of certain fish species, in certain size classes, for the waterbodies being addressed.

In determining sample size, i.e., estimation of the number of replicate composite samples per site (n) and the number of individuals per composite (m), the calculations and tables found in U.S.EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume I: Fish Sampling and Analysis*, Third Edition (USEPA 2000), page 6-27 and subsequent pages were consulted.

The starting point was the formulation of the null hypothesis and the related alternative hypothesis:

$H_0$  = the target analyte concentrations of the replicate composite samples at a site are equal to or less than the screening value (SV), or  $x_i = SV$

$H_a$  = the target analyte concentrations are greater than the screening value (SV), or  $x_i > SV$

In order to conduct the test of the null hypotheses at a known level of confidence, the following considerations were included in the study:

- 1) Address the need to achieve a minimum detectable difference between the site-specific mean target analyte concentration and the selected SV.
- 2) Ensure enough statistical power for testing the hypothesis, or defining the probability of detecting a true difference when one does exist (a type II error happens when one does not reject the null hypothesis of no difference between the mean target analyte concentration and the screening value when a difference does exist, or a “missed detection”, or “acquitting a guilty person”). Define the level of significance, i.e., define the probability of rejecting the null hypothesis of no difference between the mean target analyte concentration and the screening value when a difference does not exist (a type I error, or a “false alarm”, or “convicting a not guilty person”). A potential solution for this type of error is to lower the probability of rejecting the hypothesis, for example from 5% to 1%.
- 3) Estimate the population variance, i.e., the variance in target analyte concentrations among the individuals of the same species. This is usually not readily available.
- 4) Considerations of cost and protection of the fisheries.

The division has used the following assumption, when designing the study: that there is a need to gather enough information about the true distribution of the concentration of the target analyte in the fish population so that the statistical power to test the hypothesis is between 70 and 80 percent. This is to say that the probability of detecting a true difference between the mean target analyte concentration and the screening value when one truly exists is 7 or 8 in 10. Another way to say this is that we are willing to accept saying that, in 2 to 3 cases out of 10, the mean target analyte concentration is equal to or less than the screening value, when in reality it is higher. In this case, in 20 to 30% of the samples, the division will fail to see high levels of contaminants in the fish tissue. The best way to increase the statistical power of the test is to increase the number of replicate composite samples, which also increases cost.

The division also used the assumption that the true mean of the site-specific composite target concentrations is 50% higher than the screening value and that the estimated population standard deviation to the screening value (i.e.,  $\sigma/SV$ , similar to a coefficient of variation) is 100%. These are conservative assumptions (protective of public health) that indicate the need to have a higher number of replicate composite samples in order to achieve the above statistical power. As we know more about the variance in analyte concentration in the fish population, we might be able to decrease the number of replicates and still keep the statistical power.

Under all these assumptions, *the number of fish per composite (m) is 6 and the number of replicate composite samples (n) is 5, per class size and per species.*

#### 7.4 Sample Type

Replicate composite samples of fish tissue extracted from fillets of the target species are collected for total mercury, total selenium and total arsenic analyses. A total amount of

approximately 1.0 gram is collected for each composite samples. A fillet, collected from the right side of the six fish specimens that make up each composite, is extracted from each fish. Small cores are collected from each fillet, composited, weighed and placed in a laboratory-appropriate container.

Fish used in a composite sample must meet the following criteria: a) all be of the same species; b) be of similar size so that the smaller specimen is no less than 75% of the total length of the largest individual, c) be collected at the same time, and d) be collected in sufficient numbers to provide the necessary minimum amount for analysis.

### 7.5 *Sampling Period*

Sampling period will coincide with Division of Wildlife's regular population survey sampling efforts, as often as possible. In some cases Division of Wildlife may collaborate with the Water Quality Control Division in a special project and the scheduling is done separately from the regular population surveys.

### 7.6 *Testing the Hypothesis*

Comparing the target analyte concentrations with the screening values:

- 1) Calculate the the mean target analyte concentration of the observed replicate composite samples ( $z$ ) at each site:

$$z = \sum z_i / n, \text{ where } z_i = \text{the concentration of the } i^{\text{th}} \text{ replicate, and } i = 1, 2, 3 \dots n$$

The summation occurs over the  $n$  composite samples.

- 2) Calculate the estimate of the variance of  $z$  (mean),  $s^2$ :

$$s^2 = [\sum (z_i - z(\text{mean}))^2] / [n(n-1)], \text{ where the summation occurs over the } n \text{ composite samples.}$$

- 3) Calculate the test statistic:

$$t_c = (z(\text{mean}) - SV) / s$$

- 4) The null hypothesis of no difference is rejected in favor of the alternate hypothesis of exceedance if,

$t_c > t_{\alpha, n-1}$ , where  $t_{\alpha, n-1}$  is the tabulated value of the student-t distribution corresponding to the level of significance  $\alpha$  and  $n-1$  degrees of freedom. (The inequality is in one direction ( $>$ ) since it is exceedance of the SV that is of interest).

The level of significance will be 0.05 (5% or 95%). The test will be applied to each site, for each fish species and for each class size separately. Combining any of the above introduces variance components that are not accounted for in the procedures and assumptions described in this protocol.

### 7.7 *Limitations of this Study*

Based on the targeted sampling design method, it is very important to define very clearly that the conclusions that this study will generate will be limited by the constraints of the design. As such, there will be no inferences made about the general population of fish or about the general population of lakes. The results and conclusions will be made specifically about each lake, the fish species sampled and will apply only to the size classes and analyte of concern, as addressed in the sampling design.

## 8.0 Sampling Methods

### 8.1 Target Species

EPA recommends three options for selecting target species and size classes: 1) single composite sample for each of three size classes of each target species; 2) replicate analyses of one class size, and 3) replicate analyses of three size classes. These recommendations are based on the need to keep the statistical power of the significance test strong, but also account for limited resources and fisheries management concerns. The State of Colorado has selected to use replicate analyzes of two target species for each lake. The target population of fish to be examined by this study will vary amongst the waterbodies, so that, whichever the prevalent fish are in a certain lake or reservoir, they will be sampled. An attempt will be made to collect two fish species at each location, but it may not be possible in every instance. The decision will be made in consultation with local professionals, such as the Division of Wildlife fisheries biologist, County and City Health Departments.

Whenever possible, again, allowing for local conditions, the two larger size classes will be sampled. In the cases when concentrations of the target analytes are found above the screening level, the decision might be made to sample for smaller size classes. This will depend also on whether a smaller size class is being harvested.

The following criteria will be applied when choosing the fish at each site:

- 1) Collect fish that are preferred by the population.
- 2) Collect fish that are most common at each location.
- 3) Collect fish that have the highest probability of being contaminated.

Every effort will be made to collect the desired species and number of fish; however, the outcome of field sampling efforts will ultimately depend on the natural diversity and abundance of fish in the study areas.

Accurate taxonomic identification is essential in assuring and defining the organisms that have been composited and submitted for analysis. Under no circumstances should individuals from different species be used in a single composite sample. Ideally, the target species composite should focus on the larger individuals commonly harvested by the local population.

### 8.2 *Composite Sampling*

The Colorado Fish Tissue Study uses replicate composite sampling of the target species. Composite samples are cost-effective for estimating average tissue concentration of target analytes in target species populations and compositing ensures adequate sample mass for analysis of all target analytes.

### 8.3 *Sample Collection*

Fish collection methods can be divided into two major categories, active and passive. Each has advantages and disadvantages. Active collection methods employ a wide variety of sampling devices including electrofishing units, seines, trawls and angling equipment (hook and line). Passive collection methods employ a wide array of sampling equipment including gill nets, fyke nets, trammel nets, hoop nets, pound nets and d-traps. Selection of the most appropriate gear type(s) for a particular target lake will be at the discretion of the experienced on-site fisheries biologist. Division of Wildlife provides the sampling collection equipment. Water Quality Control Division provides sample packaging and shipping supplies. Sample collection, packaging, and shipping methods are presented in the Standard Operating Procedures.

#### 8.4 *Equipment and Supply List for Fish Tissue Sampling*

1) Sampling vessel (including boat, motor, trailer, oars, gas and all required safety equipment)
2) Electrofishing equipment, nets and/or angling equipment
3) Coast guard-approved personal floating devices
4) Maps of target lakes and access routes
5) Global Positioning System unit – GPS
6) Livewell and/or buckets
7) Measuring boards (millimeter scale)
8) Ice chests
9) Extra Heavy-duty Aluminum Foil
10) Large plastic (composite) bags (Ziploc type)
11) Knife or scissors
12) Plastic holding trays
13) Clean nitrile gloves
14) Field Record Forms
15) Sample Identification Labels
16) Chain-of-Custody Forms
17) Ice
18) Black ballpoint pens and waterproof markers
19) Clipboard
20) Packing/Strapping tape
21) First aid kit and emergency telephone numbers

As soon as fish are obtained, they should be given their common name. The scientific name will be provided later. This should be conducted only by experienced personnel knowledgeable of the taxonomy of species in the waterbodies. The individual specimens selected will be rinsed in ambient water to remove any foreign materials; they should be handled using clean nitrile gloves, and placed in clean holding containers (livewell, bucket, etc.). Each fish is then measured, weighted, and observed for any noticeable anomalies. The information is recorded in the Field Record Form.

Ideally, the habitat suitable for target species will be determined for each lake and up to three locations of that habitat will be randomly selected for sampling in the waterbody. For example, in a reservoir, the three locations may be in habitat near the inflow, middle and outflow of the reservoir. The composite is intended to estimate the mean fish tissue contaminant concentration for the waterbody.

#### 9.0 Sample Handling and Custody Requirements

##### 9.1 Sample Handling

The division uses two methods of handling sample collection in the field. This is so that there is flexibility on how to collect all the fish necessary for each waterbody study, and

still adjust for each particular situation. One method describes sample handling and processing in the field. The other method describes sample handling in the field and processing at the laboratory.

In the case that samples are collected and processed in the field, the following procedures are used. Fish are brought ashore in buckets and rinsed in ambient water. Every fish specimen is then measured, weighed and filleted. The information is recorded on the Field Record Form; a Sample Identification Label is prepared and each fillet is wrapped individually in extra heavy-duty aluminum foil, with the label inside. The labeled fish fillets are placed in ice chest with ice in sufficient quantity to keep them frozen for the time it takes to take them to the state laboratory. Monitoring Unit staff personnel is responsible for transporting the samples to the laboratory, ensuring that the samples are kept frozen and that they are placed in the freezer as soon as possible. Upon arrival at the Laboratory, the samples are kept in the Monitoring Unit's freezer at  $\leq 20^{\circ}$  C until the time of sample preparation, to preserve tissue and cell integrity.

In the case that samples are collected in the field and processed at the laboratory, the following procedures are used. Fish are brought ashore in buckets and rinsed in ambient water. Division of Wildlife staff personnel records the sample collection information, the number of fish per species and the total number of fish collected. Fish are placed inside heavy-duty plastic bags, carefully to ensure integrity of the fish, and placed in ice chests with ice in sufficient quantity to keep them frozen for the time it takes to take transfer them to the Monitoring Unit personnel or to the Division of Wildlife freezer, until the time that they are finally transferred to Monitoring Unit personnel. Monitoring Unit staff personnel is responsible for transporting the samples to the laboratory, ensuring that the samples are kept frozen and that they are placed in the freezer as soon as possible. Upon arrival at the laboratory, the samples are kept in the Monitoring Unit's freezer at  $\leq 20^{\circ}$  C until the time of sample preparation, to preserve tissue and cell integrity.

## 9.2 Sample Integrity

Sample integrity is maintained by preventing the loss of contaminants that might be present in the sample and by taking precautions to avoid possible introduction of contaminants during handling. The loss of contaminants can be prevented in the field by ensuring that the sample collected remains intact, i.e., minimal to no lacerations of fish skin. Once a sample is collected, sample integrity is maintained through careful and controlled sample handling, storage and preservation procedures. (see Section 9.1)

In order to minimize the effects of other potential sources of contamination in the field, the following preventive measures should be taken: a) collection equipment should be free of potential contaminants; b) boats should be positioned so that engine exhaust does not fall on the deck area where samples are being handled; c) ice chest and other storage containers are scrubbed clean with detergent and rinsed with distilled water prior to use;

d) samples are wrapped in aluminum foil and placed inside a plastic bag, before storage in the ice; e) use of proper gloves when handling the samples.

### 9.3 Custody Requirements

Each sample is identified and tracked with a unique numbering scheme as described in Section 6.0. The same unique number is used in all documentation including the Field Record Form, the Sample Identification Label, and the Sample Preparation Record Form, for each fish specimen.

Detailed information about the samples collected in the field and about the collection location is recorded on the Field Record Form. Two copies are made of this form: one accompanies the samples to the laboratory and one copy is kept in the Monitoring Unit, in a project-dedicated binder. Samples are transported in sealed ice chests, and are kept under custody of the project manager until final deposit in the laboratory's freezer.

### 10.0 Analytical Methods Requirements

Composite samples are analyzed for total mercury, total arsenic and total selenium. The state laboratory conducts the analyses, using EPA methods. The results are reported in parts per million, as wet weight. Analytical methods and requirements are addressed by the Quality Assurance Project Plans and Standard Operating Procedures developed by the State of Colorado's laboratory. The Fish Tissue Study might expand to analyzing for other constituents, including organic compounds, depending on availability of resources.

### 11.0 Quality Control Requirements

Data quality is addressed, in part, by consistent performance of valid procedures documented in the standard operating procedures, in Appendix G. It is enhanced by training and experience of project staff and documentation of project activities. This Quality Assurance Project Plan, a field sampling plan and training materials are distributed to all personnel involved in the implementation of the project's field work. The Water Quality Control Division project manager ensures that personnel have the training materials and that a training session is undertaken by all involved.

### 12.0 Equipment Testing, Inspection and Maintenance Requirements

All field equipment is inspected prior to sampling activities to ensure proper conditions.

### 13.0 Data Acquisition Requirements (Non-direct Measurements)

Non-direct measurements includes identification and/or verification of each sample location. This is accomplished by preparing maps and routes to each sampling location, which are attached to the field sampling plan and placed in the field documentation binder.

## 14.0 Data Management

Samples are documented and tracked via sample identification labels, field record forms and chain of custody forms. The Water Quality Control Division project manager is responsible for reviewing all completed field forms. Corrections are noted, initialed and dated by the reviewer. Forms and raw data reports are kept in a project-dedicated binder. After all the sampling is done for each year, all data are entered in an Access database and kept in the Monitoring Unit's "J:/" drive. Eventually the data will become available through U.S.EPA STORET system.

### *C Assessment/Oversight*

## 15.0 Assessment and Response Actions

The Water Quality Control Division project manager is on-call throughout the duration of the sampling effort. In the majority of the cases, the Water Quality Control Division project manager is the quality assurance officer in the field; depending on field logistics, there are other staff trained to be the quality assurance officer in other concomitant sampling efforts. In the event that quality problems or other difficulties arise in the field, the project manager is contacted and attempts to resolve the difficulty and determine the appropriate corrective action to be taken; he or she also makes notes of this. The project manager has the authority to stop work on the project if problems affecting data quality are identified that will require extensive efforts to resolve.

## 16.0 Reports to Management

A summary of the work conducted every year is prepared, and presented to the Monitoring Unit Manager. Additionally, a report addressing each lake is prepared, which includes historical information on previous studies, if any, data summary, results evaluating the relationship found between results and action levels, conclusions and recommendations.

### *D Data Validation and Usability*

## 17.0 Data Review, Validation and Verification Requirements

All field record forms and chain of custody forms are reviewed by the Water Quality Control Division project manager for completeness and correctness. Data are entered and assessed by the Monitoring Unit staff personnel by comparing entered data with the original forms. The project manager determines whether to accept, reject or qualify the entered data. A report is then prepared for submittal to the Monitoring Unit Manager.

## 18.0 Validation and Verification Methods

The project manager conducts a review of the laboratory's data results and reports, verifying that methods and protocols were followed. Checks are made that instrument calibrations, quality control and calculations are consistent, correct and complete. Other validation and verification steps include verification that procedural blanks, spike sample analyses and laboratory control sample analyses were appropriate. Based on these assessments, the data are either accepted, accepted with qualifiers or rejected. In the last case, re-analysis is considered.

## 19.0 Reconciliation with Data Quality Objectives

As soon as possible following the completion of the sample collection for each year, precision, accuracy and completeness measures are assessed by the project manager and compared with the criteria discussed in Section 4.0. This will represent the final determination of whether the data collected are of the correct type, quantity and quality to support the intended use for this project, as stated in section 4.0. Any problems encountered in meeting the performance criteria (or uncertainties and limitations in the use of the data) are discussed with the Water Quality Control Division Monitoring Unit Manager, and will be reconciled, if possible.

## ***References and Literature Used***

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