GENERAL PROTOCOL FOR SPORT FISH SAMPLING AND ANALYSIS

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Erratum

We discovered a typographical error on page 4 that created potential confusion regarding the size of fish in composite samples and the “75 percent rule.” We revised the text on December 14, 2006 to clarify the point.

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INTRODUCTION

This paper describes the general protocol developed by the Office of Environmental Health Hazard Assessment (OEHHA) to guide the design of fish sampling plans and analysis specifically for assessing mercury and/or methylmercury concentrations in freshwater fish and shellfish. The analytical results are intended for use in conducting human health evaluations and developing public health advisories (fish consumption guidelines), when appropriate. In many cases there are specific circumstances unique to the study location(s) that warrant specific considerations and can affect the sampling plan. Consequently, agencies or organizations planning for fish sampling and analysis should contact and consult with OEHHA as part of the planning process to ensure that the plan is tailored to address specific needs.

TYPES OF STUDIES FOR SAMPLING

Sampling to provide data to support development of fish consumption guidelines can be done in two stages: 1) a screening study conducted as a small preliminary sampling study, and 2) an intensive or comprehensive larger study to characterize all popular sport fish in a water body. For the screening study, it is advisable to begin by sampling a small number (e.g., 3-5) of one or two indicator or sentinel species (i.e., species that tend to accumulate a lot of one or more chemical contaminants) from a water body to initially identify species and chemicals of concern. More intensive sampling for a comprehensive list of species that incorporates larger sample sizes (e.g., nine or more) is needed to provide sufficient data to evaluate health risks and develop advisories. For both types of studies, reasonable choices need to be made about target species (including shellfish), number and type of sample, fish size, timing of sampling, sample preparation, chemical analyses, and data evaluation. These are discussed below.

TARGET SPECIES

The first step is to identify the fish species that should be sampled. The types of fish and shellfish that are most commonly caught and consumed by sport fishers and their families are the focus of sampling (U.S. EPA, 2000). In addition, those species that are more likely to accumulate chemicals of concern (e.g., mercury) may be prioritized from a longer list of potential target species. Black bass species (e.g., largemouth and smallmouth bass) are good sentinel species for mercury because they are top predators and accumulate it through the food web. Other long-lived predator species (e.g., striped bass and pikeminnow) may also be good sentinel species in some water bodies. Carp and catfish are good sentinel species for chlorinated organic chemicals (e.g., DDT and PCBs) because of their high lipid content and feeding habits. Sampling and analysis of species that might be lower in chemical contamination (e.g., trout and salmon) are also a priority so that options for selecting fish that are lower in contaminants can be provided in guidelines for fish consumers.

For screening studies, U.S. EPA (2000) recommends collecting and analyzing at least one high trophic level species for mercury and other trace metals and a bottom feeding species with high lipid content for pesticides and chlorinated organic chemicals. Ideally, both species should be tested for all common bioaccumulative fish contaminants to identify chemicals of concern. When the measured chemical concentration in any fish sample from a screening study exceeds the screening value, that chemical is of potential health concern. U.S. EPA (2000) defined screening values (SVs) as “concentrations of target analytes in fish or shellfish tissue that are of potential public health concern and that are used as threshold values against which levels of contamination in similar tissue collected from the ambient environment can be compared. Exceedance of these SVs should be taken as an indication that more intensive site-specific monitoring and/or evaluation of human health risk should be conducted” (U.S. EPA, 2000). OEHHA established SVs for a number of chemicals that bioaccumulate in fish (Brodberg and Pollock, 1999). OEHHA issues fish consumption guidelines when mercury concentrations in fish tissues exceed 0.08 parts per million (ppm), the Guidance Tissue Level (GTL) for consumption of 12 meals a month by women of childbearing age mercury (Klasing et al., 2005). Therefore, a SV of 0.08 ppm should be used to identify fish with mercury concentrations that pose a potential public health concern. Additional GTLs are being developed by OEHHA for other chemicals that accumulate in fish.

Intensive monitoring of a variety of fish species should be undertaken in water bodies to characterize the average concentrations of the chemical(s) of concern in sport fish when screening studies indicate that concentrations in sport fish are above levels of concern. For the purposes of developing advisories, sufficient samples (see later discussion) of all sport fish species caught and consumed by fishers from a water body should be collected and analyzed so that if a health advisory is warranted, it will be inclusive of all species that fishers usually catch for a water body. When the sampling plan has been designed to focus only on mercury or methylmercury concentrations in fish, consideration should nevertheless be given to performing analyses of the samples for organic chemicals such as pesticides and PCBs (polychlorinated biphenyls) and other trace metals (e.g., selenium) that accumulate in fish. Throughout California, the more important chemicals that accumulate in fish to levels where advisories have been considered are chlordane, DDTs, dieldrin, mercury, PCBs, and selenium. Other chemicals may be important in some places and new accumulative chemicals (e.g., polybrominated diphenylethers) should be considered for future monitoring. Although most fish consumption advisories in California have been issued to protect consumers from potential adverse health effects related to exposure to mercury, it is best to provide consumers information on all potential risks to adequately protect human health. Sampling therefore should include at least one species at a location that is likely to accumulate organic contaminants if they are present. If the samples cannot be analyzed for organic chemicals under the current program, samples should be archived for future analysis under a different program or funding source.

Target species can be identified from data and/or anecdotal information available on what fishers catch and eat most commonly from the study location. Information can be obtained from creel surveys, such as those conducted for some water bodies by the

California Department of Fish and Game (DFG); or from local fishers, water body managers, wardens or other staff from DFG, and also from sport fishing organizations. In some cases, fish population studies may indicate which species are common in a water body, and monitoring data from other studies in the region (such as for other locations on a stretch of river) can identify potential target species. Common freshwater species that should be considered as target species for comprehensive studies when present in a water body are listed in Appendix I.

NUMBER AND TYPE OF SAMPLES

A minimum number of samples is required to perform health evaluations for issuing fish consumption guidelines; however, the more samples that can be collected, the greater the confidence in the results being reliable and representative of the fish populations and the water body being studied. The following criteria based on guidance from U.S. EPA (2000) should be used:

- Sufficient samples of all sport fish species caught and consumed by fishers and their families from a water body should be collected and analyzed so that fish consumption advisories can be developed. For small- and moderate- sized lakes and reservoirs (approximately 2000 surface acres or less), at least nine legal and/or edible-sized fish per species should be sampled and analyzed as individuals or as three composite samples to support developing advisories. Additional fish should be sampled and analyzed for larger lakes and those with multiple arms. Multiple sampling sites for large water bodies may be obtained on the basis of north/south designations, collected from different arms of a reservoir, or simply collected from multiple locations where fish are most accessible to fishers. For small- and moderate- sized creeks and river segments (approximately 25 miles in length), at least nine legal and/or edible-sized fish per species should be sampled and analyzed as individuals or as three composites to support developing advisories. Additional fish should be sampled and analyzed from fishing areas spread along larger rivers.

- In some cases, especially when analyzing for mercury, individual fish can be run because analysis of mercury is relatively inexpensive and more information (e.g., on individual variation and correlations between size and chemical concentration) can be obtained at minimal cost. To make efficient use of resources, priority species may be analyzed as individuals and secondary species as composites.

- Composite samples are often analyzed. Composite samples include a uniform amount of muscle tissue (aliquot) from each of a designated number of fish from a given species; the tissues are homogenized and analyzed together as one sample. Composite samples provide a measure of average concentrations of chemicals in fish at a reduced cost (compared to analyses of individual fish).

- Composite samples should include a minimum of three fish each; but composites containing five fish each are preferred. In some cases (i.e., large fish such as striped bass or sturgeon), three fish per composite, or nine individuals in total, are considered acceptable. In other cases (i.e., small fish such as sunfish), more fish might be needed to provide enough tissue for chemical analysis, particularly if the samples are also to be analyzed for organic chemicals. Different species should never be combined in composites but should be analyzed separately.

- All composite samples must follow the "75 percent rule." the length of the smallest fish in a composite should be at least 75 percent of the length of the largest fish in the composite. For example, if the largest fish in the composite is 200 mm, the smallest fish must be at least 150 mm. When possible, a narrower size range is preferred for the fish included in each composite sample. Size should be measured as total length (TL). This is especially important when there is a legal size limit for the species; legal size requirements apply to TL (DFG, 2005).

- A minimum of three composites per location is also necessary to compare sites if one wants to test for site differences. In most cases, fish will move among sites, and site differences, even if observed in analytical results, are not considered appropriate for use in advisories designed to protect human health when the same (contaminated) fish might also be caught at other locations in the water body. In a few cases, sites may be sufficiently far apart geographically and differences in habitats could account for differential use by subpopulations of the fish species. Evaluating this possibility requires examination of the movement patterns and migratory behavior of the fish species.

FISH SIZE

Fish that are sampled must meet any legal requirements for minimum and/or maximum sizes established by DFG in their Sport Fishing Regulations. (Note that regulations may change from year to year.) Additionally, fish without specific legal size requirements must be of "edible" size. Fish should be sampled from sizes that are typically caught and consumed by fishers so that measures of contaminant levels will be representative of consumer exposures. Sampling a range of fish that fishers catch will also provide a more representative estimate of their likely exposure. The same sources used to determine target species may also provide information on the sizes of fish fishers catch. Past sampling data can also be used to determine typical catch sizes. OEHHA estimates minimum edible sizes by reviewing the literature on species life histories including growth rates and size at maturity, and selecting best estimates of the minimum adult size. OEHHA's current minimum legal and/or edible sizes for freshwater species are shown in Appendix I.

Sampling a broad range of fish sizes or multiple size classes is recommended when adequate resources are available to fund analyses (U.S. EPA, 2000). This is especially useful for fish with a large range of sizes (e.g., striped bass) and long-lived species, since these species may change prey types among their life stages (e.g., larger older adults may feed at a higher trophic level and/or on larger prey). Sampling and analyzing a range of individuals could provide data to examine the correlation between fish size and mercury concentration and this can be used to estimate mercury concentration for a specific size or sizes if desired. Sampling multiple size classes could also be used to support different consumption guidelines for different sized fish.

SHELLFISH

For the most part, finfish are the target for sampling and analysis for human health evaluation, but in some locations, shellfish can also be popular among consumers and should be sampled as well. The preparation of the tissues for analysis will vary depending on the species, but should include the consumable portions. Depending on the species and how much edible tissues are contained in each individual, a larger number of individuals may be needed to comprise a composite. For example, small clams may require 20 individuals per composite.

When mercury is a target analyte, it is essential to analyze shellfish for methylmercury because the ratio of methylmercury to total mercury can be considerably lower in shellfish than it is in finfish. Furthermore, the proportion of methylmercury varies by species and within species. Methylmercury concentrations are needed to perform exposure assessments and health evaluations because methylmercury is the more toxic and prevalent form of mercury in fish and shellfish. For finfish, it is assumed that 100 percent of the total mercury is methylmercury since nearly all the mercury in finfish is in the form of methylmercury (Bloom, 1992). For shellfish, however, the proportion of methylmercury is highly variable. For example, Lasorsa and Allen-Gil (1995) reported the methylmercury to total mercury ratio to be as low as three percent in mussel samples, and the ratio in lobster samples ranged from 20 to 80 percent. Data submitted to OEHHA from DFG showed the percentage of methylmercury in clams to range from 14 to 65 percent (Gassel et al., 2004). Lasorsa and Allen-Gil (1995) also reported differences in age and location of individual invertebrate samples that corresponded to differences in the ratio of methylmercury to total mercury within the same species. Therefore, it is important to analyze all shellfish samples for methylmercury.

TIMING OF SAMPLING

Ideally, the timing of sampling programs should remain consistent between years (e.g., every summer). This is especially necessary when samples are collected from the same water body over several years to build up data for a comprehensive evaluation of representative samples of all species for consumption guidelines. For some chemical contaminants, body burdens have been shown to vary seasonally in fish (Greenfield et al., 2005; 2003; Hose et al., 1989; SCCRWP, 1986). This is more likely to be the case for lipophilic chemicals, such as pesticides or PCBs, than for mercury, because chemicals stored in fat deposits can be shed during spawning or the production of eggs. Therefore, in general, sampling should be performed during non-spawning seasons (U.S. EPA, 2000). However, some fish species may only be available during certain times of the year, in which case, the ability to collect them must take priority. U.S. EPA (2000) recommends sampling fish during the period when they are most commonly harvested.

Sampling periods (e.g., every fall) should be used consistently when ongoing monitoring is conducted.

Selection of a sampling interval for ongoing monitoring depends on the objectives of the sampling program. Monitoring for temporal trends requires sampling over very long time periods because changes in mercury concentrations are not likely to be detected on a one-year, three-year, or even five-year time frame. Sampling a water body on an annual basis for two to three years, however, can provide information on interannual variation and would provide data representative of average exposures over time.

FISH COLLECTION

Target species may be collected by electroshocking, gill or fyke nets, hook and line or spear fishing. Fish should be maintained in the field in a live-well until they can be frozen on wet or dry ice. The total length of fish should be measured in the field and they should be wrapped in aluminum foil, Teflon, or placed in clean plastic bags for transport to the laboratory using chain-of-custody procedures (U.S. EPA, 2000).

PREPARATION OF SAMPLES

Tissue samples should be prepared in a laboratory clean-room environment using non-contaminating techniques (U.S. EPA, 2000). All fish should be prepared for analysis as "fillets," comprised of muscle tissue. Total weight of individuals should be determined and recorded prior to dissection. Most fish species should be prepared for laboratory analysis as skin-off fillets. A few species that are small can be prepared skin-on, e.g., bluegill, pumpkinseed, and redear sunfish. Fillets prepared skin-on prior to mercury or methylmercury analysis tend to yield lower reported mercury concentration. However, preparing fillets skin-off prior to analysis of organic chemicals tends to lower the reported concentration of these chemicals. Skin-off preparations are preferred and consistent with the methods used in past programs (e.g., the Toxic Substances Monitoring Program). Different amounts of tissues are needed for different chemical analyses and are established by individual analytical laboratories. The procedures used by DFG laboratories for tissue preparation as outlined in the Coastal Fish Contamination Program Quality Assurance Project Plan (DFG, 2002) are acceptable and consistent with U.S. EPA (2000) guidelines.

Composite samples are prepared from equal amounts of tissue from the individual fish (all of the same species) to be included in the composite. Composites are generally formed from three to five individuals, although for small species it may be necessary to use up to 20 individuals in order to obtain enough tissue for some chemical analyses. Muscle tissue samples from individuals or composites are homogenized prior to all chemical analyses. Preparation of composite samples is described in U.S. EPA (2000).

CHEMICAL ANALYSES

Fish tissues should be analyzed for mercury using cold vapor atomic absorption spectrometry (CVAA) (e.g., a Perkin Elmer Flow Injection Mercury System). The analytical method should be capable of a method detection limit (MDL) of 0.01 mg/kg (total mercury). Fish tissues can be analyzed for total mercury to estimate methylmercury concentration because nearly all the mercury in finfish is in the form of methylmercury (Bloom, 1992). As was discussed above, all shellfish species should be analyzed for methylmercury and total mercury. Samples to be analyzed for organic chemicals are generally tested for a suite of analytes identified by U.S. EPA (2000) as chemicals of concern in fish tissues, and recently, analysis for PBDEs (polybrominated diphenyl ethers or flame retardants) has been added to the list of analytes. Results should be reported as wet weights and moisture and lipid concentration should also be measured and reported. These procedures used by DFG laboratories for chemical analyses and Quality Assurance as outlined in the Coastal Fish Contamination Program Quality Assurance Project Plan (DFG, 2002) are acceptable and consistent with U.S. EPA (2000). Samples for which mercury has not been detected (non-detects) should be counted as one half the MDL.

DATA EVALUATION

Samples of fish species with mean mercury concentrations that exceed the OEHHA SV for mercury of 0.08 ppm (e.g., Klasing et al., 2005) indicate that further study is warranted. When sufficient sample sizes have been collected by following this general protocol, the data generated can be used in an evaluation to develop consumption guidelines for a health advisory.

To develop health advisories, OEHHA evaluates the concentrations of methylmercury (as mercury) in fish by comparing them to the reference dose (RfD) for methylmercury. The RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (IRIS, 1995). The measured mean mercury concentrations at a water body for each fish species are compared to the RfD, and the number of meals that can be eaten in a given time period without exceeding the RfD is determined. To streamline the development of fish consumption guidelines for future advisories, this process has been used to develop GTLs for chemicals in fish, which relate the number of recommended fish meals to mercury concentrations found in fish. Meal sizes are based on a standard eight-ounce (227 grams) portion of uncooked fish (approximately 6 oz. after cooking) for adults who weigh approximately 70 kilograms (equivalent to 154 pounds). OEHHA compares measured fish tissue concentrations to the GTLs to determine appropriate meal frequencies for fish consumption guidelines (health advisories). Use of a standard meal size that is proportional to body weight allows for adjustments up or down in the quantity of fish consumed for consumers weighing more or less than 70 kilograms, respectively, and thereby maintains equivalent exposure across consumers with different body weights.

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APPENDIX I
TARGET SPECIES AND
LEGAL AND/OR EDIBLE SIZE CRITERIA

Species Common Name	Minimum Size (mm) Total Length (TL)	Maximum Size (mm TL)
Black Bullhead	170	
Black Crappie	150	
Bluegill	100	
Brook Trout	200	
Brown Bullhead	200	
Brown Trout	200	
Carp	200	
Channel Catfish	200	
Chinook (king) Salmon	No minimum	
Coast Cutthroat Trout	200	
Crayfish[1]	30	
Eagle Lake Trout	250	
Flathead Catfish	200	
Goldfish	200	
Green Sturgeon	1168	1829
Green Sunfish	100	
Hardhead	250	
Hitch	150	
Kokanee	200	
Lahontan Cutthroat Trout	200	
Lake Trout	350	
Largemouth Bass	305	
Northern Crayfish	30	
Rainbow Trout	200	
Red Swamp Crayfish	50	
Redear Sunfish	130	
Sacramento Blackfish	200	
Sacramento Perch	250	
Sacramento Pikeminnow	250	
Sacramento Sucker	200	
Signal Crayfish	50	
Smallmouth Bass	305	
Spotted Bass	305	
Steelhead Rainbow Trout	200	
Striped Bass[2]	457	

[1] All crayfish measured as carapace length
[2] Per CDFG. There is no minimum size for the Colorado River District, the Southern District, and New Hogan, San Antonio and Santa Margarita lakes. Regulations may change yearly.

Species Common Name	Minimum Size (mm) Total Length (TL)	Maximum Size (mm TL)
Tilapia	200	
White Bass	250	
White Catfish	200	
White Crappie	150	
White Sturgeon	1168	1829
Yellow Bullhead	200	