Draft Guidance for Implementing the January 2001 Methylmercury Water Quality Criterion

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4 Monitoring and Assessment

4.1 What are the analytical methods for detecting and measuring methylmercury concentrations in fish and water?

Over the last 2 decades, EPA and other organizations have developed several analytical methods for determining mercury and methylmercury concentrations in fish and water. In 2001, EPA conducted a literature review to assess the availability of different protocols and to determine which of these protocols would be most useful for implementing the new methylmercury criterion. After its review, EPA concluded that nearly all current research on low level concentrations of mercury and methylmercury is being performed using techniques that are based on procedures developed by Bloom and Crecelius (1983) and refined by Bloom and Fitzgerald (1988), Bloom (1989), Mason and Fitzgerald (1990), and Horvat et al. (1993).

EPA Methods 1630 and 1631, developed by EPA's Office of Water, reflect the techniques developed by these researchers for analyzing methylmercury and mercury in water, respectively. Appendix A to Method 1631 (64 FR 10596) details the researcher's techniques for determining total and dissolved mercury in tissue, sludge, and sediments. These methods, which are written in EPA Environmental Monitoring Management Council (EMMC) format, include all quality control elements that EPA's Office of Water considers necessary to adequately define data quality.

In Appendix C, Table C1 summarizes these and other methods that EPA knows have been used to analyze mercury and methylmercury in fish tissue, and Table C2 summarizes methods available for the analysis of mercury and methylmercury in water and other nontissue matrices. Each table identifies the forms and species targeted by each method, estimated or known sensitivity, the techniques employed in the method, and any known studies or literature references that use the techniques employed in the method.

Modifications to Method 1630 described in Table C1 (see Appendix C) and in Horvat et al. (1993) allow for measurement of methylmercury in tissue as low as 0.001 to 0.002 mg/kg, well below the water quality criterion for methylmercury in tissue (0.3 mg/kg). EPA recommends use of these techniques when direct measurements of methylmercury in tissue are desired.

Because researchers have found that nearly all mercury in fish tissue is in the form of methylmercury (USEPA 2000c), EPA also suggests that analysis of tissue for mercury, as a surrogate for methylmercury, is a useful means for implementing the methylmercury criterion. If mercury concentrations in tissue exceed the criterion, further investigation of the methylmercury component might be desired. Appendix A to Method 1631 allows for measurement of mercury in tissue at approximately 0.002 mg/kg, well below the tissue criterion.

Several options are also available for measuring mercury concentrations in water (Table D2). Because Method 1631 has already been promulgated for use in CWA applications,

EPA strongly recommends use of this method when measuring all species of mercury in water, especially when low-level measurements are expected. When measuring methylmercury in water, three options are Method 1631, developed by the Office of Water (USEPA 2002d); UW-Madison's SOP (Hurley et al. 1996), used by the Great Lakes National Program Office for its Lake Michigan Mass Balance Study; and a recently released USGS method (DeWild et al. 2002). All these procedures are based on the same techniques, and each can meet the most stringent (i.e., Great Lakes Guidance) mercury water quality criterion of 1.3 ng/L for wildlife protection in water. While any of these methods are acceptable, EPA recommends the use of Method 1631, which is documented in EMMC format and includes all quality control criteria considered necessary to define data quality.

In summary, on the basis of the available information, EPA believes that the most appropriate methods for measuring compliance with new or revised methylmercury criteria are Method 1631 (mercury in water by cold vapor atomic fluorescence spectrometry (CVAFS)), Method 1630 (methylmercury in water by CVAFS), Appendix A to Method 1631 (mercury in tissue by CVAFS), and modifications to Method 1630 for handling tissues (described in Table C1—see Appendix C). EPA recommends these procedures for the following reasons:

- Methods 1630 and 1631 were developed by EPA to support implementation of water quality criteria for mercury and methylmercury. Both are already in the appropriate EPA format and include all standardized quality control (QC) elements needed to demonstrate that results are reliable enough to support permitting and enforcement programs.

- Appendix A to Method 1631 was developed by EPA to support its National Study of Chemical Residues in Fish Tissue. Appendix A provides information on preparing a fish tissue sample for analysis using Method 1631. The method was validated by Brooks Rand (USEPA 1998b) and is currently being used by Battelle Marine Sciences to analyze more than a thousand tissue samples collected during EPA's National Fish Tissue Survey (USEPA 2000j). Successful use of these techniques also has been widely reported in the literature. This history, combined with the fact that Appendix A supplements the already well-characterized and approved Method 1631, makes this method a good candidate for use with the new fish tissue criterion.

- Method 1630 already has been used in several studies including EPA's Cook Inlet Contaminant Study (USEPA 2001g) and the Savannah River TMDL study (USEPA 2001e). The techniques described in the method and in the recommended method modifications also have been successfully applied in numerous studies described in the published literature. The procedures in Method 1630 also are nearly identical to those given in the USGS method and in the University of Wisconsin SOP, listed in Table D2 (Hurley et al. 1996). The University of Wisconsin SOP was used in EPA's Lake Michigan Mass Balance Study (USEPA 2001f).

4.1.1 What is Method 1631 for determination of mercury in water?

In May 1998, EPA proposed Method 1631 at 40 CFR Part 136 for use in determining mercury concentrations at AWQC levels in EPA's CWA programs, and subsequently published a Notice of Data Availability (64 FR 10596) that included additional data supporting application of the method to effluent matrices. On June 8, 1999, EPA responded to numerous public comments on the proposed method and promulgated EPA Method 1631, Revision B: *Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry* at 40 CFR Part 136 for use in EPA's CWA monitoring programs. EPA promulgated the method on the basis of extensive validation of the procedures, including four single-laboratory studies and an interlaboratory validation involving 12 participating laboratories and 1 referee laboratory. The highest method detection limit (MDL) determined by all laboratories in reagent water was 0.18 ng/L, indicating that this method is capable of producing reliable measurements of mercury in aqueous matrices at AWQC levels.

EPA has revised Method 1631 after its promulgation to clarify method requirements, increase method flexibility, and address frequently asked questions. The current method (Method 1631, Revision E) includes recommendations for use of clean techniques contained in EPA's *Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels* (USEPA 1996b). The benefits of using Method 1631 are that it is an approved method under EPA's CWA monitoring programs, has been fully validated, and numerous laboratories are routinely using this method. However, Method 1631 measures only mercury (total and dissolved) in aqueous samples and is not capable of measuring the methylmercury species.

Method 1631, Appendix A was developed for processing fish tissue samples to be analyzed for mercury using the previously validated and approved Method 1631 analytical procedures. The procedures are expected to be capable of measuring mercury in the range of 2 to 5,000 ng/g (0.002 to 5.0 mg/kg). The expected method detection limit for mercury in fish tissue is 0.002 mg/kg, well below the new water quality criterion for methylmercury. The procedures in the appendix are not published in the *Code of Federal Regulations*, but were implemented in EPA's National Study of Chemical Residues in Fish Tissue (USEPA 2000j). Although Appendix A of Method 1631 has not been fully validated (i.e., via an interlaboratory validation study), it was validated by EPA in a single laboratory study, and the techniques have been widely reported in the literature. Also, as discussed above, the analytical component of the method (Method 1631) has been fully validated and approved for measurement of total or dissolved mercury in aqueous matrices.

4.1.2 What analytical methods are available for determination of methylmercury?

EPA has not published an analytical method specifically for measuring methylmercury. As technical guidance to assist States and authorized tribes in their selection of an analytical method to use, Tables C1 and C2 in Appendix C include four methods that EPA has seen investigators successfully use for the determination of methylmercury. Other methods may be acceptable for use under the appropriate circumstances. As written, all four of the methods are specific to aqueous matrices and are based on almost

identical analytical procedures (i.e., distillation, ethylation, GC separation, and CVAFS detection). These methods have been or are being used in several national or regional studies, but none are yet published in 40 CFR Part 136. Modifications to adapt these procedures for fish tissue have been reported in the literature (e.g., Bloom 1989, and modified by Horvat et al. 1993) and used in EPA's Cook Inlet contaminant study (USEPA 2001g), the 4-year Lake Michigan Mass Balance study (USEPA 2001f), and an extensive study of the Everglades (USEPA 2000b).

Because the four methods are nearly identical, they are expected to produce very similar results with sensitivity as low as 0.002 mg/kg in tissue and 0.01 to 0.05 ng/L in water. These levels are well below the methylmercury criterion for fish and the most stringent (i.e., Great Lakes Guidance) mercury water quality criterion of 1.3 ng/L for wildlife protection in water.

4.2 What is the recommended guidance on field sampling plans for collecting fish for determining attainment of the water quality standard?

EPA has published guidance providing information on sampling strategies for a fish contaminant monitoring program in Volume 1: Fish Sampling and Analysis (2000c) of a document series, *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* (USEPA 2000c). This guidance provides scientifically sound recommendations for obtaining a representative sample for issuing fish consumption advisories and, thus, offers EPA's current guidance for obtaining a representative sample for determining attainment. This guidance also includes recommendations for quality control and quality assurance considerations. In all cases, states should develop data quality objectives for determining the type, quantity, and quality of data to be collected (USEPA 2000h).

4.2.1 What fish species should be monitored?

EPA's fish sampling guidance (USEPA 2000c) provides recommendations for selecting finfish and shellfish species for monitoring to assess human consumption concerns. According to the guidance, the most important criterion is that the species are commonly eaten in the study area and have commercial, recreational, or subsistence fishing value. Fish creel data (from data gathered through surveying anglers) from state fisheries departments is one justifiable basis for estimating types and amounts of fish consumed from a given waterbody. States and authorized tribes should ensure that the creel data are of sufficient quality and are representative of the local population of people who eat fish.

The fish sampling guidance also identifies recommended target species for inland fresh waters and for Great Lakes waters. Seabass, walleye, king mackerel, tilefish, and largemouth bass have been identified as accumulating high levels of methylmercury. Reptiles such as turtle species and alligators are recommended as target species for mercury if they are part of the local diet. Larger reptiles can also bioaccumulate environmental contaminants in their tissues from exposure to contaminated sediments or via consumption of contaminated prey.

The fish sampling guidance recommends that the size range of the sampled fish ideally should include, from the species of fish that people in the area eat, the larger fish individuals harvested at each sampling site, because larger (older) fish within a population are generally the most contaminated with methylmercury (Phillips 1980, Voiland et al. 1991). This means that small fish such as minnows should be avoided as target species. In addition, the methylmercury concentrations in migratory species are likely to reflect exposures both inside and outside the study area, and the state or authorized tribe should take this into account when determining whether to sample these species. For migratory species, EPA's fish sampling guidance recommends, for migratory species, that neither spawning populations nor undersized juvenile stages be sampled in fish contaminant monitoring programs (USEPA 2000c). Sampling of target finfish species during their spawning period should be avoided as contaminant tissue concentrations may decrease during this time and because the spawning period is generally outside the legal harvest period.

If states and authorized tribes do not have local information about the types of fish present that people eat, the following two options provide an alternative for identifying which fish to sample:

Match assumed or known consumption pattern to sampled species—If the state has some knowledge of the fish species consumed by the general population, a monitoring sample could be composited to reflect this knowledge. For example, a state might decide that 75 percent of the fish consumed by the general population are trophic level 4 species, 20 percent are trophic level 3 species, and 5 percent are trophic level 2 species. A composite sample would reflect the determined trophic level breakout. Fish creel data (from data gathered through surveying anglers) from state fisheries departments is one justifiable basis for estimating types and amounts of fish consumed from a given waterbody. States and authorized tribes should ensure that the creel data are of sufficient quality and are representative of the local population of people who eat fish. The state or authorized tribe should decide which approach to use.

Trophic level 4 fish only—Predator species (e.g., trout, walleye, largemouth bass, smallmouth bass) are good indicators for mercury and other persistent pollutants that are biomagnified through several trophic levels of the food web. Increasing mercury concentrations correlate with an increase in fish age, with some variability, so that consumption of higher trophic level species correlates with greater risks to human health. (This correlation is less evident in estuarine and marine species.) Therefore, targeting trophic level 4 species should serve as a conservative approach (depending upon the species most frequently consumed by anglers) for addressing waterbodies with highly varying concentrations of methylmercury.

4.2.2 What sample types best represent exposure?

EPA recommends using composite samples of fish fillets from the types of fish people in the local area eat because methylmercury binds to proteins and is found primarily in fish muscle. Using skinless fillets is a more appropriate approach for addressing mercury

exposures for members of the general population and most recreational fishers because fish consumers generally eat the fillets. Because mercury is differentially concentrated in muscle tissue, leaving the skin on the fish fillet actually results in a lower mercury concentration per gram of skin-on fillet than per gram of skinless fillet (USEPA 2000c). Analysis of skinless fillets might also be more appropriate for some target species such as catfish and other scaleless finfish species. However, some fish consumers do eat fish with the skin on. In areas where the local population eats fish with the skin, the state or authorized tribe should consider including the skin in the sample.

Composite samples are homogeneous mixtures of samples from two or more individual organisms of the same species collected at a site and analyzed as a single sample. Because the costs of performing individual chemical analyses are usually higher than the costs of sample collection and preparation, composite samples are most cost effective for estimating average tissue concentrations in target species populations. Besides being cost effective, composite samples also ensure adequate sample mass to allow analyses for all recommended contaminants. In compositing samples, EPA recommends that composites be of the same species and of similar size so that the smallest individual in a composite is no less than 75 percent of the total length (size) of the largest individual (USEPA 2000c). Composite samples can also overcome the need to determine how nondetections will be factored into any arithmetical averaging because the composite represents a physical averaging of the samples. However, depending upon the objectives of a study, compositing might be a disadvantage because individual concentration values for individual organisms are lost. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1*, at sections 6.1.1.6 and 6.1.2.6 provides additional guidance for sampling recommendations.

4.2.3 What is the recommended study design for site selection?

To address spatial variability of methylmercury levels in fish, EPA recommends that states and tribes design a probabilistic sampling by randomly selecting sites or sampling locations. This approach allows statistically valid inferences to be drawn on an area as a whole.

Ideally, samples should be collected over a geographic area that represents the average exposure to those who eat fish from the waterbody. However, if there are smaller areas where people are known to concentrate fishing, these areas should be used as the sampling area. Fish sampled in locations with mercury point sources should be included in the average concentration if fishing occurs in these areas but not included if the area is not used for fishing.

4.2.4 How often should fish samples be collected?

EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1*, (USEPA 2000c) at section 6.1.1.5 provides recommendations for how frequently to sample fish tissue. If sufficient program resources exist, this guidance recommends biennial sampling of fish in waterbodies where recreational or subsistence harvesting is commonly practiced. If biennial screening is not possible, waterbodies

should be screened at least once every 5 years. Also, the state or authorized tribe should sample during the period when the target species is most frequently harvested or caught.

In fresh waters, the guidance recommends that the most desirable sampling period is from late summer to early fall (i.e., August to October). Water levels are typically lower during this time, thus simplifying collection procedures. Also, the fish lipid content is generally higher, thus allowing these data to also provide information for other contaminant levels. The guidance does not recommend the late summer to early fall sampling period if it does not coincide with the legal harvest season of the target species or if the target species spawns during this period. However, if the target species can be legally harvested during its spawning period, sampling to determine contaminant concentrations should be conducted during that time. In estuarine and coastal waters, the guidance recommends that the most appropriate sampling time is during the period when most fish are caught and consumed (usually summer for recreational and subsistence fishers).

EPA recommends that states and tribes sample consistently in a season to eliminate seasonal variability as a confounding factor when analyzing fish monitoring data. Additionally, focused seasonality studies could be used both to assess the impact of seasonal variability on fish concentrations and to normalize concentrations to a standard season(s). Several studies have measured seasonality in fish-fillet muscle mercury concentrations in estuaries and reservoirs (Kehrig et al. 1998, Park and Curtis 1997, Szefer et al. 2003). In these studies, concentrations were generally higher in cold seasons by as much as a factor of two to three times that in warm seasons. Slotten et. al. (1995) showed that the uptake of methylmercury in zooplankton and fish increased dramatically during the fall mixing of Davis Creek Reservoir, a California reservoir contaminated by mercury mining activities.

No studies of seasonality in fish mercury were found for rivers or natural lakes. On the basis of literature reported fish-mercury depuration rates, EPA does not expect seasonal fluctuations in fish mercury. Though reported mercury elimination half-lives cover a wide range of rates, from a few days to several years, the central tendency is 100–200 days (Giblin and Massaro 1973, Rodgers and Beamish 1982, Huckabee et al. 1979 [literature review], Burrows and Krenkel 1973, McKim et al. 1976). Such slow depuration rates are expected to dampen strongly any fluctuations in methylmercury concentrations in fish. Instead, season variations in fish tissue are likely linked to seasonal nutrition variability that impact fish body conditions but not mercury body burden.

EPA recommends that states and tribes routinely collect both weight and length data when assessing the potential influence of fish nutritional state on mercury concentration, and potentially for normalizing fish concentrations to a standard body condition. Greenfield et al. (2001), Cizdziel et al. (2002, 2003), and Hinners (2004) reported a negative correlation between fish body condition (a ratio of weight to cubed length) and fish tissue mercury concentration. These studies support the concept of *starvation concentration*—whereby loss of muscle mass during periods of starvation occurs quicker than loss of mercury. Burrows and Krenkel (1973) found mercury elimination rate to be the same for fish that were starved relative to nonstarved fish. The converse phenomenon of *growth dilution*, where lower fish-mercury concentrations correlate with higher growth

rates, has been described by a number of researchers (Simoneau et al. 2005, Doyon et al. 1998, Park and Curtis 1997). The authors of the first two papers hypothesize that slower-growing fish allocate more energy towards maintenance and less to flesh production while faster growing fish add flesh at a lower energy cost and, thus, with proportionally less mercury intake. Park and Curtis (1997) proposed an alternative hypothesis that growth dilution occurs when high growth coincides with periods of low methylmercury concentration. Regardless of the exact mechanism, body condition offers a useful method to explain variability in fish mercury.

4.2.5 How many samples should be collected?

EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1*, (USEPA 2000c) at section 6.1.2.7.1 provides information to help determine the number of composite samples for comparing fish tissue information to a target value. This guidance does not recommend a single set of sample size requirements (e.g., number of replicate composite samples per site and the number of individuals per composite sample) for all fish contaminant monitoring studies, but rather presents a more general approach that is both scientifically defensible and cost effective. The guidance provides the means for determining an optimal sampling design that identifies the minimum number of composite samples and of individuals per composite necessary to detect a minimum difference between a target (in this case, the water quality criterion) and the mean concentration of composite samples at a site. Under optimal field and laboratory conditions, at least two composite samples are needed at each site to estimate the variance. To minimize the risk of a destroyed or contaminated composite sample preventing the site-specific statistical analysis, a minimum of three replicate composite samples should be collected at each site.

4.2.6 What form of mercury should be analyzed?

Because of the higher cost of methylmercury analysis (two to three times greater than for mercury analysis), states and authorized tribes should first measure mercury in fish tissue. This approach assumes that all mercury in fish tissue is methylmercury and is, thus, a conservative assessment. This approach does not pose a risk of a false positive decision (considering the tissue to exceed the criterion when it does not) where the measured mercury in fish tissue is less than the 0.3 mg/kg criterion (or a site-specific criterion adopted by a state) nor should it pose a realistic risk of a false positive when the measured mercury exceeds the criterion by 10 percent. Appendix E summarizes seven studies of the relative proportion of the mercury concentration in North American freshwater fish that is in the form of methylmercury. In six of the seven studies, methylmercury, on average, accounted for more than 90 percent of the mercury concentration in fish tissue. If the measured mercury level is within 10 percent of the methylmercury criterion, states might wish to repeat the sampling (if sufficient tissue is not left) and analyze for methylmercury.

4.3 How should waterbody impairment be assessed for listing decisions?

Section 303(d)(1) of the CWA requires states and authorized tribes to identify and establish priority ranking for waters that do not, or are not expected to, achieve or maintain water quality standards with existing or anticipated required controls. In accordance to this ranking, a TMDL for such waters must then be established. For purposes of determining impairment of a waterbody and whether to include it on section 303(d) lists, states and authorized tribes must consider all existing and readily available data and information (see 40 CFR 130.7).

States and authorized tribes determine attainment of water quality standards by comparing ambient concentrations to the numeric AWQC. EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1*, at section 6.1.2.7.1 recommends using the t-test to determine whether the mean concentration of mercury in composite fish tissue samples exceeds the screening value. This involves a statistical comparison of the mean of all fish tissue data to the criterion. If the t-test statistic of the mean exceeds the water quality standards, there is an exceedence. EPA recommends that this procedure also be used for determining impairment. States and authorized tribes might also want to consider the guidance in Appendices C and D of the *Consolidated Assessment and Listing Methodology*, *Toward a Compendium of Best Practices* (USEPA 2002b). Ultimately, the method that states choose depends on how they express their water quality standards.

4.3.1 How should nondetections be addressed?

When computing the mean of mercury in fish tissue, a state or authorized tribe might encounter a data set that includes analyzed values below the detection level. EPA does not expect this to occur frequently for two reasons. First, if the samples are physically composited (see section 4.2.2.), the composite itself provides the average, and there will be no need to mathematically compute an average. Second, the newer analytical Methods 1630 and 1631 are able to quantify mercury at 0.002 mg/kg, which should be lower than the observed mercury in fish tissue samples being analyzed.

However, if a state or authorized tribe is mathematically computing an average of a data set that does include several values below the detection level, the water quality standards and/or assessment methodology should discuss how it will evaluate these values. The convention recommended in EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 1*, at section 9.1.2, is to use one-half of the method detection limit for nondetects in calculating mean values (USEPA 2000c). This guidance also recommends that measurements that fall between the method detection limit and the method quantitation limit be assigned a value of the detection limit plus one-half the difference between the detection limit and quantitation limit. EPA notes, however, that these conventions provide a biased estimate of the average concentration (Gilbert 1987), and where the computed average is close to the criterion, might suggest an impairment when one does not exist or, conversely, suggest no impairment when one does exist.

States or tribes can calculate the average of a data set that includes values below the detection level using other statistical methods (e.g., sample median and trimmed means)

(Gilbert 1987). EPA has published a review of several methods and analyzed the potential bias each can introduce into the calculation of the mean (USEPA 2001i).

One approach that a state or authorized tribe could take is to conduct a sensitivity analysis to ascertain the consequence of what value is used to quantify samples below the detection level. In a sensitivity analysis, the state or authorized tribe would compute the mean concentration using first the value of the detection level to quantify samples below the detection level and then again using a zero value for samples below the detection level. If both calculated means are either above or below the criterion, it is clear that the choice of how to quantify samples below the detection level does not affect the decision. However, if one calculated mean is below the criterion and the other is above, it is clear that the choice of how to quantify samples below the detection does affect the decision, and a more sophisticated approach such as the ones in *Robust Estimation of Mean and Variance Using Environmental Data Sets with Below Detection Limit Observations* (USEPA 2001i) should be used.

All methods have advantages and disadvantages. A state or authorized tribe should understand the consequences of which method it uses, especially if the choice makes a difference as to whether a waterbody is considered impaired or not. Furthermore, a state or authorized tribe should be clear about which approach it used.

4.3.2 How should data be averaged across trophic levels?

If target populations consume fish from different trophic levels, the state or authorized tribe should consider factoring the consumption by trophic level when computing the average methylmercury concentration in fish tissue. To take this approach, the state or authorized tribe would need some knowledge of the fish species consumed by the general population so that the state or authorized tribe performs the calculation using only data for fish species that people commonly eat. (For guidance on gathering this information see section 3.2.1.2) States and authorized tribes can choose to apportion all the fish consumption, either a value reflecting the local area or the 17.5 grams fish/day national value for freshwater and estuarine fish if a local value is not available, to the highest trophic level consumed for their population or modify it using local or regional consumption patterns. Fish creel data from state fisheries departments are one reasonable basis for estimating types and amounts of fish consumed from a given waterbody. The state or authorized tribe must decide which approach to use.

As an example of how to use consumption information to calculate a weighted average fish tissue concentration, see Table 3.

Table 3. Example data for calculating a weighted average fish tissue value

Species	Trophic Level	Number of Samples	Geometric Mean Methylmercury Concentration (mg/kg)
Cutthroat Trout	3	30	0.07
Kokanee	3	30	0.12
Yellow Perch	3	30	0.19
Smallmouth Bass	4	95	0.45
Pumpkinseed	3	30	0.13
Brown bullhead	3	13	0.39
Signal crayfish	2	45	0.07

These concentrations are used to compute a weighted average of tissue methylmercury concentrations for comparison to the 0.3 mg/kg criterion. All fish measured are classified as trophic level 3 except for signal crayfish, which are trophic level 2, and smallmouth bass, which are trophic level 4. The mean methylmercury concentration in trophic level 3 fish in this example is 0.15 mg/kg. This is calculated by weighting the geometric mean methylmercury concentration in each trophic level 3 species by the number of samples of each of the trophic level 3 species, and then averaging the weighted geometric means. Had the concentrations been averaged without weighting for the number of samples, the average concentration would be 0.18 mg/kg, and would have given more weight to the methylmercury concentrations in brown bullhead than the concentrations in the other species. (Note that this averaging approach does not consider that the trophic level 3 fish in this sample are of different sizes, or that some fish might be consumed more or less frequently than is represented by the number of samples.) Equation 4 shows how the total (all trophic levels) weighted concentration is calculated using the 0.15 mg/kg value as representative of trophic level 3 fish and the default consumption for each trophic level:

$$C_{avg} = \frac{3.8 * C_2 + 8.0 * C_3 + 5.7 * C_4}{(3.8 + 8.0 + 5.7)} = 0.23 \text{ mg/kg} \qquad \text{(Equation 4)}$$

Where:

C_2 = average mercury concentration for trophic level 2
C_3 = average mercury concentration for trophic level 3
C_4 = average mercury concentration for trophic level 4

This calculation is based on apportioning the 17.5 grams/day national default consumption rate for freshwater and estuarine fish and shellfish by trophic level (5.7 grams/day of trophic level 4 fish, 8.0 grams/day of trophic level 3 fish, and 3.8 grams/day of trophic level 2 fish[16]). However, as noted throughout this document, the consumption pattern of the target population should be used if available

[16] The values for each trophic level are the same as discussed in section 3.2.1.2., and are found in *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health* (USEPA 2000e).

If fish tissue data from a trophic level are missing, one would drop the consumption factor for that trophic level from both the numerator and denominator. For example, if there were no data for trophic level 2 fish in the previous example, Equation 5 shows the revised calculation:

$$C_{avg} = \frac{8.0 * C_3 + 5.7 * C_4}{(8.0 + 5.7)} = 0.27 \text{ mg/kg} \qquad \text{(Equation 5)}$$

This revised calculation preserves the relative contribution of each trophic level to consumption patterns. However, this approach should not be used if there are no data for trophic level 4 fish, which is the type of fish that is most often eaten. Instead, the state or authorized tribe should collect information to determine the consumption rate for fish in trophic level 4. If the state or authorized tribe finds that no trophic level 4 fish are eaten, the approach can be applied to trophic level 4.

If the state or authorized tribe has developed a site-specific fish consumption rate for the criterion, then the state or authorized tribe should incorporate this site-specific rate in Equation 4 above. In this case, the state or authorized tribe would replace the values of 5.7 grams/day of trophic level 4 fish, 8.0 grams/day of trophic level 3 fish, and 3.8 grams/day of trophic level 2 fish with the values that the state or authorized tribe developed.

As an alternative approach, states or authorized tribes might wish to translate fish tissue sample data to a standard size, length, or species of fish that is more commonly consumed or are representative of the risk considerations of the state. Regression models have been developed for this purpose (Wente 2003, Rae 1997). An inherent assumption is that concentrations will differ between samples of two different species/lengths/sample cuts in a fixed equilibrium distribution relationship among all fish. If this relationship is known and at least one tissue sample concentration is measured from a species/length/sample cut that is accurately described by this relationship, fish consumption risk analyses could be performed for any species/lengths/sample cuts described by the relationship at this site.

Such regression models may include independent variables that account for species, aquatic environment (e.g., lotic vs. lentic, or other waterbody characteristics), sample cut (e.g., whole fish, skin-on fillet, skinless fillet), specific characteristics (e.g., age and retention time) of reservoirs, temporal trends, and fish length. The response variable is fish mercury concentration, which is typically assumed log-normally distributed. In a graphic sense, the model shows the covariance of each combination of nominal scale variables (e.g., whole fish, lentic waterbody) with fish length, with the slope representing the concentration/length ratio. Regression slopes can vary from lake to lake resulting in models that inappropriately retain some fish-size covariation (Soneston 2003).

EPA used the USGS National Descriptive Model of Mercury and Fish to analyze two data sets for use in analysis supporting the CAMR (USEPA 2005a). This model is a statistical model related to covariance and allows the prediction of methylmercury concentrations in different species, cuts, and lengths of fish for sampling events, even when those species, lengths, or cuts of fish were not sampled during those sampling

events. This model can also prove useful to states and authorized tribes in averaging fish tissue across trophic levels.

4.3.3 How should older data be assessed?

For purposes of determining waterbody impairment and inclusion on section 303(d) lists, states and authorized tribes must consider all existing and readily available water-quality related data and information (40 CFR 130.7). Ideally, a state or authorized tribe would have collected fish tissue information within the last 5 years, as recommended in section 4.2.4. However, such information might not be available, and states and authorized tribes will often consider mercury from samples collected and analyzed several years in the past. Although the state and authorized tribe should consider this information, they should also determine the reliability of this information and its accordance with applicable data collection or quality assurance/quality control (QA/QC) program requirements before using these data for listing assessments.

4.3.4 How should fish consumption advisories be used to determine impairment?

On October 24, 2000, EPA issued guidance on the use of fish advisories in CWA section 303(d) listing and 305(b) reporting decisions (USEPA 2000g). This guidance notes EPA's general interpretation that fish consumption advisories on the basis of waterbody specific information can demonstrate impairment of CWA section 101(a) "fishable" uses. Although the CWA does not explicitly direct the use of fish consumption advisories to determine attainment of water quality standards, states and authorized tribes must consider all existing and readily available data and information to identify impaired waterbodies on their section 303(d) lists. For purposes of determining waterbody impairment and inclusion on a section 303(d) list, EPA considers a fish consumption advisory and the supporting data as existing and readily available data and information.

A state or authorized tribe should include on its section 303(d) list, at a minimum, those waters where waterbody-specific data that was the basis of a fish or shellfish consumption advisory demonstrates nonattainment of water quality standards. EPA believes that a fish or shellfish advisory would demonstrate nonattainment when the advisory is based on tissue data, the data are from the specific waterbody in question, and the risk assessment parameters of the advisory or classification are cumulatively equal to or less protective than those in the water quality standards.[17] For example, consider a state or authorized tribe that bases its water quality criterion on eating two fish meals a month. If the state or authorized tribe finds fish tissue information showing that the level of mercury is at a level where it decides to advise people to not eat more than one fish meal a month and all other risk assessment factors are the same, the advisory also may serve to demonstrate a water quality standard exceedence and that the waterbody should be placed on the 303(d) list. In contrast, if this same state or authorized tribe finds the level of mercury in fish in another waterbody is at a level where it would advise people to

[17] The October 2000 EPA guidance assumes that the fish tissue monitoring that supports the advisory is sufficiently robust to provide a representative sample of mercury in fish tissue. EPA's fish tissue guidance (USEPA 2000c) provides recommendations on how public health officials can collect sufficient information about contaminants in fish.

eat no more than 8 meals a month, and all other risk assessment factors are the same, the advisory is not necessarily the same as an impairment, and the waterbody may not need to be listed.

When reporting water quality conditions under CWA sections 303(d) or 305(b) on the basis of a fish advisory for a migratory fish species, the state or authorized tribe should include the waters where the migratory fish are known to inhabit because these are the waters where the fish would become potentially exposed to mercury. In addition, a state or authorized tribe has the discretion to include any other water having a fish consumption advisory as impaired on its section 303(d) list if the state or authorized tribe believes it is appropriate.