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Derivation of Numeric Wildlife Targets for Methylmercury in the Development of a Total Maximum Daily Load for the Guadalupe River Watershed

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I. Introduction

The State of California's Regional Water Quality Control Board – San Francisco Bay Region (RWQCB) is in the process of drafting a Total Maximum Daily Load (TMDL) for mercury in the Guadalupe River Watershed, Santa Clara County, California. Five waterbodies in this watershed (Guadalupe River, Guadalupe Creek, Alamitos Creek, Guadalupe Reservoir, Calero Reservoir) are listed as impaired by mercury, in accordance with the guidance in Section 303(d) of the Clean Water Act (CWA). According to the RWQCB, the primary reason for listing these waterbodies was because mercury concentrations in the watershed's fish were found to be substantially above the U.S. Environmental Protection Agency's (EPA) new CWA Section 304(a) human health criterion for methylmercury (U.S. Environmental Protection Agency, 2001). This criterion was developed as a fish tissue methylmercury concentration of 0.3 mg/kg, wet weight. The criterion exceedances observed in the watershed have resulted in the posting of fish consumption advisories for the county.

In its development of this TMDL effort, the RWQCB has also noted that elevated fish tissue mercury concentrations may pose a threat to piscivorous wildlife in the watershed. As the support and preservation of Wildlife Habitat is one of the designated beneficial uses for surface waters in this watershed, the high fish tissue concentrations observed in the watershed mean that this beneficial use is also impaired. To remove this impairment, the RWQCB is planning to propose numeric targets for the TMDL, expressed as fish tissue methylmercury concentrations, which will be protective of piscivorous wildlife.

As part of an interagency agreement between the U.S. Fish and Wildlife Service (Service) and California's State Water Resources Control Board (SWRCB) (Agreement #02-196-250-0), the Service was funded to develop mercury targets for wildlife protection in various San Francisco Bay watersheds to support TMDL development. It was agreed upon by all parties (*i.e.*, Service, SWRCB, RWQCB) that the Guadalupe River Watershed TMDL would be the first to have these targets developed. This report presents the results of that effort.

II. Methodology

The methodology used in deriving these wildlife targets stemmed from the Service's evaluation of the EPA's human health methylmercury criterion (U.S. Fish and Wildlife Service, 2003). That evaluation was a joint effort between Service and EPA scientists, and the methodology employed to evaluate the criterion was a modification of the procedure used to develop wildlife criteria for the Great Lakes Initiative (GLI) (U.S. Environmental Protection Agency, 1995). The methodology was further refined by the Service and the RWQCB – Central Valley Region during the development of TMDLs for the Cache Creek and Sacramento-San Joaquin Delta Watersheds (U.S. Fish and Wildlife Service, 2004), and this refined version was the basis for the Guadalupe TMDL targets.

The full wildlife target methodology will be developed as a generic TMDL model that may be used for mercury-impaired waters in other California watersheds. That model, a deliverable under the aforementioned interagency agreement, will be presented to the SWRCB under separate cover. This Guadalupe TMDL target report does not provide the detailed, step-by-step

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The full wildlife target methodology will be developed as a generic TMDL model that may be used for mercury-impaired waters in other California watersheds. That model, a deliverable under the aforementioned interagency agreement, will be presented to the SWRCB under separate cover. This Guadalupe TMDL target report does not provide the detailed, step-by-step instructions for applying the generic model, rather it presents the fundamental components of the model and the input parameters specific to the Guadalupe River watershed.

II.A. Selection of Species of Concern

Wildlife currently thought to be most likely at risk from mercury in an aquatic environment are terrestrial species that are primarily or exclusively piscivorous, ingesting methylmercury that has bioaccumulated and biomagnified in their aquatic prey (Wiener *et al.*, 2002). While a variety of aquatic-dependent terrestrial species (*e.g.*, reptiles and amphibians) may be exposed to methylmercury through their diets, research into the effects of methylmercury on wildlife has generally focused on birds and mammals that prey directly on fish and other aquatic organisms. This focus has likely been due to the fact that piscivorous birds and mammals are generally higher order predators than aquatic-dependent reptiles and amphibians, which may result in a greater potential for dietary exposure and subsequent toxicity.

There are two piscivorous mammals common to California; mink (*Mustela vison*) and river otter (*Lutra canadensis*). It is likely that both of these species are present in the Guadalupe watershed and forage in the mercury-impaired waterbodies. However, neither species was included in this analysis because of the reasonable assumption that targets necessary to protect them from adverse dietary exposure would only be equivalent to, or higher than, targets necessary to protect the area's piscivorous birds. This assumption was based on the findings from the aforementioned Cache Creek TMDL effort, and no site-specific information was found for the Guadalupe effort suggesting model input parameters for these mammal species would be different than those used for Cache Creek. In effect, fish tissue targets which are protective of the watershed's piscivorous birds should also protect the mink and river otter.

In researching the Guadalupe watershed's wildlife, it became clear that many piscivorous birds frequent the area at some time during the year (*see* http://www.scvas.org/pdfs/checklist.pdf; http://home.att.net/~redknot/almadenbirdlist.htm). Since the avian toxicological endpoint of interest for mercury is reproductive impairment (see section III.A.), our focus was on those species that forage in the watershed and are resident in or around the watershed during their breeding season. Based both on observational data from the websites provided above and on the opinions of two expert birders familiar with the watershed (personal communications with John Mariani, Birds West Website [http://home.att.net/~redknot/index.htm]; and Bill Bousman, Santa Clara Valley Audubon Society), the following species were selected for fish tissue target development:

Common Merganser (*Mergus merganser*)
Osprey (*Pandion haliaetus*)
Belted Kingfisher (*Ceryle alcyon*)
Great Blue Heron (*Ardea herodias*)
Forster's Tern (*Sterna forsteri*)

Both birding authorities above indicated that common mergansers breed at the Guadalupe watershed's reservoirs, and the observational data provided in the above websites show that belted kingfishers and great blue herons are common year-round residents in the watershed.

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Consideration was also given to whether any State- or Federally-listed threatened and endangered species are present in the Guadalupe watershed, with a focus on those species that are aquatic or aquatic-dependent. The only listed aquatic-dependent bird known to forage in the watershed is the bald eagle (*Haliaeetus leucocephalus*). However, in the personal communications cited above, both birding authorities indicated that bald eagles are only winter visitors to the watershed's reservoirs, and are not known to breed near the watershed.

Another listed aquatic-dependent bird, the California least tern (*Sterna antillarum browni*), is known to live and breed in the San Francisco Bay area (Goals Project, 2000). Mercury from the Guadalupe watershed may contribute to fish tissue concentrations in the south San Francisco Bay area, an area in which California least terns are known to forage. However, least terns were not considered in this target report because they are likely not foraging directly in the Guadalupe watershed, and because they are being considered under the mercury TMDL for San Francisco Bay (California Regional Water Quality Control Board, San Francisco Bay Region, 2004).

Both the Central California Coast Steelhead (*Oncorhynchus mykiss*) and Fall-Run Chinook Salmon (*Oncorhynchus tshawytscha*) are known to use the Guadalupe River (pers. comm., Joseph Dillon, NOAA Fisheries). The steelhead is federally listed as threatened. The fall-run Chinook is not listed; however, it is regulated by NOAA Fisheries under the Magnuson-Stevens Fishery Conservation and Management Act. While both species are large, predatory fish, they were not considered in this target report for two reasons: 1) being anadromous, neither species spends considerable amounts of time in the river over the course of a year, and 2) our evaluation of the EPA's human health methylmercury criterion indicated that fish tissue targets necessary to protect piscivorous birds and mammals were likely sufficient to protect fish (U.S. Fish and Wildlife Service, 2003).

There are other non-listed aquatic-dependent wildlife species present in the watershed (*e.g.*, green heron [*Butorides virescens*], black-crowned night-heron [*Nycticorax nycticorax*], western grebe [*Aechmophorus occidentalis*], snowy egrets [*Egretta thula*]) for which fish constitute a substantial portion of the diet. However, the five species selected are more likely to have a diet comprised entirely of fish. It is assumed that a fully piscivorous diet, rather than one which includes a substantial percentage of lower trophic level invertebrates or terrestrial organisms, represents a maximum exposure to mercury in the aquatic ecosystem.

II.B. Average Concentration Trophic Level Approach

Once a decision has been made to develop wildlife targets based on dietary exposure to methylmercury, a relatively simple equation can be used to calculate a protective concentration for the overall diet of a given species. Given sufficient methylmercury toxicity data to determine

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$$\mathbf{WV} = \frac{\mathbf{RfD \times BW}}{\mathbf{FIR}} \tag{1}$$

where: WV = Wildlife Value (mg/kg in diet)
RfD = Reference Dose (mg/kg of body weight/day)
BW = Body Weight (kg) for species of concern
FIR = Total Food Ingestion Rate (kg of food/day) for species of concern

The WV represents the concentration of methylmercury, as an average in all the prey consumed, necessary to keep the organism's daily ingested amount at or below a sufficiently protective reference dose. Reference doses (RfD) may be defined as the daily exposure to a toxicant at which no adverse effects are expected. As discussed in further detail below in Section III.A., the adverse effect associated with methylmercury and birds is impaired reproductive success. In effect, Equation 1 converts a protective RfD into an overall dietary concentration (in mg/kg in diet) needed to prevent reproductive toxicity.

For certain piscivorous species, a calculated WV may be acceptable to use as the protective wildlife target. This situation exists when the food consumed by the species of concern is sufficiently uniform so that methylmercury concentrations in the individual prey items are expected to be roughly equivalent. One example of this situation is when the species' diet is comprised of equivalently sized fish from the same trophic level. Trophic levels are general classifications applied to the various biotic components of a food chain, and organisms are placed in these classifications depending on what they consume. Stated in its most simplistic form, trophic level 1 plants are consumed by trophic level 2 herbivores, which are consumed by trophic level 3 predators, which are then consumed by the top predators in trophic level 4. Although the bioaccumulation of methylmercury may vary between fish species, it may be assumed that those fish occupying similar ecological niches (*e.g.*, trophic level 3 fish feeding on the same trophic level 2 prey base) will contain similar tissue concentrations of methylmercury. Trophic levels used in this evaluation were based on definitions provided in Volume II of *Trophic Level and Exposure Analyses for Selected Piscivorous Birds and Mammals* (U.S. Environmental Protection Agency, 2002a):

Trophic Level 1 - Aquatic Plants (*e.g.*, periphyton, phytoplankton)
Trophic Level 2 - Herbivores and Detritivores (*e.g.*, copepods, water fleas)
Trophic Level 3 - Predators on trophic level 2 organisms (*e.g.*, minnows, sunfish, suckers)
Trophic Level 4 - Predators on trophic level 3 organisms (*e.g.*, adult trout, bass, pike)

In contrast to wildlife species with uniform diets, many predators that feed from aquatic ecosystems are more opportunistic and will consume prey from more than one trophic level. Because of methylmercury biomagnification, aquatic food chains do not attain a steady-state condition whereby aquatic biota from all trophic positions exhibit the same tissue concentrations. Instead, organisms higher on the aquatic food chain contain greater concentrations than those a dietary dose at which no adverse effects to an organism are expected, a protective methylmercury concentration in the overall diet can be calculated using Equation 1, based on information about that organism's body weight and daily food consumption:

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The Average Concentration Trophic Level (TL) Approach is one method by which the principles of trophic transfer can be used to estimate trophic level-specific limiting concentrations for these wildlife species. Food web dynamics in real-world ecosystems are generally more complex than the simple linear food chain model described above (*i.e.*, TL1 → TL2 → TL3 → TL4); however, the methodology employed in this approach is based on the assumption that the general concepts underlying this food chain model remain valid for considering the trophic transfer of methylmercury in aquatic biota.

As mentioned above, the WV represents an average concentration of methylmercury in the overall diet necessary to keep the organism's daily ingested amount at or below a sufficiently protective reference dose. Another way the WV can be expressed is by the equation:

$$\mathbf{WV = (\%TL2 \times FDTL2) + (\%TL3 \times FDTL3) + (\%TL4 \times FDTL4)} \qquad \mathbf{(2)}$$

where: %TL2 = Percent of trophic level 2 biota in diet
%TL3 = Percent of trophic level 3 biota in diet
%TL4 = Percent of trophic level 4 biota in diet
FDTL2 = concentration in food (FD) from trophic level 2
FDTL3 = concentration in food from trophic level 3
FDTL4 = concentration in food from trophic level 4

Determining the dietary percentage for the various trophic levels may be accomplished by reviewing the scientific literature for a particular species, or extrapolated from information about a similar species. However, before all the trophic level concentrations can be determined, Equation 2 must be rearranged so that it can be solved for one of the trophic levels (*e.g.*, FDTL2). This requires that the other trophic level components of the equation be expressed as a function of the one to be solved (*i.e.*, FDTL3 = FDTL2 × some linkage value). With methylmercury, these linkage values can be derived from the relationships of bioaccumulation and biomagnification between trophic levels, expressed as **food chain multipliers** (FCM_x):

FCM3 = Food chain multiplier from TL2 to TL3 biota
FCM4 = Food chain multiplier from TL3 to TL4 biota

The FDTL3 and FDTL4 terms can then be expressed as functions of FDTL2:

FDTL3 = FDTL2 × FCM3
FDTL4 = FDTL2 × FCM3 × FCM4

This allows Equation 2 to be rearranged, substituting food chain multiplier equivalents, as:

$$\mathrm{WV} = (\%\mathrm{TL2} \times \mathbf{FDTL2}) + (\%\mathbf{TL3} \times \mathbf{FDTL2} \times \mathbf{FCM3}) + (\%\mathbf{TL4} \times \mathbf{FDTL2} \times \mathbf{FCM3} \times \mathbf{FCM4}) \qquad \mathbf{(3)}$$

This equation can then be solved for the concentration in the lowest trophic level:

$$\mathbf{FDTL2} = \mathbf{WV} / [(\%\mathbf{TL2}) + (\%\mathbf{TL3} \times \mathbf{FCM3}) + (\%\mathbf{TL4} \times \mathbf{FCM3} \times \mathbf{FCM4})] \qquad \mathbf{(4)}$$

Once the concentration in trophic level 2 is determined, the remaining trophic level concentrations can be calculated using the food chain multiplier relationships:

$$\mathbf{FDTL3} = \mathbf{FDTL2} \times \mathbf{FCM3} \qquad \mathbf{(5)}$$

$$\mathbf{FDTL4} = \mathbf{FDTL3} \times \mathbf{FCM4} \qquad \mathbf{(6)}$$

Food chain multipliers can be determined several ways, depending on the information available. For example, bioaccumulation factors (BAFs) are numeric values showing the amount of contaminant uptake into biota, relative to concentrations in the water column. These BAFs can be determined for each trophic level of aquatic biota. The food chain multiplier for any given trophic level is the ratio of the BAF for that trophic level to the BAF for the trophic level directly below.

For example: BAF for water to trophic level 4 = 680,000
BAF for water to trophic level 3 = 160,000

FCM4 = 680,000/160,000 = 4.25

Any methylmercury concentration estimated for trophic level 3 biota can then multiplied by the FCM4 to estimate the expected concentration in trophic level 4 biota.

If sufficient data on existing fish tissue methylmercury concentrations are available, food chain multipliers can also be established using the ratio of these concentrations between trophic levels.

For example: Average tissue concentration in TL4 fish = 0.15 mg/kg
Average tissue concentration in TL3 fish = 0.05 mg/kg

FCM4 = 0.15/0.05 = 3

Both of these approaches to determining food chain multipliers assume there is a direct consumption link between the trophic levels, with methylmercury concentrations in the higher trophic level fish resulting from ingesting the concentrations found in fish from the next lower trophic level. Because of this assumption, using either approach to calculate methylmercury targets for specific trophic levels requires that the resultant limiting concentrations be applied to the appropriate food chain cohorts (*e.g.*, a limiting concentration for TL3 must be applied to the species and size class of fish that would be consumed by larger predatory TL4 fish). This distinction is important because some TL3 fish will grow as large or larger than co-occurring

TL4 fish, and the relationship between these fish may not be one of predator and prey. For example, a 250 mm sunfish (TL3) is too large to be consumed by a 250 mm smallmouth bass (TL4).

Therefore, using existing fish tissue data to calculate a concentration ratio between trophic levels may not necessarily represent a food chain multiplier. In the example mentioned above, the ratio between methylmercury concentrations in same-size bass and sunfish (*i.e.*, TL4 concentration/ TL3 concentration) would only represent the concentration relationship between similarly sized fish feeding at different positions in the food chain. In effect, these data simply provide a **trophic level ratio** (TLR) rather than a food chain multiplier. However, substituting trophic level ratios in place of food chain multipliers in the Average Concentration TL Approach (*e.g.*, WV = [%TL3 × FDTL3] + [%TL4 × FDTL3 × TLR]) is an equally valid way to develop fish tissue targets, with the following caveats: 1) the fish prey of the wildlife species of concern must be approximately the same size, regardless of trophic level, and 2) the resultant limiting concentrations calculated with these trophic level ratios are applied to the appropriate size classes of fish (*i.e.*, using the example of bass and sunfish provided above, the limiting concentration for TL3 must be applied to fish 250 mm or larger, *not* to the small individuals that would be preyed upon by large TL4 fish).

Both caveats stem from the general trend of increasing tissue methylmercury concentrations with increasing fish size (Wiener and Spry, 1996). Because of this size-concentration relationship, a trophic level ratio based on the concentrations in similarly sized TL3 and TL4 fish will be smaller than a ratio based on concentrations in small TL3 fish and the TL4 fish that prey on them (*i.e.*, a food chain multiplier). If the wildlife target is based on a concentration ratio between large, similarly sized TL3 and TL4 fish, but the TL3 component of the wildlife species' diet is actually comprised of small fish, the contribution to the daily ingested dose from the TL3 component is overestimated. This overestimation would then result in a target concentration for TL3 that is larger than it should be for the small prey fish consumed, and a target concentration for TL4 that is smaller than it should be for the large predatory fish consumed.

Food chain multipliers and trophic level ratios are only necessary when determining targets for those wildlife species that feed from different trophic levels. As discussed above, trophic level ratios may be more appropriate when the wildlife species' prey base is comprised of similarly sized fish, regardless of the trophic level. In contrast, food chain multipliers may be more appropriate when the wildlife species consumes a broad size range of fish, including small TL3 fish and the larger TL4 fish that prey on them.

III. Calculating Wildlife Targets with Average Concentration TL Approach

In order to perform the Average Concentration TL Approach for the Guadalupe River Watershed TMDL wildlife species, WVs for each species were generated. This required species-specific information on average adult female body weights (kg) and daily food ingestion rates (FIR *in* kg of food/day). It also required determining a protective avian RfD. Once the WVs were determined, information about the dietary composition and prey size for each species allowed for the calculation of trophic level- and size-specific fish tissue methylmercury targets. All of these parameters are discussed below.

III.A. Reference Doses

In order to calculate the Guadalupe wildlife targets, the Service used an avian methylmercury RfD of **0.021 mg/kg-bw/day**. This RfD is based on a test dose generated by controlled feeding studies using mallard ducks, in which the toxicological endpoint was impaired reproductive success (Heinz, 1979). Reference doses are derived by applying various uncertainty factors to test doses to estimate the daily exposure at which no adverse effects are expected. A full discussion of the development of this RfD can be found in the Service's evaluation of the human health methylmercury criterion (U.S. Fish and Wildlife Service, 2003).

III.B. Adult Female Body Weights

Because the most sensitive endpoints for methylmercury toxicity in birds relate to reproduction, the focus of the Average Concentration TL Approach is to establish WVs based on preventing adverse reproductive impacts from maternally ingested methylmercury. As body weight influences the estimation of total food ingestion, with both factors affecting the estimation of WVs, it is most appropriate to use the best available information for adult female body weights.

Table 1. Adult Female Body Weights (kg) for Guadalupe Watershed Wildlife Species

Species	Weight (kg)
Great Blue Heron	2.20
Osprey	1.75
Common Merganser	1.23
Forster's Tern	0.16
Belted Kingfisher	0.15

The values for great blue heron, common merganser, Forster's tern, and belted kingfisher come from female body weight means presented by Dunning (1993). The mean value for female osprey in that reference is 1.568 kg; however, the Service used a slightly higher value of 1.75 kg. Our value is based on an approximation from data presented in the more recent compilation by Poole *et al.* (2002), and on the fact that female osprey may be close to 25 percent larger than males. No information was found in the scientific literature indicating a California-specific variation in body weight was required for any of the species.

III.C. Food Ingestion Rates

All five of the species of concern for this effort are predominantly piscivorous, although each may occasionally consume non-fish prey (*e.g.*, benthic invertebrates, amphibians, small mammals) (U.S. Environmental Protection Agency, 2002b). When considering each species, it is reasonable to assume that, at any given time, the diet may be comprised solely of fish prey. Although some non-fish aquatic prey such as crayfish may bioaccumulate methylmercury at higher rates than fish in a similar trophic position, a diet comprised solely of fish may represent a maximum potential exposure to methylmercury in the aquatic ecosystem. For this reason, we based our estimates of daily food ingestion rates (FIR) on the assumption that each species of concern consumed only fish.

Allometric calculations to determine FIRs for numerous wildlife species have been developed by Nagy (1987 and 2001), based on measurements of free-living metabolic rates (FMR) and the metabolizable energy (ME) in various foods (*e.g.*, fish, birds, mammals). Generic allometric equations from Nagy (1987) to calculate FIRs for broad categories (*e.g.*, all birds, passerines, seabirds) were presented in the *Wildlife Exposure Factors Handbook* (U.S. Environmental Protection Agency, 1993). These equations provide FIR in grams of dry matter per day, which can then be converted to wet weight based on percent moisture in the food. More recent work by Nagy (2001) expanded on the development of generic allometric equations, providing both dry weight and wet weight calculations for a broader range of distinct wildlife categories (*e.g.*, Charadriiformes, Galliformes, Insectivorous Birds, Carnivorous Birds). Since all the generic allometric equations are based on the compilation of metabolic data from a wide range of species, they may not provide the most accurate estimate of FIRs for specific species of concern. If available, estimates of FMR, dietary composition, and assimilation efficiency (AE) for the species of concern should be considered, as this information will provide a more accurate estimate of daily food requirements.

For the Guadalupe TMDL effort, we followed this latter procedure to determine FIRs, which were calculated by dividing each species' FMR by the ME of its food. According to the *Wildlife Exposure Factors Handbook* (U.S. Environmental Protection Agency, 1993), ME equals the gross energy (GE) of the food in kcal/g wet weight times the assimilation efficiency (AE) of the consumer for that food. The EPA Handbook gives a GE value of 1.2 kcal/g for bony fishes, while an AE of 79 percent is given for eagles and seabirds consuming fish. With these two values, **an ME value of 0.948 kcal/g fish** was calculated (*i.e.*, 1.2 kcal/g × 0.79 = 0.948). The species-specific FMRs were calculated using Nagy's (1987) equation for all birds:

$$\text{FMR (kcal/day)} = 2.601 \times \text{body weight (g)}^{0.640}$$

Similar equations were presented for sub-groups of the 'all bird' category (*i.e.*, passerines, non-passerines, seabirds, non-seabirds). The 'all bird' equation was derived from a broad dataset, rather than from smaller subsets of data, and the Service felt that none of these sub-groups provided a better fit for the Guadalupe species of concern. Using an ME value of 0.948 kcal/g fish, adult female body weights from Table 1, and the equation above to calculate FMRs, species-specific FIRs were calculated using the following equation and are presented below in Table 2:

$$\text{FIR (g/day)} = \text{FMR (kcal/day)} \div \text{ME (kcal/g fish)}$$

Table 2. Total Food Ingestion Rates (expressed as *kg/day*) for Guadalupe Watershed Wildlife Species

Species	FMR (kcal/day)	FIR = FMR / ME
Great Blue Heron	358	0.378
Osprey	310	0.327
Common Merganser	247	0.261
Forster's Tern	67	0.071
Belted Kingfisher	64	0.068

III.D. Calculation of Wildlife Values

Having determined the appropriate RfD, as well as the body weights and FIRs for all species of concern, the next step in the Average Concentration TL Approach was to calculate WVs for each species, presented in Table 3. This was done using Equation 1, described previously:

$$\mathbf{WV} = \frac{\mathbf{RfD \times BW}}{\mathbf{FIR}}$$

Table 3. Wildlife Values (mg/kg in diet) for Guadalupe Watershed Wildlife Species

Species	RfD (mg/kg/day)	Body Weight (kg)	FIR (kg/day)	WV (mg/kg in diet)
Great Blue Heron	0.021	2.20	0.378	0.122
Osprey	0.021	1.75	0.327	0.112
Common Merganser	0.021	1.23	0.261	0.099
Forster's Tern	0.021	0.16	0.071	0.047
Belted Kingfisher	0.021	0.15	0.068	0.046

III.E. Trophic Level Dietary Composition and Prey Size

As discussed previously, the trophic level composition of a wildlife species' diet is a critical factor influencing how much methylmercury is ingested on a daily basis. While WVs provide information about the methylmercury concentration in the overall diet necessary to maintain the daily ingested amount at a protective reference dose, an understanding of the animal's dietary composition is essential for determining what the concentrations need to be in the prey from each trophic level.

It must also be noted that numeric trophic levels are artificial constructs, and the trophic position of higher order aquatic organisms is often more complex than a single assigned TL number. For example, a fish species is considered TL3 if it eats TL2 zooplankton. But the species may also consume other small TL3 fish and aquatic insects. By including these higher order prey items in its diet, the species is behaving as a partial TL4 consumer. When the entire diet is considered, the TL of the species may be some value between 3 and 4 (*e.g.*, 3.5).

This dietary elasticity in higher order aquatic organisms can complicate the process of determining the TL composition of a piscivorous wildlife species' diet. When a piscivorous animal eats a variety of fish that occupy intermediate trophic positions (*e.g.*, TL3.5, TL2.2), it can be difficult to assign portions of the diet to discrete numeric trophic levels. Adding to this difficulty is the fact that the aquatic prey of a wildlife species can vary both temporally and spatially. Feeding ecology studies conducted for a specific waterbody or watershed can minimize these difficulties; however, such studies remain rare for the vast majority of piscivorous species and geographic locations.

In an effort to determine the dietary composition for each of the five wildlife species considered, presented below in Table 4, the scientific literature was searched for any pertinent feeding ecology studies. This effort included Internet searches (*e.g.*, Searchable Ornithological Research Archive [http://elibrary.unm.edu/sora/]; Google Scholar), and examination of the EPA's *Trophic Level Analyses for Selected Piscivorous Birds and Mammals, Volume III: Appendices* (U.S. Environmental Protection Agency, 2002b). No studies specific to the Guadalupe watershed were found, and studies specific to California were only found for two of the five species (osprey and Forster's tern).

The EPA's *Trophic Level Analyses* document provides detailed accounts on dietary studies from all over North America, including assessments of the average trophic level of the wildlife species' prey. Each of the five wildlife species considered in the Guadalupe watershed have dietary accounts in this document, including the California-specific studies for osprey and Forster's tern, and most are based on numerous individual studies. For all the Guadalupe wildlife species, the average prey trophic level in all these individual studies is right around TL3, with minor variations in both directions (*e.g.*, TL2.9; TL3.2).

Because of the difficulty in developing targets based on intermediate TL numbers, as discussed previously, and because the deviations in the EPA average prey trophic levels were small, the Service assigned a dietary composition of 100 percent TL3 fish for four of the five Guadalupe wildlife species: great blue heron, common merganser, belted kingfisher, and Forster's tern. For the osprey, we assigned a dietary composition of 90 percent TL3 and 10 percent TL4. This decision was based on the fact that osprey are known to occasionally capture large TL4 predatory fish such as bass (*Micropterus* spp.), and because the fish assemblages in Guadalupe watershed reservoirs are heavily centrarchid-influenced (pers. comm., Thomas Grieb, Tetra Tech, Inc.). While the actual osprey diet in the watershed may consist of more TL4 fish, we believe a 10 percent contribution is a reasonable assumption.

Table 4. Trophic Level Compositions (% of overall diet, expressed as decimal fractions) for Guadalupe Watershed Wildlife Species

Species	TL3	TL4
Great Blue Heron	1.00	--
Osprey	0.90	0.10
Common Merganser	1.00	--
Forster's Tern	1.00	--
Belted Kingfisher	1.00	--

Prey size is another important dietary consideration when determining wildlife targets, although size information is not essential for determining limiting trophic level concentrations. There can be wide variations in the sizes of prey fish consumed by wildlife, even though all the prey fish may occupy the same trophic position. For example, the Forster's tern generally consumes TL3 fish less than 50 mm in length (McNicholl *et al.*, 2001), while a great blue heron may mainly capture TL3 fish between 150-300 mm (Butler, 1992). Although it is conceivable that these two sizes of prey fish have bioaccumulated equal amounts of methylmercury in their tissues, there is a greater likelihood that the larger fish have built up higher tissue levels over longer lifespans.

This proportional bioaccumulation can also increase the complexity in developing targets for multiple wildlife species. For example, taking the WV (0.047 mg/kg) calculated for small TL3 fish consumed by Forster's terns and applying it to larger TL3 prey of the great blue heron may be overly stringent. Conversely, taking the WV (0.122 mg/kg) calculated for TL3 fish consumed by great blue herons and applying it to the smaller TL3 fish (<50 mm) would allow concentrations that may place the Forster's tern at risk for adverse effects from methylmercury toxicity. An understanding of proportional bioaccumulation specific to the watershed may prevent these two extremes when setting appropriate wildlife targets.

By collecting data on the methylmercury concentrations in prey fish size classes appropriate for the wildlife species of concern, one may find that a target for the larger TL3 prey of great blue herons will be attained naturally if the target for the smaller TL3 Forster's tern prey is met. In essence, there may only be a need for one compliance monitoring target, even though targets are developed for several wildlife species. This determination cannot be made without first determining the appropriate size classes for the wildlife prey and then performing waterbody-specific fish tissue sampling and analyses to examine the degree of proportional bioaccumulation present in the system.

For the Guadalupe wildlife species, the Service again consulted the scientific literature to determine the appropriate prey size classes. As mentioned above, great blue herons are known to capture fish up to 300 mm in length or longer; however, they also consume fish less than 50 mm long (Butler, 1992; U.S. Environmental Protection Agency, 2002a). The critical period when considering the potential for reproductive effects from ingested methylmercury is immediately prior to egg formation and laying. The period from courtship to egg laying for great blue herons in California is early January to mid-March (Butler, 1992). According to a feeding ecology study conducted in New Jersey, the majority of fish captured by great blue herons in November and December were between 50-150 mm in length (Willard, 1977). Based on this information, the Service recommends that the target for protecting great blue herons be applied to prey fish in the size range of 50-150 mm.

As mentioned previously, osprey are known to capture large fish. The osprey species account by Poole *et al.* (2002), from *The Birds of North America* series, states that prey fish generally measure about 250-350 mm in length. Other studies have shown a broader range, with a substantial percentage of the osprey diet consisting of fish around 150 mm in length (U.S. Environmental Protection Agency, 2002a). In several cases, fish less than 150 mm or fish greater than 350 mm contributed to the osprey diet; however, these contributions were relatively minor to the overall diet. Three of the studies summarized in this EPA reference were from California locations; two coastal locations where the osprey fed on marine species like anchovies and surf smelt (*Hypomesus pretiosus*), and one inland lake location (Eagle Lake) where osprey fed on salmonids, cyprinids, and suckers. The average length of fish from this inland study was 310 mm. At one of the coastal locations (Humboldt Bay), over 75 percent of the fish caught were between 100-300 mm long. At the second coastal location, osprey nested on and fed from the river (Usal Creek), or nested near and fed from the ocean. Fish prey from the river nests were between 230-410 mm, while those from the ocean nests were only between 130-150 mm long. After reviewing this information, the Service recommends that the target for protecting osprey be applied to prey fish in the size range of 150-350 mm.

According to the common merganser account from *The Birds of North America* series, these birds generally eat fish between 100-300 mm long, and fish up to 360 mm are commonly consumed (Mallory and Metz, 1999). This reference also states that common mergansers will "...choose disproportionately more large fish compared with available sizes," and that there are reports of mergansers capturing and consuming eels up to 550 mm long. Based on this information, the Service recommends that the target for protecting common mergansers also be applied to the prey fish size range of 150-350 mm.

As noted above, the Forster's tern generally consumes fish less than 50 mm in length, although the prey size can range from 10-100 mm (McNicholl *et al.*, 2001). One feeding study was found that examined the prey of Forster's terns in Monterey County, California (Baltz *et al.*, 1979). Although the prey species were from the marine environment (*e.g.*, northern anchovy [*Engraulis mordax*]), as opposed to the freshwater environment of the Guadalupe watershed reservoirs, the majority of prey items examined were less than 50 mm long. For this reason, the Service recommends that the target for protecting Forster's terns be applied to prey fish less than 50 mm in length.

Although kingfishers generally capture fish less than 102 mm, and fish longer than 127 mm are thought to be difficult for kingfishers to swallow (Hamas, 1994), they can occasionally consume fish as long as 180 mm (U.S. Environmental Protection Agency, 2002a). Based on the results from several feeding ecology studies, it appears that the majority of prey fish consumed by kingfishers are between 50-100 mm, with both smaller and larger fish contributing to the diet

(U.S. Environmental Protection Agency, 2002a). As the contribution from larger prey fish (>100 mm) increases the potential dietary exposure to methylmercury, the Service recommends that the target for protecting belted kingfishers be applied to prey fish in the size range of 50-150 mm.

IV. Determining Trophic Level- and Size-Specific Methylmercury Targets

As discussed earlier in Section II.B., there are several ways that the Average Concentration TL Approach can be used to develop limiting methylmercury concentrations protective of wildlife, each one dependent on the dietary habits of the species of concern. When the diet of a species consists of similar prey from the same trophic level, a WV calculated with Equation 1 is sufficient to use as the protective target. In contrast, when the diet consists of prey from different trophic levels, multiple targets must be determined by considering the dietary trophic level composition and by incorporating either food chain multipliers (FCM) or trophic level ratios (TLR) into the model. It may also be necessary to form a hybrid calculation, combining information about FCMs and TLRs in one equation. Two of these iterations were necessary, each discussed below, to develop targets for the various Guadalupe watershed wildlife species examined here. Values used in all target calculations were not rounded; however, all final targets were rounded to two significant digits.

IV.A. Using Wildlife Values Only to Determine Wildlife Targets

Four of the Guadalupe watershed wildlife species (great blue heron, common merganser, Forster's tern, and belted kingfisher) were assumed to have diets comprised solely of TL3 fish. For this reason, the WVs calculated with the appropriate reference dose, body weights, and food ingestion rates (from Table 3) can serve as protective wildlife targets.

To sufficiently protect the great blue heron, **TL3 fish between 50-150 mm in length should have methylmercury concentrations no greater than 0.12 mg/kg, wet weight.**

To sufficiently protect the common merganser, **TL3 fish between 150-350 mm in length should have methylmercury concentrations no greater than 0.10 mg/kg, wet weight.**

To sufficiently protect the Forster's tern, **TL3 fish less than 50 mm in length should have methylmercury concentrations no greater than 0.05 mg/kg, wet weight.**

To sufficiently protect the belted kingfisher, **TL3 fish between 50-150 mm in length should have methylmercury concentrations no greater than 0.05 mg/kg, wet weight.**

IV.B. Using Trophic Level Ratios to Determine Wildlife Targets

In contrast to the four species above, the osprey feeds on large TL3 and TL4 fish. The size of fish consumed by adult osprey does not vary significantly, regardless of which trophic position the fish occupy. It is likely there is no predator-prey relationship between the TL4 and TL3 fish consumed, as TL3 fish of this size are likely too large to be preyed upon by similarly sized TL4 fish. Therefore, it is more appropriate to use the TLR iteration of the Average Concentration TL Approach in developing targets for this species.

As described earlier in Section II.B., substituting TLRs in place of FCMs in the Average Concentration TL Approach (*e.g.*, WV = [%TL3 × FDTL3] + [%TL4 × FDTL3 × **TLR**]) is an equally valid way to develop fish tissue targets, with the following caveats: 1) the fish prey of the wildlife species of concern must be approximately the same size, regardless of trophic level, and 2) the resultant limiting concentrations calculated with these trophic level ratios are applied to the appropriate size classes of fish (*i.e.*, the limiting concentration for TL3 must be applied to fish in the size range used to calculate the target, *not* to the smaller TL3 individuals that would be preyed upon by large TL4 fish).

In order to follow the TLR approach, the concentration relationship between similarly sized fish from both trophic levels must be determined. This requires data on tissue concentrations in fish from both trophic levels, preferably from the waterbodies of interest. In 2004, an attempt was made by the TMDL stakeholders (EPA, RWQCB, Santa Clara Valley Water District) to obtain tissue concentration data on large TL3 and TL4 fish from the watershed; however, they did not collect sufficient numbers of TL3 fish to determine a rigorous concentration relationship.

As there is no way to extrapolate methylmercury concentrations in the large TL3 fish from the tissue concentration data for large TL4 fish generated from the sampling attempt, some other means to determine the concentration relationship between the two trophic levels had to be used. A default FCM could be used, as was done in our evaluation of the EPA human health criterion (U.S. Fish and Wildlife Service, 2003). The FCMs from that effort were based on the ratios between draft national BAFs. However, FCMs represent the difference between tissue concentrations in the organisms from one trophic level and tissue concentrations in the lower trophic level organisms they consume. Because there is likely no predator-prey relationship between the TL4 and TL3 fish consumed by osprey, the option of using an FCM from our previous evaluation effort to develop the Guadalupe targets would have been inappropriate.

A large fish tissue dataset was presented by the Central Valley RWQCB in the previously mentioned Cache Creek TMDL effort, and was used by the Service in our evaluation of proposed wildlife targets (U.S. Fish and Wildlife Service, 2004). Based on fish tissue data from six sub-watersheds in the Cache Creek area, the Service calculated that the average TLR between TL3 and TL4 fish greater than 180 mm in length was 1.7. We did not include in our calculations data from fish between 150-180 mm in length, nor did we restrict the dataset to fish 350 mm or less. It is unknown what effect incorporating a size range of 150-350 mm on that dataset analysis would have had on the final average TLR. While all these data were from a different watershed than the Guadalupe's, it is reasonable to assume that a TLR for the Guadalupe watershed's fish would be similar in magnitude. Some of the Cache Creek sub-watershed TLR calculations resulted in TLRs of 2 or above, and because our osprey prey size range for the Guadalupe watershed includes fish between 150-180 mm in length, the Service selected a TLR of 2 to develop protective targets for osprey.

The osprey's WV is 0.112 mg/kg and its dietary composition is assumed to be 90 percent TL3 fish and 10 percent TL4 fish. Because TL2 fish are not a component of the osprey's diet, Equation 3 can be modified as:

$$WV = (\%TL3 \times FDTL3) + (\%TL4 \times FDTL4)$$

Substituting the TLR equivalent, this can further be arranged as:

$WV = (\%TL3 \times FDTL3) + (\%TL4 \times FDTL3 \times TLR)$

Then, substituting the above values for WV and dietary composition:

$0.112 = (0.90 \times FDTL3) + (0.10 \times FDTL3 \times 2)$

Solving this equation for FDTL3:

$FDTL3 = 0.112/[(0.90) + (0.2)]$
FDTL3 = 0.112/1.1 = **0.1018 mg/kg**

Once the FDTL3 concentration is calculated, the FDTL4 concentration can be determined using the TLR relationship:

$FDTL4 = FDTL3 \times TLR$
FDTL4 = 0.1018 mg/kg × 2
FDTL4 = 0.2036 mg/kg

Thus, to sufficiently protect osprey, **TL3 fish and TL4 fish between 150 - 350 mm in length should have methylmercury concentrations no greater than 0.10 and 0.20 mg/kg, wet weight, respectively.**

V. Summary of the Guadalupe River Watershed Wildlife Targets

Using various iterations of the Average Concentration TL Approach and all the various exposure parameters described above, protective targets for the five wildlife species of concern in the Guadalupe River watershed are presented below in Table 5.

Table 5. Protective Targets (*in* mg/kg, wet weight) for Guadalupe Watershed Wildlife Species

	TL3 Fish < 50 mm	TL3 Fish 50-150 mm	TL3 Fish 150-350 mm	TL4 Fish 150-350 mm
Great Blue Heron		0.12		
Osprey			0.10	0.20
Common Merganser			0.10	
Forster's Tern	0.05			
Belted Kingfisher		0.05		

As some of the species and prey fish size classes overlap, a closer examination of the suite of targets allows for target recommendations to protect all five species. The target for belted kingfisher (0.05 mg/kg), which should be applied to TL3 fish between 50-150 mm long, is sufficient to also protect the great blue heron. It should also be protective of the Forster's tern, due to the concept of proportional bioaccumulation, which suggests that fish smaller than 50 mm long should have less methylmercury than those between 50-150 mm. The target for common mergansers (0.10 mg/kg), which should be applied to TL3 fish between 150-350 mm in length, is also the TL3 fish target concentration determined protective of osprey. If methylmercury is bioaccumulating proportionally in the watershed, it may be that the ratio between the kingfisher target and the merganser/osprey target (*i.e.*, 0.10 ÷ 0.05 = 2) would be naturally attained. Similarly, the TLR between TL3 and TL4 fish in the 150-350 mm size range may also occur naturally. However, both assumptions should be verified through appropriate fish tissue monitoring.

Therefore, the Service recommends methylmercury values of 0.05 mg/kg in TL3 fish between 50-150 mm long and 0.10 mg/kg in TL3 fish between 150-350 mm long be set as the protective wildlife targets for the Guadalupe River watershed. In addition, a fish tissue monitoring plan should be developed to determine whether the assumptions about proportional bioaccumulation between these two size classes and about the TL4/TL3 ratio are valid for the watershed. Should both assumptions hold, it would be reasonable to assign one target concentration (*i.e.*, 0.10 mg/kg in 150-350 mm TL3 fish) that would be protective of all wildlife species in the watershed.

The recommendations and Service-derived numeric targets presented in this evaluation are intended to assist the RWQCB in its development of a final TMDL for the Guadalupe River watershed. The Service targets were based, in part, on a variety of assumptions regarding the wildlife species of concern and fish tissue methylmercury concentration relationships in the watershed. We recognize that additional data from the watershed may result in changes to these assumptions and the subsequent wildlife targets.

Throughout the development of this evaluation, staff from the Service and RWQCB have worked closely to share information and insights on the approaches presented. We believe this cooperative effort has been an invaluable asset toward achieving the goal of protective wildlife targets. The Service remains available to assist the RWQCB as it completes the TMDL for the Guadalupe River watershed.

VI. Target Location Based on Habitat Type

Having developed targets that should be protective of all wildlife in the watershed, the Service considered how and where the five species of concern might forage to see if the targets should be applied on a habitat-specific basis. The Guadalupe watershed has three distinct habitat types (upper watershed creeks, reservoirs, Guadalupe River), and the five wildlife species likely do not forage in these habitats with equal intensity. Species accounts from *The Birds of North America* series for each of the five birds were evaluated to determine whether they had preferential foraging habitats.

Butler (1992) reported that the great blue heron is a sight predator, waiting or wading in shallow water until prey is located. They are known to forage in a variety of microhabitats, such as wetlands, ponds, lakes, and riverbanks. Great blue herons in the Guadalupe watershed are likely to forage in slow, shallow segments of the Guadalupe River, shallow edges of the reservoirs, and possibly in the upper watershed creeks.

The foraging habitats used by osprey are similarly broad, including rivers, marshes, reservoirs, and natural ponds and lakes (Poole *et al.*, 2002). Osprey capture prey in both deep water and in shallow littoral zones, although likely not as shallow as the great blue heron, due to the fact that osprey forage by diving into the water and grasping fish with their talons. Osprey are likely to forage in both the Guadalupe River and in the watershed's various reservoirs. They may forage in the upper watershed creeks, if the streams are larger and slower moving.

Mallory and Metz (1999) reported that common mergansers are visual pursuit predators, needing clear water in streams, rivers, and littoral zones of lakes, coastal bays, and estuaries. Mergansers prefer to feed in water less than 4 meters deep, chasing fish underwater and grasping with serrated bills. Like the osprey, mergansers are likely to forage in both the River and the reservoirs, probably with a preference for the latter.

The Forster's tern is also a sight predator, hunting by flying low over water until a prey fish is located, then plunging toward prey and grasping with the bill (McNicholl *et al.*, 2001). They are known to forage in marshes, lakes, channels, estuaries, and coastal areas. Based on the fact that Forster's terns are known in the watershed from their summer presence at Calero Reservoir (pers. comm., Bill Bousman, Santa Clara Valley Audubon Society), this is likely the bird's preferential foraging habitat in the watershed.

Belted kingfishers forage in much the same manner as the Forster's tern, searching out prey fish and snatching them from the water with their bills (Hamas, 1994). Kingfishers hunt in shallow water, or in somewhat deeper water on fishes swimming close to the surface. Most fishes are caught less than 60 cm below the surface. Kingfishers are likely to forage in all three of the watershed's habitat types, preferring the edges rather than the more open water in the center of the River and reservoirs.

Based on this information on the foraging habitats of these five species, along with our analysis of the potential for proportional bioaccumulation, the Service does not feel that habitat type-specific application of the proposed wildlife targets is necessary. None of the species forage exclusively in one habitat; such as if the great blue heron was the only one of the five to use the upper watershed creeks. Therefore, the Service believes that the two size class-specific targets described above should be applied to all three habitat types in order to fully protect all wildlife.

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