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Evaluation of the Clean Water Act Section 304(a) Human Health Criterion for Methylmercury: Protectiveness for Threatened and Endangered Wildlife in California

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EXECUTIVE SUMMARY

Introduction

In January 2001, the U.S. Environmental Protection Agency (EPA) developed a new recommended water quality criterion for methylmercury, under section 304(a) of the federal Clean Water Act. The criterion, a tissue residue concentration (TRC) of 0.3 milligrams per kilogram wet weight (mg/kg, ww) of methylmercury in edible portions of fish and shellfish, was designed to protect human health against adverse effects of methylmercury toxicity. The EPA intends to propose this human health criterion in California in order to fulfill consultation obligations under the federal Endangered Species Act (ESA) stemming from promulgation of the California Toxics Rule in 2000. As part of that ESA consultation, the EPA agreed that the human health criterion should be sufficient to protect federally listed aquatic and aquatic-dependent wildlife species in California. In proposing this criterion, the EPA must complete a biological evaluation of the effects of the proposed action on federally listed and proposed threatened and endangered species and critical habitat within California.

To facilitate this biological evaluation, the EPA's Region 9 entered into an Intergovernmental Agreement (IAG) with the U.S. Fish and Wildlife Service's (Service) Sacramento Fish and Wildlife Office, Environmental Contaminants Division (ECD). The primary objective of this IAG was to conduct the analyses necessary to determine whether the TRC may affect any federally listed species in California. This document presents the risk assessment methodology, developed collaboratively by scientists from both the Service and EPA, used to perform these analyses. This document also provides the ECD's interpretation of the results and our conclusions regarding the TRC's effect on the species evaluated. **These conclusions do not represent the results of consultation under Section 7 of the ESA, rather they were based solely on our current understanding of methylmercury's behavior in aquatic ecosystems and the toxicological foundation from which the risk assessment methodology was developed.** The results of these analyses may be used by the EPA in making ESA-related effects determinations for the subsequent biological evaluation. Any such determinations are solely the responsibility of the EPA.

Evaluating Wildlife Protection

The 0.3 mg/kg TRC represents a generic dietary concentration intended to be the maximum allowable concentration of methylmercury in freshwater and estuarine fish and shellfish that would protect human consumers, based on an average consumption of 17.5 grams of fish and shellfish per day. It is possible to develop similar dietary concentrations for wildlife species, provided sufficient life history and toxicity data exist. However, the protection of wildlife cannot be evaluated by simply comparing a protective generic dietary concentration determined for any given species with the generic dietary concentration proposed as the human health criterion.

One of the primary principles in constructing a risk assessment to evaluate wildlife protection is the need to consider the food chains of aquatic ecosystems in terms of trophic levels. Food chains, defined in their most simplistic form, start with trophic level 1 (TL1) plants. These plants are consumed by trophic level 2 (TL2) herbivores, which are consumed by trophic level 3 (TL3) predators, which are then consumed by the top predators in trophic level 4 (TL4). Consideration of trophic levels is necessary because methylmercury is a highly bioaccumulative pollutant which concentrates in biological tissues and biomagnifies as it moves up through successively higher trophic levels of a food chain. Organisms higher on the food chain contain greater methylmercury concentrations than those lower on the food chain. If fish and shellfish from TL2 contain tissue methylmercury concentrations of 0.3 mg/kg, then biota from TL3 and TL4 will have higher tissue concentrations. Conversely, if TL4 biota have tissue concentrations of 0.3 mg/kg, biota from TL2 and TL3 will have lower tissue concentrations.

There are numerous challenges in taking a trophic level approach to evaluating the TRC for its protectiveness of multiple listed fish and wildlife species. Most predators that feed from aquatic food webs are opportunistic and will consume prey from more than one trophic level. These dietary habits vary widely among different species and can change seasonally. Thus, methylmercury concentrations in any trophic level that may be protective of one species may place another consumer from the same water body at increased risk. In addition, different species of wildlife vary in their sensitivity to methylmercury toxicity. Since the toxicological literature contains dosing studies from very few species of wildlife, most ecological risk assessment methodologies, including this one, use uncertainty factors to account for unknown variations in sensitivity among species.

Consideration of these food chain dynamics in a risk assessment for wildlife requires trophic level-specific methylmercury concentrations. The manner in which the TRC is to be implemented for protection of human health will determine the limiting concentrations of methylmercury in the various trophic levels. Under a strict interpretation of the criterion (*i.e.*, no fish tissue exceeding the TRC), and given an understanding of biomagnification relationships between trophic levels, it is possible to set the TRC as the limiting concentration for TL4 biota and then estimate the tissue concentrations expected for biota in TLs 2 and 3. However, if a specific human population consumes only TL2 or TL3 fish from a water body, then the TRC could be applied to just those trophic levels. This would result in methylmercury concentrations in TL4 biota that are higher than the TRC and increase the exposure risks for wildlife.

For this evaluation, two approaches were used to determine trophic level-specific methylmercury concentrations that could be expected from the TRC. The Average Concentration TL Approach estimated these concentrations based on the human consumption rate of 17.5 g per day, with a defined trophic level composition (*i.e.*, a certain percentage from each trophic level). The Highest TL Approach set the TRC as the limiting concentration for TL4 biota, and then estimated the subsequent concentrations for TLs 2 and 3. Both approaches required assumptions about the relationships of bioaccumulation and biomagnification between trophic levels.

Average Concentration Trophic Level Approach

This approach estimated the methylmercury concentrations in each trophic level consumed by humans that, when combined, would correspond to the overall dietary concentration of 0.3 mg/kg. The EPA's human health methylmercury criterion document presented a national average intake rate of 17.5 grams of fish per day based on an assumed percentage from each individual trophic level: TL2 - 21.7% (3.8 g), TL3 - 45.7% (8.0 g), TL4 - 32.6% (5.7 g), for a total of 100% (17.5 g).

Based on national bioaccumulation data, it was determined that methylmercury concentrations in TL4 biota are generally 4.0 times those seen in TL3 biota. Concentrations in TL3 biota are generally 5.7 times those seen in TL2 biota. Using these methylmercury biomagnification factors and the assumed trophic level composition of the average human diet, the concentration of methylmercury in TL2, TL3, and TL4 fish and shellfish that will maintain an overall human dietary concentration of 0.3 mg/kg methylmercury can be calculated. The resulting concentrations are: TL2 - **0.029 mg/kg**; TL3 - **0.165 mg/kg**; and TL4 - **0.660 mg/kg**.

Highest Trophic Level Approach

This approach would set the proposed TRC of 0.3 mg/kg as the limiting concentration in TL4 biota. Concentrations expected in TLs 2 and 3 were then estimated by dividing by the appropriate biomagnification factors (*i.e.*, TL3 = TL4 concentration divided by 4, TL2 = TL3 concentration divided by 5.7). The resulting concentrations are: TL4 - **0.3 mg/kg**, TL3 - **0.075 mg/kg**; and TL2 - **0.013 mg/kg**.

This approach is the most conservative (*i.e.*, protective) method of establishing trophic level concentrations with the TRC. This is because it eliminates the possibility of different human populations exceeding the protective reference dose, assuming the national average consumption rate remains constant. Thus, a diet of 100 percent TL4 fish would maintain the overall dietary concentration of 0.3 mg/kg. Any other combination of trophic level foods in the diet (totaling 17.5 g per day) will maintain a dietary concentration at or below the protective level.

The trophic level methylmercury values for the two approaches were then used, along with dietary intake information for each species of concern, to evaluate the protectiveness of the TRC for aquatic and aquatic-dependent wildlife species at greatest risk from exposure to methylmercury.

Selection of Species

Based on the information available in the scientific literature, and given consideration of methylmercury's capacity to bioaccumulate and biomagnify in the aquatic food chain, this evaluation assumed that upper trophic level wildlife species (*i.e.*, predatory birds and mammals)

have the greatest inherent risk from exposure to methylmercury. In California these species are:

Southern Sea Otter (*Enhydra lutris nereis*)
California Least Tern (*Sterna antillarum brownii*)
California Clapper Rail (*Rallus longirostris obsoletus*)
Light-Footed Clapper Rail (*Rallus longirostris levipe*)
Yuma Clapper Rail (*Rallus longirostris yumaensis*)
Western Snowy Plover (*Charadrius alexandrinus nivosus*)
Bald Eagle (*Haliaeetus leucocephalus*)

The scientific literature was also reviewed to see whether the listed fish, reptile, and amphibian species may be protected under either trophic level approach. For fish species, the risk assessment was based solely on adverse effects associated with tissue methylmercury concentrations. The scientific literature contains little information on methylmercury risk to reptiles and amphibians.

Wildlife Values and Predicted Dietary Concentrations

A Wildlife Value (WV) represents the overall dietary concentration of methylmercury necessary to keep the daily ingested amount at or below a level at which no adverse effects are expected. The WV is analogous to the TRC for the human health criterion. For each species of concern, a WV was determined using body weight, total daily food ingestion rate, and a protective reference dose.

A predicted dietary concentration (DC) also represents an overall concentration in the diet, but is determined using the trophic level methylmercury concentrations expected under each TL approach and the trophic level composition of the species' diet. In effect, the percentage of each trophic level consumed is multiplied by the concentration expected for that trophic level. The resulting products are then summed to provide the total concentration of methylmercury in the diet.

The predicted DC for each species of concern was then compared to the WV determined to be protective for that species. If the predicted DC was at or below the WV then it was assumed that the species is not at risk from dietary exposure to methylmercury under that scenario. If the predicted DC is higher than the WV, it was assumed that the species would likely have a dietary exposure that may place it at risk for adverse effects from methylmercury toxicity.

Results of the Evaluation

Average Concentration Trophic Level Approach

Based on the analyses conducted for this evaluation, applying the TRC with the estimated trophic level methylmercury concentrations under the Average Concentration TL Approach may be

sufficiently protective for only two of the seven species considered: southern sea otter and Western snowy plover. **The five other species examined (California least tern; California, light-footed, and Yuma clapper rails; bald eagle) would likely have dietary exposures under this approach that may place them at risk for adverse effects from methylmercury toxicity**.

Highest Trophic Level Approach

This approach, with its lower estimated trophic level methylmercury concentrations, would provide a greater degree of protection than the Average Concentration TL Approach. Applying the TRC under the Highest TL Approach should be sufficiently protective for four of the seven species considered: southern sea otter, California clapper rail, Western snowy plover, and bald eagle. **Two of the species examined (California least tern and Yuma clapper rail) would likely have dietary exposures under this approach that may place them at risk for adverse effects from methylmercury toxicity**. The least tern may be at an elevated risk for methylmercury toxicity because of its small body size and its diet of exclusively TL3 fish. Although methylmercury concentrations for all three trophic levels are expected to be substantially lower under this approach, the estimated TL3 concentration of 0.075 mg/kg would still not be low enough to remove the potential risk of adverse effects from dietary methylmercury exposure for the least tern. The evaluation for the Yuma clapper rail, regardless of the WV used in the analysis, indicates this subspecies would likely have a dietary exposure under this approach that may place it at risk for adverse effects from methylmercury toxicity.

At this time, no conclusion can be drawn regarding the light-footed clapper rail. If this subspecies' sensitivity to methylmercury is the same as the California clapper rail and the analysis of its dietary composition is correct, the light-footed rail would likely have dietary exposures under this approach that may place them at risk. However, if other biological characteristics (*e.g.*, a greater ability to detoxify ingested methylmercury, lower diet-to-egg transfer efficiency) indicate a lower sensitivity to methylmercury, the evaluation results suggest this TL approach should be sufficiently protective for the light-footed rail. Research should be initiated to answer questions surrounding the relative sensitivity of this subspecies and to determine the appropriate trophic level methylmercury concentrations to provide sufficient protection against toxicity.

Fish

None of the data examined provided definitive answers regarding the level of protection for fish afforded by the TRC. **The methylmercury concentrations expected from applying the TRC under both trophic level approaches appear to be well below observed adverse effects concentrations; however, the trophic level concentrations expected under the Average TL Approach are much closer to these adverse effects concentrations.** Increasing emphasis on examining more subtle methylmercury-induced effects may reveal even lower tissue-based threshold effects concentrations for fish.

Reptiles and Amphibians

Too little is presently known about mercury bioaccumulation in reptiles and amphibians to allow for any comparative risk prediction capability based on bioaccumulation in fish. **The available scientific literature strongly suggests that both reptiles and amphibians can bioaccumulate methylmercury, although possibly less so than piscivorous birds and mammals with a greater daily reliance on aquatic prey. Until the appropriate toxicological data are generated, no definitive conclusions can be drawn about the protectiveness of either trophic level approach for the California red-legged frog, San Francisco garter snake, or giant garter snake.**

Discussion

The Service's Environmental Contaminants Division believes the analyses presented in this document represent the most current state of knowledge regarding the risk to California's listed species from dietary methylmercury. Conclusions about the protectiveness of the TRC for each species evaluated by the two trophic level approaches are summarized in Executive Summary (ES) Table 1. Of the two approaches evaluated, the Highest TL Approach affords a greater degree of protection for California's listed bird and mammal species than the Average TL Approach. The best currently available data on mercury toxicity in fish suggest that the TRC under either approach should be sufficiently protective of all listed fish in California; however, the trophic level concentrations expected under the Average TL Approach would be much closer to observed adverse effects concentrations described in the scientific literature. Although a lack of relevant data precludes any conclusions regarding the potential impact of the TRC on the reptile and amphibian species considered, the lower trophic level concentrations expected under the Highest TL Approach would afford a greater measure of protection than those expected under the Average TL Approach. **We believe that the TRC would not adequately protect all listed species in California; however, applying the TRC under the Highest TL Approach would reduce the number of species at risk.**

These conclusions reflect the interpretation of the evaluation results by the Service's Environmental Contaminants Division only, and are not intended to represent the views of those EPA or Service scientists who helped develop the risk assessment methodology. In addition, these conclusions do not constitute the results of consultation under Section 7 of the ESA.

Finally, it must be noted that the risk assessment methodology presented in this document was not applied to any wildlife species other than the federally listed species. Other non-listed wildlife may be potentially at risk under the TRC, due to their dietary dependence on aquatic ecosystems. **Using the same approach followed in this effort, regulatory agencies should be able to determine whether concentrations of methylmercury in fish tissue under the TRC may also pose a risk to non-listed wildlife species.**

ES Table 1. Protectiveness of EPA's Methylmercury Tissue Residue Criterion for Seven Federally Listed California Species.

Is the TRC Protective for...	Southern Sea Otter	Ca. Least Tern	Ca. Clapper Rail	Light-footed Clapper Rail	Yuma Clapper Rail	Western Snowy Plover	Bald Eagle
Under the Average TL Approach?	Yes	**No**	Yes	**No**	**No**	Yes	**No**
-with interspecies uncertainty factor of 3*	na	na	**No**	**No**	**No**	Yes	na
Under the Highest TL Approach?	Yes	**No**	Yes	Yes	**No**	Yes	Yes
-with interspecies uncertainty factor of 3*	na	na	Yes	**No**	**No**	Yes	na

(na - not applicable)

* - discussion of uncertainty is presented in Section III.D. of document

I. INTRODUCTION

I.A. Background

In January 2001, the U.S. Environmental Protection Agency (EPA) developed a new recommended water quality criterion for methylmercury, under section 304(a) of the federal Clean Water Act (CWA; 33 U.S.C. 1251 - 1376, as amended). The criterion, a tissue residue concentration (TRC) of 0.3 milligrams per kilogram wet weight (mg/kg, ww) of methylmercury in edible portions of fish and shellfish, was designed to protect human health against adverse effects of methylmercury toxicity. In order to fulfill consultation obligations under the federal Endangered Species Act (ESA; 16 U.S.C. 1531-1544, as amended) stemming from promulgation of the California Toxics Rule in 2000, the EPA intends to propose this criterion in the State of California. While EPA intends to propose this TRC as a human health criterion, the Agency agreed as part of the California Toxics Rule ESA consultation that the human health criterion should be sufficient to protect federally listed aquatic and aquatic-dependent wildlife species. As part of the proposal process, the EPA must complete a biological evaluation of the effects of the proposed action on federally listed and proposed threatened and endangered species (see Appendix) and critical habitat within California.

To facilitate this biological evaluation, the EPA's Region 9 entered into an Intergovernmental Agreement (IAG) with the U.S. Fish and Wildlife Service's (Service) Sacramento Fish and Wildlife Office, Environmental Contaminants Division (ECD). The primary objective of this IAG was to conduct the analyses necessary to determine whether the TRC may affect any federally listed species in California. This document presents the risk assessment methodology, developed collaboratively by scientists from both the Service and EPA, used to perform these analyses. The results of these analyses may be used by the EPA in making ESA-related effects determinations for the subsequent biological evaluation. Any such determinations are solely the responsibility of the EPA. However, this document also provides the ECD's interpretation of the analytical results and our conclusions regarding the TRC's effect on the species evaluated. These conclusions do not represent the results of consultation under Section 7 of the ESA, rather they were based solely on our current understanding of methylmercury's behavior in aquatic ecosystems and the toxicological foundation from which the risk assessment methodology was developed.

I.B. Evaluating Wildlife Protection

When sufficient methylmercury toxicity data exist to determine a dietary dose at which no adverse effects to an organism are expected, then it becomes a relatively simple process to calculate a protective methylmercury concentration in the overall diet, based on information about that organism's body weight and daily food consumption. The 0.3 mg/kg[1] TRC represents just such a generic dietary concentration for humans. The TRC is intended to be the maximum

[1] All concentrations are reported on a wet weight basis unless otherwise noted.

allowable concentration of methylmercury in freshwater and estuarine fish and shellfish that would protect human consumers, based on an average consumption of 17.5 grams of fish and shellfish per day.

However, the protection of wildlife cannot be evaluated by simply comparing a protective generic dietary concentration determined for any given species with the generic dietary concentration proposed by the human health criterion. One of the primary principles in constructing a risk assessment methodology to evaluate wildlife protection was the need to consider aquatic ecosystems in terms of trophic levels. 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. Predator-prey relationships in real-world ecosystems are generally more complex than this simple linear model, with a tendency for higher order predators to include prey from more than one trophic level in their diets. However, the risk assessment methodology employed in this evaluation was based on the assumption that the general concepts underlying the simple linear food chain model remain a valid approach for considering the trophic transfer of methylmercury in aquatic biota. Trophic levels used in this evaluation were based on definitions provided in Volume I of *Trophic Level and Exposure Analyses for Selected Piscivorous Birds and Mammals* (U.S. Environmental Protection Agency, 1995a):

Trophic Level 1 - Plants and detritus
Trophic Level 2 - Herbivores and detritivores
Trophic Level 3 - Predators on trophic level 2 organisms
Trophic Level 4 - Predators on trophic level 3 organisms

This consideration of trophic levels was necessary because methylmercury is a highly bioaccumulative pollutant which concentrates in biological tissues and biomagnifies as it moves up through successively higher trophic levels of a food chain. The TRC was not derived by assuming specific methylmercury concentrations in any particular trophic level. Instead, 0.3 mg of methylmercury per kg of fish and shellfish tissue in a daily consumed average of 17.5 g was assumed to be protective for human populations eating from various trophic levels, rather than from any particular trophic level. However, due to the characteristics of methylmercury described above, aquatic food chains do not attain a steady-state condition wherein aquatic biota from all trophic positions exhibit the same tissue concentrations. Instead, organisms higher on the food chain contain greater concentrations than those lower on the food chain. For example, if fish and shellfish from trophic level 2 (*e.g.*, herbivorous fish) contain concentrations of 0.3 mg/kg, then biota from trophic levels 3 and 4 (*e.g.*, predatory fish) will undoubtedly have higher tissue concentrations. Conversely, if aquatic biota from the highest trophic level in the system have tissue methylmercury concentrations of 0.3 mg/kg, examination of lower order biota will show substantially lower tissue concentrations. Consideration of methylmercury's propensity to bioaccumulate and biomagnify as it is passed up the aquatic food chain was critical in this

evaluation as many higher order predators (*e.g.*, piscivorous birds and mammals) eat aquatic biota from a variety of trophic levels.

There are several challenges in evaluating the TRC for its protectiveness of multiple listed fish and wildlife species. The first involves determining the dietary characteristics of the species of concern (*e.g.*, ratio of daily food ingestion rate to body weight; trophic level composition of diet). Most predators that feed from aquatic food webs are opportunistic and will consume prey from more than one trophic level. Furthermore, the distribution of prey types they consume may vary seasonally. While an overall dietary methylmercury concentration can be calculated that will protect any given species, the amount of prey consumed from each trophic level is the driving factor influencing the amount of methylmercury ingested on a daily basis. The methylmercury concentration in the overall diet for any species is dependent on both the trophic level composition of its diet *and* the methylmercury concentrations in each of the trophic levels from which the species feeds. Without an understanding of this dietary composition, it is impossible to determine the limiting concentrations for each trophic level that will result in any calculated overall dietary concentration.

A second challenge is that these dietary characteristics vary widely from species to species. While one species may eat primarily from trophic level 2, another may prey predominantly on higher trophic level organisms. Methylmercury concentrations in any trophic level that may be protective of one species may place another consumer from the same water body at increased risk.

Another challenge is due to the potential for different species of wildlife to vary in their sensitivity to methylmercury toxicity. The toxicological literature contains dosing studies from very few species of wildlife, so most ecological risk assessment methodologies, including this one, use uncertainty factors to account for unknown variations in sensitivity among species. This is discussed in more detail in Section III.D., below.

In addition to the complexities of wildlife diets, another challenge involves how the TRC is to be implemented for protection of human health. Under a strict interpretation of the criterion (*i.e.*, no fish tissue exceeding the TRC), and given an understanding of biomagnification relationships between trophic levels, it may be possible to set the TRC for trophic level 4 biota and then estimate the tissue concentrations expected for biota in trophic levels 2 and 3. If the aforementioned dietary characteristics can be determined, the various trophic level methylmercury concentrations can then be used to evaluate their protectiveness for any given species. However, in implementing the criterion, adjustments may be made to account for site-specific or regional conditions regarding human consumption of fish and shellfish. These adjustments could include apportioning a fish intake rate to the highest trophic level consumed for a specific human population. This suggests that if a specific human population consumes only trophic level 2 or 3 fish from a water body, then the TRC could be applied to those trophic levels. The increased methylmercury concentrations in higher trophic levels resulting from this implementation could then increase the exposure for top wildlife predators.

II. APPROACHES TO EVALUATION

In order to evaluate the protectiveness of any given criterion expressed as a general concentration in the overall diet of a consumer eating from various trophic levels, it is first necessary to establish concentrations specific to each trophic level. As noted above, it is possible to set the human health criterion as the limiting concentration at trophic level 2, 3 or 4, depending on the particular fish consumption habits of the human population to be protected. Alternatively, varying concentrations in each trophic level could be calculated based on different combinations of the human dietary trophic level composition (*e.g.*, 90% trophic level 4 and 10% trophic level 3 vs. 50% trophic level 4, 40% trophic level 3, and 10% trophic level 2). Although a multitude of trophic level approaches are possible, this evaluation is focused on two options, each described below.

II.A. Average Concentration Trophic Level Approach

In the human health criterion development, the TRC was determined using a national average fish consumption rate of 17.5 g/day for the general population. This national average can be broken out by determining the percentage of fish and shellfish consumed from each of the three trophic levels (TL2, TL3, TL4). A trophic level breakout was presented in the human health criterion document, although this was not intended to be used in setting concentration limits for each trophic level. However, using this breakout to estimate individual trophic level concentrations that would maintain the overall dietary concentration of 0.3 mg/kg provides one way to evaluate the protectiveness of the TRC for species of concern. The following methodology describes the steps for conducting this approach.

The first step is to estimate the methylmercury concentrations in each trophic level consumed by humans that, when combined, would correspond to the overall dietary concentration of 0.3 mg/kg. In order to do this, several input parameters must first be identified:

%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
MTL3 - Food chain multiplier from TL2 to TL3 biota
MTL4 - Food chain multiplier from TL3 to TL4 biota

Food chain multipliers are values derived from relationships of bioaccumulation and biomagnification between trophic levels. These 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

MTL4 = 680,000/160,000 = 4.25

Any methylmercury concentration estimated for trophic level 3 biota can then multiplied by the MTL4 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 = 0.45 mg/kg
Average tissue concentration in TL3 fish = 0.15 mg/kg

MTL4 = 0.45/0.15 = 3

For this evaluation, food chain multipliers were calculated from draft national BAFs presented in the EPA's methylmercury criterion document. Although these values are draft only, they were empirically derived from national data. If more site-specific BAF data exist for water bodies in California, they may be used in place of the draft values to calculate food chain multipliers.

Draft national BAF for trophic level 4 = 2,700,000
Draft national BAF for trophic level 3 = 680,000
Draft national BAF for trophic level 2 = 120,000

MTL4 = 2,700,000 / 680,000 = 4
MTL3 = 680,000 / 120,000 = 5.7

Having identified the above input parameters, the following additional terms are necessary to then construct the equation for calculating trophic level concentrations necessary to maintain the overall dietary concentration:

FDTL2 - concentration in food (FD) from trophic level 2
FDTL3 - concentration in food from trophic level 3 - (equivalent to FDTL2 × MTL3)
FDTL4 - concentration in food from trophic level 4 - (equivalent to FDTL2 × MTL3 × MTL4)

The overall dietary concentration (DC) of methylmercury can be expressed in the equation:

$$\mathbf{DC = (\%TL2 \times FDTL2) + (\%TL3 \times FDTL3) + (\%TL4 \times FDTL4)} \quad \mathbf{(1)}$$

The equation can then be further arranged, substituting food chain multiplier equivalents, as:

$$\mathbf{DC = (\%TL2 \times FDTL2) + (\%TL3 \times FDTL2 \times MTL3) + (\%TL4 \times FDTL2 \times MTL3 \times MTL4)} \quad \mathbf{(2)}$$

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

FDTL2 = DC / [(%TL2) + (%TL3 × MTL3) + (%TL4 × MTL3 × MTL4)] **(3)**

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

FDTL3 = FDTL2 × MTL3 **(4)**

FDTL4 = FDTL3 × MTL4 **(5)**

As discussed above, the human health methylmercury criterion document presents a national average intake rate of 17.5 grams of fish per day for the general population. This national average was based on an average consumption of individual trophic levels as follows: TL2 = 3.8 g, TL3 = 8 g, TL4 = 5.7 g. These values correspond to: TL2 = 21.7%, TL3 = 45.7%, TL4 = 32.6%. Using these values, and substituting the TRC for the DC term in Equation 3, the concentration in trophic level 2 biota necessary to maintain the overall dietary concentration can then be calculated.

FDTL2 = TRC / [(%TL2) + (%TL3 × MTL3) + (%TL4 × MTL3 × MTL4)]

FDTL2 = 0.3 mg/kg / [(0.217) + (0.457 × 5.7) + (0.326 × 5.7 × 4)]

FDTL2 = 0.3 / 10.247

FDTL2 = 0.029 mg/kg

Then, using the previously calculated food chain multipliers from above:

FDTL2 = 0.029 mg/kg
FDTL3 = 0.029 × 5.7 = **0.165 mg/kg**
FDTL4 = 0.165 × 4.0 = **0.660 mg/kg**

Based on the trophic level breakout for the default human fish consumption rate identified in the criterion document, the above concentrations of methylmercury will result in an overall dietary concentration (DC) of 0.3 mg/kg:

DC = (%TL2 × FDTL2) + (%TL3 × FDTL3) + (%TL4 × FDTL4)

0.3 mg/kg = (.217 × 0.029 mg/kg) + (.457 × 0.165 mg/kg) + (.326 × 0.66 mg/kg)

II.B. Highest Trophic Level Approach

In contrast to the Average Concentration Trophic Level Approach, the Highest Trophic Level Approach sets the proposed human health methylmercury criterion of 0.3 mg/kg as the limiting concentration in edible portions of trophic level 4 fish. Concentrations expected in trophic levels 2 and 3 can then be estimated using a variation of the food chain multiplier approach described above. In effect, these multipliers determined by the ratios of trophic level concentration relationships become food chain dividers: 0.3 mg/kg in trophic level 4 is divided by the MTL4 to estimate the concentration in trophic level 3, which is then divided by the MTL3 to estimate the concentration in trophic level 2.

FDTL4 = 0.3 mg/kg
FDTL3 = 0.3 / 4 = **0.075 mg/kg**
FDTL2 = 0.075 / 5.7 = **0.013 mg/kg**

This approach is the most conservative (*i.e.*, protective) method of establishing trophic level concentrations with the TRC, as it eliminates the possibility of different human populations exceeding the protective reference dose, assuming the national average consumption rate remains constant. A diet of 100 percent trophic level 4 fish would maintain the overall dietary concentration of 0.3 mg/kg.

III. PROTECTIVE WILDLIFE VALUES

III.A. Selection of Species

The next step in this evaluation was to determine an overall dietary concentration of methylmercury that will protect each species of concern. Species considered in this evaluation include representatives from several taxonomic classes: birds, mammals, fish, reptiles, and amphibians (see Appendix). Initially, the taxonomic class or classes with the greatest potential risk from methylmercury concentrations in fish tissue were identified. For fish species, risk assessment was based solely on adverse effects associated with tissue methylmercury concentrations (see Section X). For non-fish species, the risk assessment was based on exposure through ingestion of methylmercury-contaminated aquatic prey.

The scientific literature contains little information on methylmercury risk to reptiles and amphibians, with no studies found that relate effects to dietary doses (see Section X). Throughout the past several decades, however, a great deal of toxicity research has been conducted on various birds, mammals, and fish. While toxicity data for fish indicate adverse effects resulting from a wide range of tissue methylmercury concentrations, the majority of this research has been conducted with tissue concentrations substantially higher than the TRC. Research on birds and mammals, particularly piscivorous species, is also extensive. Much of this work has involved oral dose studies.

Based on the information available in the scientific literature, and given consideration of methylmercury's capacity to bioaccumulate and biomagnify in the food chain, this evaluation assumed that upper trophic level wildlife species (*i.e.*, predatory birds and mammals) have the greatest inherent risk from exposure to methylmercury, compared to other biota. Wildlife Values (WV), which are the total dietary methylmercury concentrations that will protect predatory birds and mammals, were determined for these upper trophic level species. The methodology then allows for an assessment of whether these values would be exceeded based on the various trophic level concentrations estimated by the two approaches described above. After an analysis of the protection afforded to listed birds and mammals, the scientific literature was reviewed to see whether the listed fish, reptile, and amphibian species may be protected by either trophic level approach.

Listed species for which WVs were generated:

Southern Sea Otter (*Enhydra lutris nereis*)
California Least Tern (*Sterna antillarum brownii*)
California Clapper Rail (*Rallus longirostris obsoletus*)
Light-Footed Clapper Rail (*Rallus longirostris levipe*)
Yuma Clapper Rail (*Rallus longirostris yumaensis*)
Western Snowy Plover (*Charadrius alexandrinus nivosus*)
Bald Eagle (*Haliaeetus leucocephalus*)

III.B. Equation to Calculate Wildlife Values

A Wildlife Value represents the overall dietary concentration of methylmercury necessary to keep the 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. In effect, the WV converts the protective RfD into an overall dietary concentration (in mg/kg in diet). The WV is analogous to the TRC for the human health criterion. The WV is calculated using the following equation:

$$\mathbf{WV} = \frac{\mathbf{RfD} \times \mathbf{BW}}{\sum \mathbf{FIR_i}} \qquad \mathbf{(6)}$$

WV = Wildlife Value (mg/kg in diet)
RfD = Reference Dose
BW = Body Weight (in kg) for species of concern
FIR_i = Total Food Ingestion Rate (kg food/day), from the i^{th} trophic level, for species of concern

Because the most sensitive endpoints for toxicity of methylmercury in birds and mammals relate to reproduction, the focus of this methodology is to establish reference doses based on preventing adverse impacts from maternally ingested methylmercury, that could potentially affect the reproductive viability of the species. In order to establish RfDs, the scientific literature was first

reviewed to find the most appropriate toxicity test doses for avian and mammalian species. An uncertainty analysis (described below, Section III.D.) was then conducted for each test dose to arrive at the appropriate RfD. Body weights used in this approach were those of adult females for the species of concern. Total food ingestion rates for species of concern, and the trophic level breakout of the diet, were obtained from the scientific literature or estimated using allometric equations.

III.C. Determination of Test Doses

Once the taxonomic class or classes assumed to be at greatest risk were identified (*i.e.*, predatory birds and mammals), the next step in the evaluation was to identify appropriate toxicity test doses to use for determining a protective RfD for each group. As the species of concern for this evaluation are federally listed as threatened or endangered, the goal of this step was to find the lowest test doses associated with endpoints that could adversely affect the continued existence of the species or the loss of individuals from the population. Most often these toxicity endpoints were based on subtle effects concentrations (*e.g.*, reproductive success), rather than more severe effects in individuals (*e.g.*, lethality). However, if the lowest test dose was found to cause impacts that could effectively remove an individual from the population, even without any apparent effect on reproductive success, this test dose was used in the analyses.

The approach used in this methodology assesses toxicity through ingestion of methylmercury in contaminated prey, so the scientific literature was searched for all available oral test doses demonstrating observable effects concentrations. The data preferences used in this analysis were the same as outlined in the Great Lakes Initiative (GLI) *Technical Support Document for Wildlife Criteria* (U.S. Environmental Protection Agency, 1995c):

- Appropriate endpoints (reproductive or developmental success, organismal viability or growth, other parameters influencing population dynamics)
- Chemical-specific dose-response curve
- Chronic or sub-chronic study duration
- Wildlife species preferred over traditional laboratory animals
- Field studies preferred over laboratory studies
- Oral route of exposure, although other routes acceptable if possible to convert to oral dose

Many oral dose toxicity studies report test doses as the amount of contaminant in the diet of the tested species (*e.g.*, mg/kg food). Therefore, it is often necessary to convert these reported levels to a daily ingested dose (mg/kg-bw/day), using body weights and food ingestion rates for the species studied (*i.e.*, mg/kg in food × kg food consumed per kg body weight per day = mg/kg body weight per day).

For this evaluation, the scientific literature was reviewed with particular emphasis on searching for rigorous data reported since the development of water quality wildlife criteria for the GLI in

1995. For the GLI effort, two studies that best fit the data preferences were selected to calculate the mercury wildlife criteria for avian and mammalian species. These are described below, along with relevant findings from the current literature search.

Mammalian Test Dose: In developing water quality criteria for mercury in the GLI, the EPA reviewed numerous mammalian chronic and subchronic toxicity studies. Test animals studied were rats and mink. Toxicity to mink was evaluated in two subchronic studies by Wobeser *et al.* (1976a,b), and these studies formed the basis for EPA's calculation of the mammalian wildlife criterion for mercury. Each study had different exposure durations (93 and 145 days) and dosing levels. The 145 day study dosed mink with two methylmercury concentrations (0.22 and 0.33 mg/kg) in food. These concentrations corresponded to dietary doses of 0.033 and 0.05 mg/kg-bw/day, respectively, using a food ingestion rate of 0.15 kg/day and a body weight of 1 kg for captive mink. The EPA determined that no adverse effects were seen at either dose, and concluded the 0.05 mg/kg-bw/day constituted a No Observable Adverse Effects Level (NOAEL) test dose.

From the 93 day study, the EPA determined both NOAEL and LOAEL (Lowest Observable Adverse Effects Level) test doses. A concentration of 1.1 mg/kg in food caused pathological alterations in the mink nervous system (nerve tissue lesions), while concentrations of 1.8 mg/kg and higher in food resulted in clinical signs of mercury intoxication [anorexia (loss of appetite) and ataxia (loss of coordination)] and subsequent mortality. Using the same food ingestion rate and body weight converts the 1.1 and 1.8 mg/kg concentrations to dietary doses of 0.16 and 0.27 mg/kg-bw/day, respectively. The EPA concluded that the effects seen in the 0.16 mg/kg-bw/day dose group were not associated with any obvious clinical evidence of toxicity, and that this dose constituted the NOAEL test dose, despite Wobeser's conclusion that distinct clinical signs of toxicity would have resulted had the exposure period been longer. The 0.27 mg/kg-bw/day dose was designated the LOAEL.

For several years, the U.S. Department of Energy (DOE) (1993-1996) has published *Toxicological Benchmarks for Wildlife*. These documents have also used toxicity studies of rats and mink to determine the mammalian benchmarks for methylmercury compounds. In determining final NOAEL and LOAEL values for piscivorous mammals, Wobeser *et al.*'s (1976b) 93 day study was used. The DOE's evaluation of this study agreed with the EPA's conclusion that the 1.1 mg/kg concentration constituted a NOAEL; however, using a slightly different value for the mink food ingestion rate (0.137 kg/day), a dietary dose of 0.15 mg/kg-bw/day was calculated.

In 1997, the EPA published the *Mercury Study Report to Congress* (MSRC). Volume VI of this report (U.S. Environmental Protection Agency, 1997a) presented reviews of several methylmercury toxicity tests with mammalian wildlife, including both Wobeser *et al.* (1976a,b) studies. For the MSRC, the EPA concluded that the nerve tissue lesions observed in the 1.1 mg/kg concentration group from the 93 day study were relevant effects endpoints, noting the researcher's opinion that the nerve tissue damage would have become manifested as impaired

motor function had the study continued for a longer period. For this reason, the EPA assigned the 1.1 mg/kg concentration as the LOAEL. As this was the lowest dosing group in the study, a NOAEL could no longer be determined. Instead, the EPA selected the 0.33 mg/kg concentration from the 145 day study as the NOAEL. Using the food ingestion rate found in the DOE analysis (0.137 kg/day) and a body weight of 0.8 kg (as opposed to 1.0 kg used in both the GLI and DOE reports), the EPA converted the 0.33 mg/kg dose in food to a dietary NOAEL test dose of 0.055 mg/kg-bw/day for the MSRC.

The MSRC also presented findings from a long-term feeding study with domestic cats (Charbonneau *et al*., 1974). Cats were fed various doses of methylmercury, either as methylmercuric chloride in food or as methylmercury-contaminated fish, for two years. The dietary test doses of 0.046 and 0.020 mg/kg-bw/day were determined to be the LOAEL and NOAEL, respectively, based on neurological impairment effects. These values were only used for comparative purposes, however, as the intent of the MSRC effort was to derive water quality criteria that would be protective of wildlife. The NOAEL test dose from the 145 day mink study was used in the subsequent MSRC calculations to derive criteria values for mammalian wildlife.

As all the effects seen in the semi-domesticated mink and domestic cat studies involved toxicity to individual animals, an effort was made for this evaluation to find data on effects to reproductive performance. Wren *et al*. (1987) reported no effects on reproduction in mink fed a diet supplemented with 1.0 mg/kg methylmercury every other day for 150 days. In a two generation study (G1, G2) of mink fed organic mercury-contaminated diets, Dansereau *et al*. (1999) analyzed effects on reproductive performance. Dosing groups were 0.1, 0.5, and 1.0 mg/kg total mercury. Whelping percentage for the G1 females was statistically higher in the 0.1 mg/kg group than in the 0.5 or 1.0 groups. Whelping percentages for all other G1 and G2 dosing groups were low relative to reported performance of untreated female mink. The researchers suggested that the observed linear decrease of performance with increasing methylmercury exposure may have been the result of adverse effects of methylmercury on the reproductive process; however, they were unable to show a statistically significant difference. Although the study could not conclude the reproductive process itself was adversely affected, female mink from both generations in the 1.0 mg/kg suffered mortality from methylmercury intoxication. A large percentage of first generation females died at 11 months of age, after 90 days of exposure. Death occurred approximately one month after whelping the G2 offspring. Second generation females died at the same age as their mothers, but after approximately 330 days of exposure. However, the G2 females had been mated at the age of 10 months and death occurred one month later in 6 out of 7 individuals, before giving birth. The remaining individual died shortly after giving birth. The researchers concluded that "...survival and consequently the reproduction of the G2 females fed 1.0 ppm Hg diet were therefore affected."

Although the 1999 Dansereau *et al*. study could not confirm impaired reproductive performance, it is useful for validating that a concentration of 1.0 mg/kg methylmercury in food represents an observable adverse effects level, which could inhibit the overall success of a population by removing reproductively viable individuals. The researchers found no mortality or neurological

signs of toxicity in any mink in the 0.1 and 0.5 mg/kg diet groups; however, the animals were not sacrificed and examined for histopathological effects in either of these groups. A review of the available scientific literature since the GLI revealed no new data that better fits the GLI preferences or that reports lower oral dose observed effects concentrations for mammalian wildlife. Therefore, the NOAEL dose of 0.33 mg/kg in food (0.055 mg/kg-bw/day) from the 145 day study by Wobeser *et al*. (1976a) is the appropriate test dose for determining protection of piscivorous mammalian wildlife in this evaluation.

Avian Test Dose: For the GLI effort, the EPA also reviewed numerous subchronic and chronic mercury toxicity studies using avian species. Species examined in this review included domestic chicken, pheasant, Japanese quail, red-tailed hawk, zebra finch, and game farm mallard ducks. The EPA ultimately selected a study examining reproductive and behavioral effects in three generations of mallard ducks (Heinz, 1979) to determine an appropriate test dose for its avian wildlife criteria calculations.

In these studies, three generations of mallard ducks were exposed to a mercury-free control diet or one containing 0.5 mg/kg methylmercury dicyandiamide. Several measurements of reproductive success were evaluated throughout the course of the study. Statistically significant adverse effects were observed in the percentage of eggs laid outside the nest box (increase) and in the number of one-week-old ducklings produced (decrease), relative to controls. In addition, adverse behavioral effects were seen in the ducklings from the treatment group, relative to controls. The behavioral aberrations observed included a smaller percentage of ducklings approaching tape-recorded maternal calls, and an increased sensitivity to frightening stimuli, as measured by the distance traveled in avoidance.

Based on the methylmercury concentration tested (0.5 mg/kg in food) and the reported average food consumption rate for 2nd and 3rd generation mallards in the treatment group (0.156 kg/kg-bw/day), the EPA determined a dietary dose of 0.078 mg/kg-bw/day. No lower effects concentration test doses were reported in any of the other avian toxicity studies evaluated by the EPA. As there were no lower treatment concentrations in the mallard studies, the EPA assigned this dietary dose as the LOAEL to be used in avian wildlife value calculations. For the GLI, the EPA (1995b) concluded that the mallard studies best fit the data preferences, providing a chemical-specific dose-response curve and demonstrating effects that "...clearly have potential consequences on populations of mallards exposed to methylmercury."

Although mercury toxicity has been studied extensively using avian species, both before and after the GLI effort, Heinz' (1979) multi-generational mallard work has been used almost exclusively in subsequent efforts to derive water quality values for methylmercury that are protective of avian wildlife (U.S. Department of Energy, 1994-1996; U.S. Environmental Protection Agency, 1997a; Nichols *et al*., 1999; Canadian Council of Ministers of the Environment, 2000; Buchanan *et al*., 2001; California Regional Water Quality Control Board - Central Valley Region, 2001; Evers *et al*., 2002). In large part, this is because few other studies have attempted to establish oral dose-response data from long-term feeding studies. There is a

great deal of scientific literature devoted to methylmercury residues in various avian tissues (*e.g.*, muscle, liver, egg); however, these studies were generally not designed to determine chronic dietary doses. The literature search for this evaluation only revealed a few additional studies, described below, that could be used for evaluating dietary concentrations associated with subchronic or chronic effects.

In a broad survey of freshwater lakes in Canada, which were contaminated with mercury and experienced unnatural water level fluctuations and turbidity, Barr (1986) examined the population dynamics of common loons. Loons in these systems preyed on fish containing various concentrations of methylmercury. Based on his observational data, Barr concluded that adverse reproductive effects in loons (*i.e.*, reductions in egg laying, and nest site and territorial fidelity) were associated with mean fish tissue concentrations ranging from 0.3 - 0.4 mg/kg methylmercury. As this study was not designed as a controlled feeding experiment, Barr did not convert these concentrations into daily ingested doses (*i.e.*, mg/kg-bw/day). However, Barr's reported average body weights for male and female loons (~ 4.0 kg) and assumed food consumption rate of 20 percent body weight per day (0.8 kg/day) allowed for comparison with the 0.078 mg/kg-bw/day dietary dose from the Heinz (1979) mallard work. Multiplying the lowest concentration Barr associated with adverse effects (0.3 mg/kg in fish) and the assumed average food ingestion rate (0.2 kg/kg-bw/day) produces a daily dietary dose of 0.06 mg/kg-bw/day. While the limitations of the Barr study (*i.e.*, no controlled oral dose-response data) prevent the use of this daily value as the appropriate test dose for this evaluation, it serves to support the test dose selected by the EPA for the GLI effort.

Effects of controlled methylmercury dosing on captive great egret nestlings were reported in Bouton *et al*. (1999) and Spalding *et al*. (2000a,b). In these studies, 16 great egret nestlings were captured from the wild and separated into various dosing groups (0, 0.5, 5.0 mg/kg methylmercury chloride in diet) for 14 weeks. Methylmercury was administered via gelatin capsules, and doses were maintained based on daily food consumed. Although dietary concentrations were maintained, the daily amount of methylmercury consumed per kilogram of body weight varied from 0.048 to 0.135 mg/kg-bw/day. This was because nestling body weights and food consumption rates are very dynamic during this intense growth phase. The variation in daily dietary doses limited the usefulness of these studies for determining an appropriate avian test dose for this evaluation; however, analysis of effects observed in the 0.5 mg/kg dose group for each of the three studies (described below) allowed for comparison with the LOAEL concentration from the Heinz (1979) effort.

Bouton *et al*. (1999) measured behavioral effects in the captive egrets during the period of the experiment (10-14 weeks) approximate to post-fledging in wild egrets (11 weeks of age). These researchers concluded that adverse effects, including reduced activity, food intake, and willingness to hunt prey, were demonstrated in the 0.5 mg/kg dosing group. They also postulated that these behavioral effects may result in reduced juvenile survival in free-ranging birds.

Spalding *et al*. (2000a) examined the accumulation of methylmercury in tissues of the captive egrets and its effect on growth and appetite. These researchers hypothesized that nestling wading birds would be less at risk from ingested methylmercury than fledgling birds, due to depuration of the methylmercury into the rapidly growing feathers of the younger birds. Reduced appetite, and a subsequent decline in growth, was observed after the ninth week of the experiment in both the 0.5 and 5.0 mg/kg dose group, corresponding to the cessation of feather growth. Although the magnitude of weight loss was small, the study's authors concluded that the abundance of food in the controlled setting may have masked some of the effects that would have resulted had the birds been hunting on their own. The study results supported the conclusion that, relative to pre-fledging nestlings, post-fledging birds are at an elevated risk from methylmercury exposure at even the 0.5 mg/kg dietary concentration, during the period when feathers stop growing. The researchers noted that this period also coincides with the time that young birds face the multiple risk factors of having to forage on their own, leave the natal colony, and become exposed to novel predation and disease factors.

Spalding *et al*. (2000b) examined the same egrets for histologic, neurologic, and immunologic effects. Both dosing groups exhibited effects of varying magnitude. Birds in the 5.0 mg/kg dose group showed severe ataxia, as well as hematologic, neurologic, and histologic changes, with the most severe lesions in immune and nervous system tissues. The 0.5 mg/kg dosed birds also exhibited multiple effects for various endpoints, relative to birds in the control group. In comparing their findings with effects reported in studies of wild birds, the authors concluded that the thresholds for sublethal effects measured in captive birds were lower than those in wild birds. However, these researchers attributed this discrepancy to the increased detectability of effects in controlled experiments, and suggested that LOAELs from captive studies may be a more accurate predictor of effects for field situations than field-derived LOAELs applied to captive studies.

Taken together, these three studies (Bouton *et al*., 1999 and Spalding *et al*., 2000a,b) demonstrated adverse effects in juvenile piscivorous birds exposed to a diet containing 0.5 mg/kg methylmercury. The multitude of effects reported, while not directly associated with reproduction, could have significant implications for population viability. Even if the number of offspring produced is not affected by a diet containing 0.5 mg/kg methylmercury, the number of juvenile birds becoming breeding individuals may be reduced through impaired fitness or increased mortality. These studies provided validation for adverse effects to avian species resulting from a dietary concentration of 0.5 mg/kg methylmercury.

In a similar evaluation of methylmercury impacts to juvenile piscivorous birds, Henny *et al*. (2002) studied three bird species nesting in a mercury-contaminated watershed. Various tissues and endpoints from both adult and juvenile double-crested cormorants, black-crowned night herons, and snowy egrets were measured, including methylmercury concentrations in stomach contents. Based on stomach content analyses, it was determined that young of these species were fed diets averaging 0.36 - 1.18 mg/kg methylmercury through fledging. Although adult birds were exposed to the same prey pool and had higher total mercury concentrations in their livers than fledglings, the younger birds exhibited greater evidence of sublethal toxicity to their

immune, detoxification, and nervous systems. The strongest evidence of these effects was seen in the cormorants, which had the highest average methylmercury concentration reported from stomach content analysis (1.18 mg/kg). However, these effects were also observed in the other species, with average dietary concentrations of 0.36 mg/kg (snowy egrets) and 0.43 mg/kg (black-crowned night herons). No conclusions could be drawn regarding post-fledging survival, as the study concluded at about the time of fledging. However, noting that many of the fledglings remained in the watershed after leaving the nest area, the study authors suggested that the additional period of foraging in the contaminated system, coupled with the completion of feather growth, may have critically increased the body burden of mercury and its potential toxicity.

None of the studies described above (Barr, 1986; Bouton *et al*., 1999; Spalding *et al*., 2000a,b; Henny *et al*., 2002) provided a suitable avian oral test dose for methylmercury that could be used as an alternative to the one generated in the Heinz (1979) work with mallard ducks. They do, however, confirm that a concentration of methylmercury in food around 0.5 mg/kg is sufficient to cause significant adverse effects to avian reproduction and health that could have deleterious impacts at both the individual and population levels. A review of the scientific literature revealed no other dose-response studies that established appropriate oral test doses for avian species, and the Heinz (1979) work remains the most robust benchmark for evaluating impacts to birds from methylmercury in the diet.

The body of work on mercury toxicity to avian species includes a great deal of data on residue concentrations in various tissues (*e.g.*, brain, liver, feather). Often these studies have attempted to establish threshold concentrations in specific tissues correlated with adverse effects. The use of egg concentrations is often cited as a valuable endpoint in evaluating the toxicity of methylmercury, as developing embryos are more sensitive than adults (Wiener *et al*., 2002). Reviews of studies reporting data on mercury concentrations in eggs of both wild and captive birds can be found in Thompson (1996), Burger and Gochfeld (1997), Wolfe *et al*. (1998), and Eisler (2000). However, as important as these studies are for determining concentrations associated with embryotoxic effects, relatively few provide information on the dietary doses of the laying birds that resulted in the observed egg methylmercury concentrations.

The two most commonly cited studies reporting egg methylmercury concentrations and adverse effects resulting from controlled feeding studies examined pheasants (Fimreite, 1971) and mallards (Heinz, 1979). The mallard study is the same as the one discussed above, used in determining the LOAEL dietary test dose for the GLI. From a dietary concentration of 0.5 mg/kg methylmercury, Heinz (1979) reported an average concentration over three generations of 0.83 mg/kg wet weight in eggs. Although mallard embryos were not examined for signs of toxicosis, the egg concentrations reported resulted from a dietary dose causing adverse reproductive effects. Fimreite's (1971) controlled dosing experiment with ring-necked pheasants demonstrated reduced hatchability, expressed as the percentage of eggs incubated, in egg samples containing between 0.5 - 1.5 mg/kg methylmercury. This range is similar in magnitude to the average egg concentration (0.83 mg/kg) reported by Heinz (1979), and the lower end (0.5 mg/kg) is often

cited as a LOAEL for avian eggs (Wolfe *et al*., 1998). Based on the egg concentrations and associated adverse reproductive effects reported in these two studies, it is generally accepted in the scientific literature that eggs of pheasants are more sensitive to methylmercury than mallard eggs. However, the dietary concentrations (~ 2-5 mg/kg) resulting in the range of egg concentrations observed in pheasants by Fimreite (1971) were substantially higher than the 0.5 mg/kg dietary concentration causing the similar egg values reported in mallards by Heinz (1979). This indicates a substantial difference between these species in the transfer efficiency from methylmercury in the maternal diet to methylmercury in the egg.

Recent and ongoing efforts by Heinz (pers. comm., 2003) are focused on more closely examining interspecies differences in sensitivity to egg methylmercury concentrations. Through direct injection into the eggs of various bird species, different concentrations of methylmercury can be evaluated as to their effects on developing embryos. Preliminary results seem to confirm the findings from the feeding studies described above that pheasant eggs are more sensitive than mallard eggs. In addition, there appears to be a broad range of species sensitivity, both more and less sensitive than mallard eggs. While the data from these efforts, when published, will provide important information concerning the relative magnitude of sensitivity exhibited by different species, their utility for evaluating effects from dietary methylmercury is limited by two constraints. First, it requires less methylmercury to cause adverse effects in eggs when it is injected than when naturally deposited by the mother. Therefore, species-specific LOAELs for eggs cannot be determined from injected concentrations until a relationship to maternally-deposited concentrations can be accurately determined. Second, as seen with the pheasant and mallard feeding studies, there may be wide variations among species in diet-to-egg transfer efficiency. Selecting an egg LOAEL based on the most sensitive species examined in injection studies may correspond to a higher dietary concentration, relative to other species with higher egg LOAELs.

As no other toxicity data were found that could provide a more appropriate oral test dose for avian species, the results of the Heinz (1979) study with mallard ducks was used for this evaluation. However, discrepancies were noted in the scientific literature regarding how these results were used to convert the dietary concentration (mg/kg in food) to a daily dose (mg/kg-bw/day). As described above, the EPA used the average food consumption rate for 2nd and 3rd generation mallards in the treatment group (0.156 kg/kg-bw/day) to calculate a dietary dose of 0.078 mg/kg-bw/day for use in the GLI avian wildlife criterion derivation (U.S. Environmental Protection Agency, 1995d). In a departure from this approach, the U.S. Department of Energy (1993-1996) used the average food consumption rate for the study's control group (0.126 kg/kg-bw/day) to calculate a dietary dose of 0.064 mg/kg-bw/day for the derivation of toxicological benchmarks for wildlife. This lower value has been used in Wolfe and Norman (1998) and California Regional Water Quality Control Board - Central Valley Region (2001), while the higher value has been used in Nichols *et al*. (1999), Canadian Council of Ministers of the Environment (2000), Buchanan *et al*. (2001), and Evers *et al*. (2002). Further confounding the matter, the MSRC used the higher value in one volume (Vol. VI) (U.S. Environmental Protection Agency, 1997a) and the lower value in a different volume (Vol. VII) (U.S. Environmental

Protection Agency, 1997b), although the higher value was used in the Report to calculate water quality criteria.

In an effort to understand the rationale for using the control group's food consumption rate to calculate a LOAEL, the author of the 1979 mallard study was contacted (Heinz, pers. comm., 2002). Heinz stated that the difference in his reported ingestion rates for the two study groups was not due to greater wastage on the part of the treatment group, and further, that the reported rates were probably not very accurate for either group. He explained that the ability to distinguish wasted food from the debris at the bottom of test subject cages (fecal matter, undigested food, *etc*.) was insufficient to calculate feeding rates with a great degree of precision. However, based on his understanding of work subsequent to the 1979 study, Heinz believes that true mallard feeding rates are likely even lower than the rates he reported (0.1 kg/kg-bw/day vs. 0.128 and 0.156). While Heinz did not suggest a 0.1 kg/kg-bw/day ingestion rate be used to determine the LOAEL, he did caution against using the 0.156 kg/kg-bw/day rate reported for his 1979 treatment group. This conversation supported the use of the 0.064 mg/kg-bw/day LOAEL calculated with Heinz' control group feeding rate as the appropriate dietary dose for evaluating risk to avian species, with the acknowledgment that true mallard feeding rates may suggest the need for a lower LOAEL.

III.D. Determination of Reference Doses

As noted previously, a reference dose (RfD) may be defined as the daily exposure to a toxicant at which no adverse effects are expected, analogous to NOAEL doses determined from toxicity tests. However, RfDs are intended to protect all species likely to be at risk from exposure to the contaminant, from each taxonomic class for which test doses were determined. Ideally, toxicity tests to determine chronic effects of a contaminant will be of sufficient duration and dose spacing to allow for establishment of a reliable NOAEL. For a variety of reasons, the duration and dose spacing of many toxicity tests are not suitable for this, and NOAELs must be extrapolated from the test information available. In addition, any NOAELs established may only be applicable for the species tested. Extrapolating any given test dose into a RfD at which no adverse effects are expected, for potentially a broad range of species, involves some amount of uncertainty.

In order to determine the RfD for a given taxonomic group, the test dose selected to represent that group may need to be adjusted by uncertainty factors to incorporate variability in toxicological sensitivity among species and to extrapolate for duration (subchronic-to-chronic) or dose spacing (LOAEL-to-NOAEL) issues. The RfD is calculated using the following equation:

$$\mathbf{RfD} = \frac{\mathbf{TD}}{\mathbf{UF_A} \times \mathbf{UF_S} \times \mathbf{UF_L}} \qquad \mathbf{(7)}$$

RfD = Reference Dose (mg/kg-bw/day)
TD = Test Dose (mg/kg-bw/day)
UF_A = Interspecies Uncertainty Factor (unitless)
UF_S = Subchronic-to-Chronic Uncertainty Factor (unitless)
UF_L = LOAEL-to-NOAEL Uncertainty Factor (unitless)

The concept of adjusting test doses to account for these types of uncertainty has been widely used in efforts to develop avian and mammalian reference doses for methylmercury that would be protective of a range of wildlife species (U.S. Department of Energy, 1993-1996; Canadian Council of Ministers of the Environment, 2000; Buchanan *et al*., 2001; California Regional Water Quality Control Board - Central Valley Region, 2001; Evers *et al*., 2002). However, the majority of these efforts have used the same uncertainty factors originally determined in either the GLI effort (U.S. Environmental Protection Agency, 1995d) or the MSRC (U.S. Environmental Protection Agency, 1997a,b). Guidance on determining the appropriate values for each uncertainty factor can be found in two EPA documents: *Technical Basis for Recommended Ranges of Uncertainty Factors used in Deriving Wildlife Criteria for the Great Lakes Water Quality Initiative* (Draft Report) (Abt Associates Inc.,1995) and Great Lakes Water Quality Initiative *Technical Support Document for Wildlife Criteria* (U.S. Environmental Protection Agency,1995a).

Mammalian RfD: As described previously in Section IV,C (Determination of Test Doses), the EPA selected studies by Wobeser *et al*. (1976a,b), in both the GLI and the MSRC, to determine the appropriate mammalian test dose for calculating the RfD. However, the two efforts applied different assumptions and arrived at different test doses. For the GLI, a test dose of 0.16 mg/kg-bw/day was determined to be the NOAEL, while the MSRC concluded the test dose of 0.055 mg/kg-bw/day was the appropriate NOAEL. In addition to this difference, each effort then applied different uncertainty factors to each test dose to determine the RfD.

In the GLI, the UF_A and UF_L were both assigned a value of 1. This was because the experimental animal (mink) and the representative species to be protected (river otter) are closely related and assumed to be similarly sensitive, and because the study identified a NOAEL. The UF_S was set at a value of 10 because the study chosen (Wobeser *et al*., 1976b) was of subchronic duration. Applying these three combined uncertainty factors to the test dose of 0.16 mg/kg-bw/day resulted in a mammalian RfD of 0.016 mg/kg-bw/day.

For the MSRC, the UF_A and UF_L were also both assigned a value of 1, for the same reasons outlined above. However, the UF_S for this effort was set at a value of 3 because the effects observed at the subchronic NOAEL (Wobeser *et al*., 1976a) were not associated with overt signs of toxicity (Nichols *et al*., 1999). Applying these three uncertainty factors to the test dose of 0.055 mg/kg-bw/day resulted in a mammalian RfD of 0.018 mg/kg-bw/day.

So despite the discrepancy regarding the appropriate test dose for mammals, both efforts arrived at roughly the same mammalian RfD. The single mammalian species of concern for this

evaluation is the southern sea otter (*Enhydra lutris nereis*), in the same taxonomic family (*Mustelidae*) as the mink and river otter. Therefore, no further adjustments to the UF_A or UF_L were necessary. The analyses regarding the mammalian test dose and UF_S presented in the MSRC represent the most current comprehensive assessment of these Wobeser *et al*. (1976a,b) studies. As a result, **a mammalian RfD of 0.018 mg/kg-bw/day** was used in this evaluation (Table 1.).

Avian RfD: Similar discrepancies concerning uncertainty factors for the avian RfD were noted between the GLI and the MSRC. Both of these efforts agreed on an avian test dose (0.078 mg/kg-bw/day) from the three generation mallard duck study (Heinz, 1979), and both agreed that the UF_S should be assigned a value of 1 because the study was of sufficient chronic duration. However, varying assumptions regarding LOAEL-to-NOAEL relationships and interspecies sensitivity resulted in each effort assigning different UF_L and UF_A values.

Regarding the UF_L, a value of 2 was assigned for the GLI because the LOAEL identified by the EPA from the mallard study, 0.078 mg/kg-bw/day, "...appeared to be very near the threshold for effects of mercury on mallards." As explained in Nichols *et al*. (1999), a range of 1 - 10 was used to set the UF_L values in the GLI, based on an evaluation of chronic toxicity studies with wildlife species using five chemicals (cadmium, DDT, DDE, dieldrin, and mercury). This conclusion was reached after determining that 97 percent of the LOAEL-to-NOAEL ratios examined were less than or equal to 10 and 50 percent were less than or equal to 3.

In contrast, the authors of the MSRC evaluated toxicity studies with methylmercury only. Twenty LOAEL-to-NOAEL ratios were calculated, with the majority between 1 - 2 or 4 - 5 (Nichols *et al*., 1999). For the final calculations of wildlife criteria values in the MSRC, the UF_L was assigned a value of 3. The MSRC (Vol. VI) concluded that "Given the substantial uncertainties in all the values used to calculate the WC for mercury exposure, neither two nor three can be considered to be the only correct value" (U.S. Environmental Protection Agency, 1997a).

The conceptual basis for use of a UF_A is that toxicokinetic and/or toxicodynamic differences among species may result in variable responses to the same applied dose. Empirical data from acute and chronic toxicity tests with wildlife species support the use of a UF_A ranging from 1 to 100 when extrapolating toxicological effects across species. Values tending toward the lower end of this range may be justified by several factors including: 1) the amount and quality of available testing data, 2) a close taxonomic relationship between the tested species and the species of interest, 3) similarity in size of the tested species and the species of interest, and 4) toxicokinetic and / or toxicodynamic information which would suggest that the tested species is likely to be more sensitive than the species of interest.

For the GLI, a UF_A greater than 1 was recommended because of the need to extrapolate mallard data to species in different taxonomic orders, and because of the possibility that another of the species (pheasant) examined in toxicity studies might prove more sensitive if given a longer

exposure duration. However, because the analysis of suitable avian toxicity values reviewed for the GLI indicated that the mallard was possibly the most sensitive to mercury of the six species examined, the conclusion was drawn that a UF_A of 10 would likely be overly conservative. A UF_A of 3 (half-way between 1 and 10 on a log 10 scale) was therefore applied as a reasonable protection for those species that may be more sensitive than mallards.

The question of interspecies sensitivity was revisited in the MSRC. The three species selected in the GLI to represent avian wildlife (belted kingfisher, herring gull, bald eagle) are piscivorous birds. The authors of the MSRC cited literature suggesting that piscivorous birds possess, in comparison to non-piscivorous birds, a greater capacity to demethylate and thereby detoxify methylmercury. Although piscivorous birds are likely faced with the greatest exposure to methylmercury, the MSRC authors concluded that these birds are unlikely to be more sensitive than mallard ducks (an omnivorous species) to the toxic effects of methylmercury, and that application of a UF_A greater than 1 was unwarranted for piscivorous species. Research conducted since publication of the MSRC has provided additional support for the existence of a protective demethylating capability in piscivorous birds (Henny *et al*., 2002). As the species selected in the MSRC to represent avian wildlife (belted kingfisher, loon, osprey, bald eagle) are also piscivorous, the UF_A for that effort was assigned a value of 1. In summary, the uncertainty factors used in both the GLI and the MSRC to adjust the mallard test dose to an avian RfD were as follows:

	GLI	MSRC
UF_A	3	1
UF_S	1	1
UF_L	2	3

For this evaluation, two of the federally-listed avian species of concern are primarily (bald eagle) or exclusively (California least tern) piscivorous. For these species, the rationale used in the MSRC to assign a UF_A of 1 is therefore applicable. This effort differs, however, from both the GLI and MSRC efforts insofar as it includes consideration of four species (California clapper rail, light-footed clapper rail, Yuma clapper rail, and snowy plover) which feed extensively on invertebrates, including (in the case of the snowy plover) invertebrates of non-aquatic origins.

No information could be found regarding the capability of clapper rails or snowy plovers to detoxify methylmercury. Henny *et al*. (2002) provided some data indicating that adult birds whose diet consists largely of aquatic invertebrates may also possess this detoxifying capacity. In this study, Henny *et al*. examined three bird species nesting in a mercury-contaminated watershed. Examination of stomach contents for two of these species, black-crowned night herons (*Nycticorax nycticorax*) and snowy egrets (*Egretta thula*), revealed diets ranging from 100 percent fish to 100 percent large aquatic insect larvae. The diet of the third species, double-crested cormorant (*Phalacrocorax auritus*), was comprised entirely of fish. Analysis of livers from all three species indicated that hepatic demethylation, possibly in a dose-dependent

relationship, allowed adult birds to tolerate relatively high mercury concentrations without apparent adverse effects. Fledglings did not exhibit the same degree of tolerance to liver mercury concentrations; however, the study ended before it could be determined whether hepatic demethylation would become more pronounced as the fledglings matured. The results of this study lend support to the idea that even birds that are not strictly piscivorous, but still primarily consume aquatic biota, may be less sensitive to methylmercury than the non-piscivorous mallard.

However, as described previously in the section on avian test doses, there has been recent work on interspecies sensitivity to methylmercury using egg injection studies (Heinz, pers. comm., 2003). The clapper rail is one of the species examined thus far whose sensitivity to methylmercury in the egg appears to be greater than the mallard, perhaps closer in sensitivity to the pheasant. These results are preliminary only, and presently it is impossible to translate differences in sensitivity of clapper rail and mallard duck eggs to an injected dose of methylmercury into an ecologically meaningful comparison. No information was available from this work on the amount of methylmercury in food necessary to achieve any observed egg effects concentrations or on the relationship of observed effects concentrations to a maternally-deposited dose. The diet-to-egg transfer efficiency can vary widely between different species, as evidenced by the controlled feeding studies with mallards (Heinz, 1979) and pheasants (Fimreite, 1971). It would be imprudent to assume that similar sensitivities to egg concentrations between the clapper rail and the pheasant would necessarily be caused by the same dietary concentration. However, although no definitive conclusions can presently be drawn as to whether the clapper rail is more or less sensitive to methylmercury in food than the mallard, the need for a greater UF_A for this species in determining a reference dose could not be ruled out.

Based on the information outlined above, the uncertainty factors presented in the MSRC are more generally appropriate than those from the GLI for determining the avian reference dose. However, because several of the bird species considered in this effort are not obligate piscivores, the argument presented in the MSRC for using a UF_A of 1 may not be appropriate for these species. For this reason the derivation and subsequent assessment of WVs was based on a UF_A of 1 for piscivorous avian species (least tern and bald eagle) and UF_As of both 1 and 3 for the snowy plover and clapper rails. The UF_A of 3 was selected using the same rationale from the GLI (*i.e.*, half-way between 1 and 10 on a log scale). The alternative reference doses generated by the two UF_As provided for a comparative analysis of protection afforded by both evaluation approaches.

Based on the avian TD of 0.064 mg/kg-bw/day from the Heinz (1979) mallard duck study, and the uncertainty factors from the MSRC, **an avian RfD of 0.021 mg/kg-bw/day** was used in this evaluation (Table 1.). An **alternative avian RfD of 0.007 mg/kg-bw/day** was also presented for the three clapper rail subspecies and the snowy plover.

Table 1. Test Doses, Uncertainty Factors, and Reference Doses for Birds and Mammals

	Mammals	All Birds	Clapper Rails / Snowy Plover
Test Dose	0.055 mg/kg-bw/day	0.064 mg/kg-bw/day	0.064 mg/kg-bw/day
UF_A	1	1	3
UF_S	3	1	1
UF_L	1	3	3
RfD	0.018 mg/kg-bw/day	0.021 mg/kg-bw/day	0.007 mg/kg-bw/day

IV. CALCULATING WILDLIFE VALUES: BODY WEIGHTS, DIETARY COMPOSITION, FOOD INGESTION RATES

Once the RfDs for each taxonomic group were determined from the appropriate test doses, species-specific WVs were calculated (Equation 6; see page 7). This required information on average adult female body weights (kg) and species-specific daily food ingestion rates (FIR *in* kg food/day). References for body weights are provided in each species account below.

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). However, because 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.

Dietary composition, the amount of each food type consumed on a daily basis, is a critical component in determining FIR, as different foods provide different amounts of gross energy (*e.g.*, kcal/g food matter) to the consumer. For example, the gross energy (GE) available from aquatic invertebrates is greater than that available from aquatic algae (U.S. Environmental

Protection Agency, 1993). The AE values for different foods may also vary substantially. For example, a bird eating aquatic invertebrates assimilates the available energy at a substantially higher efficiency (77%) than if it were eating aquatic vegetation (23%) (U.S. Environmental Protection Agency, 1993). Therefore, the amount of aquatic invertebrate food necessary to fulfill the energetic requirements of a bird consumer would be substantially less than the amount of aquatic vegetation needed to meet the same requirements.

In addition to providing the percentages of each food type in a wildlife consumer's diet, feeding ecology studies can establish the trophic level composition of the diet. While this information is not necessary for calculating WVs, it is essential for evaluating whether either of the TRC trophic level approaches presented here will result in an exceedance of the WVs. Ideally, dietary information on both food type amounts and trophic level composition can be determined in percent biomass, as this provides the most accurate representation of actual ingestion. However, due to the difficulty inherent in determining the exact daily dietary composition of any free-living animal, dietary studies often rely on frequency of feeding observations or analysis of prey remains or a combination of both. These types of data pose less of a problem if the prey species are the same kind (*e.g.*, all fish) and roughly the same size. As the diversity of the prey base increases, however, the relative contribution from each prey item to the daily ingested biomass can be over- or under-represented if reported on the basis of occurrence frequency. For example, observations of predation may indicate an animal consumes small crabs and clams in equal amounts (*i.e.*, 50% clams:50% crabs). However, clams may provide more biomass per animal consumed than crabs, indicating the need for a different dietary ratio (*e.g.*, 70% clams:30% crabs) in estimating food ingestion rates and determining whether WVs will be exceeded.

The following accounts present the best available information regarding dietary composition and FIRs for the species of concern in this evaluation. When species-specific information regarding metabolic needs and assimilation efficiencies for various food types was not available, FIRs were determined using the most appropriate allometric equations from Nagy (2001). When this information was available, FIRs were determined using equations to estimate FMR (Nagy, 1987) and the methodology described in the *Wildlife Exposure Factors Handbook* (U.S. Environmental Protection Agency, 1993). The reader is directed to the three references mentioned for a complete explanation of the allometric methodology.

As the goal of the evaluation was to consider potential effects to animals living and breeding in California, every attempt was made to find the most rigorous dietary data for resident animals. For some species, few detailed feeding studies have been conducted. As a result, some of the following dietary information is based on only one or two studies, some conducted several decades ago. Until new data are generated, however, these studies remain the best source for dietary information.

IV.A. Southern Sea Otter (*Enhydra lutris nereis*):

Sea otters are the largest member of the Mustelidae family but one of the smallest marine mammals (Riedman and Estes, 1988). Based on length measurements of dead sea otters in California, the predicted average weights of healthy animals are 29.0 kg (males) and 19.8 kg (females) (Riedman and Estes, 1990). Although individual body weights may vary from these values, the predicted **average weight for female otters (19.8 kg)** was used for the calculation of wildlife values in this evaluation.

Information on southern sea otter diet was taken primarily from Riedman and Estes (1988, 1990). The diet of southern sea otters rarely or never includes fish, instead being comprised almost exclusively of benthic macroinvertebrates. Over 60 different invertebrate species have been identified as prey items of southern sea otters. However, sea otter diet is influenced by prey species availability, length of time otters have occupied an area, habitat type, and time of year.

Southern sea otters are primarily associated with subtidal habitats characterized by rocky substrata, although they are also found in areas with soft-sediment substrata. The main prey items in rocky subtidal habitats are abalones (*Haliotis* spp.), rock crabs (*Cancer* spp.), and red sea urchins (*Strongylocentrotus* spp.) (Riedman and Estes, 1988). Abalones and sea urchins are predominantly herbivorous, while rock crabs (*e.g.*, red crab, Dungeness crab) are carnivorous on small crustaceans, clams, and oysters (Morris *et al*., 1980). Sea otters in soft-sediment substrata also rely heavily on bivalve molluscs (*e.g.*, Pismo, Washington, and gaper clams), although the 13 soft-sediment species identified as prey in these habitats include rock crabs and the Lewis's moon snail (*Polinices lewisii*) (Kvitek and Oliver, 1988). The moon snail is primarily a predator on clams (Morris *et al*., 1980).

In addition to the aforementioned invertebrates, southern sea otter diets can include a wide variety of prey: kelp crabs (*Pugettia* spp.), turban snails, mussels (*Mytilus* spp.), octopus (*Octopus* spp.), barnacles (*Balanus* spp.), scallops (*Hinnites* spp.), fat innkeeper worms, sea stars (*Pisaster* spp.), and chitons (*Cryptochiton* spp.) (Riedman and Estes, 1990). Seasonal abundance can also play a role in determining important food items. Squid, spawning during fall and spring in Monterey Bay, constitute a large component of some sea otter diets (Riedman and Estes, 1990). Sea otters also occasionally prey on various seabirds, including western grebes (*Aechmophorous occidentalis*), surf scoters (*Melanitta perspicillata*), cormorants (*Phalacrocorax* spp.), common loons (*Gavia immer*), and gulls (*Larus* spp.). However, observations of this foraging behavior suggest that it is rare and that male otters may be responsible for the majority of seabird predation (Riedman and Estes, 1990).

The diet of southern sea otters may include a number of species considered trophic level 3 organisms (*e.g.*, octopus, squid, rock crab, moon snail, sea stars), although trophic level 2 organisms (*e.g.*, abalones, clams, mussels, urchins) appear to be the predominant prey. However, diet and foraging strategy appear to vary between individual otters, even within the same foraging habitat (Riedman and Estes, 1988). Sea otters appear to specialize on certain available

prey species, and these preferences may be maintained for several years. Observations of tagged female sea otters in Monterey Bay provided examples of this specialization, with one female preferentially eating kelp crabs, turban snails, and purple urchins, while another female foraged on abalones and rock crabs (Riedman and Estes, 1988).

This apparent foraging specialization, coupled with the diverse array of prey known to be consumed by sea otters, makes it difficult to assign a particular dietary trophic level composition. In a study of foraging in soft-sediment habitats, clams (trophic level 2) were captured and eaten on more than 75 percent of successful foraging dives (Kvitek and Oliver, 1988). Crabs considered trophic level 3 organisms (*Cancer* spp.) appeared to account for only a small percentage (~ 4%) of the diet, with other, lower trophic level crabs (*e.g.*, mole crab, kelp crab) and molluscs comprising the remainder. No comparable estimations of dietary composition were found for otters in rocky habitats, although it appears generally accepted that trophic level 2 organisms like abalones and sea urchins account for the majority of food consumed by these otters. However, based on the availability of a variety of trophic level 3 prey and the potential for individual otters to specialize on certain species, the dietary composition used for evaluating the TRC trophic level approaches for sea otters was **20 percent trophic level 3, 80 percent trophic level 2**. These are not static values and further research may indicate the need for an alternate estimation of dietary composition.

It has been estimated that free-ranging adult sea otters may consume food equivalent to 23-33 percent of their body weights per day (Riedman and Estes, 1990). Using the high end of this range (*i.e.*, 33%) as a conservative approach to represent the assumed higher metabolic needs of a breeding female sea otter, and the predicted average female weight of 19.8 kg results in a daily food ingestion rate of 6.5 kg/day. This estimate of FIR is substantially higher than what would be expected using any of the allometric equations described previously. However, this apparent discrepancy may be explained by considering the sea otter's metabolism and energetic requirements. Sea otters are small relative to other marine mammals, and lack the blubber layer which provides insulation and an energy reserve. Sea otters compensate for the thermal stress of a marine existence by maintaining a high level of internal heat production; 2.4 - 3.2 times that expected for a terrestrial mammal of similar size (Riedman and Estes, 1990). Based on the otter's elevated energetic requirements, it has been estimated that a 20 kg adult would need between 4,295 and 5,750 kcal/day (Riedman and Estes, 1990), roughly twice the FMR estimated using Nagy's allometric equation for all placental mammals (U.S. Environmental Protection Agency, 1993).

FIR for southern sea otter = 6.5 kg wet weight/day

IV.B. California Least Tern (*Sterna antillarum browni*):

The least tern is the smallest of the tern species that nest on open beaches and islands free of vegetation (Thompson *et al*., 1997). Adult female body weights presented in this reference range from 36 - 62 g; however, this range includes three geographic subspecies: *S. a. antillarum* (U.S.

Atlantic/Gulf coasts, West Indies); *S. a. athalassos* (interior U.S.); and *S. a. browni* (California coast, west coast of Mexico). The mean weight for *S. a. antillarum* is 49.3 g, while that of *S. a. athalassos* is 42.5 g. The reported weight for *S. a. browni* (39.8 g) was only based on one specimen. Dunning (1993) reported a mean weight of 43.1 g (unknown sex) for breeding birds in Kansas (most likely *S. a. athalassos*). Using the mean weights reported in Thompson *et al.* (1997) for the two coastal subspecies results in an **average adult female body weight of 45 g**.

Although other subspecies' diets include small crustaceans and insects (Thompson *et al.*, 1997), the California least tern appears to be strictly piscivorous (Massey, 1974). Breeding colonies may form on beach sites along the coast or on suitable alternative substrates set back from the ocean (U.S. Fish and Wildlife Service, 1985a). Colonies are generally located either near the coast, or near lagoons, estuaries, or rivers (Thompson *et al.*, 1997).

Individuals from three breeding colonies near the coast, that had little or no freshwater or estuarine habitats nearby, were found to forage almost exclusively in relatively shallow, nearshore ocean waters in the vicinity of major river mouths (Atwood and Minsky, 1983). Terns were observed to feed on three primary forage fish species: northern anchovy (*Engraulis mordax*) and two species in the silversides family - topsmelt (*Atherinops affinis*) and jacksmelt (*Atherinopsis californiensis*). Prey size at two coastal colonies varied for each tern age class, with chicks consuming smaller fish than adults or juveniles. However, 73 percent of the three primary forage fish species eaten by all age classes were less than 5 cm in length (Atwood and Kelly, 1984).

In contrast to tern colonies which foraged mainly in nearshore ocean waters, terns from breeding colonies located near estuarine habitats fed primarily in shallow saltmarsh channels and tidal estuaries (Atwood and Minsky, 1983; Atwood and Kelly, 1984). The dominant forage fish species in these waters, and the majority (82%) of fish dropped at a colony in Anaheim Bay, were the topsmelt and California killifish (*Fundulus parvipinnis*). Atwood and Kelly (1984) found that fish dropped at breeding tern colonies, either accidentally or from lack of hunger, were generally valid indicators of the principal prey species consumed. Two other forage fish, deepbody anchovies (*Anchoa compressa*) and slough anchovies (*Anchoa delicatissima*), were the most abundant prey dropped at two southerly colonies, although no distinction was made as to where terns from these colonies foraged (Atwood and Kelly, 1984). Although a total of 49 forage fish species, all represented by individuals less than 1 year old, were found at 10 breeding tern colonies, Atwood and Kelly (1984) concluded that five fish (northern anchovy, topsmelt, jacksmelt, deepbody anchovy, slough anchovy) represented the main food items at least tern breeding colonies in California.

Foraging ecology for a tern breeding colony located near San Francisco Bay has been monitored for numerous years, providing a long-term assessment of the colony's dietary preferences (Elliott and Sydeman, 2002). Prey fish dropped at the colony by foraging birds were collected and identified from 1981-1982, 1984-1995, and 2000-2001. Although minor variations in forage fish species abundance were reported between years, the combined data from all years revealed that

three fish (topsmelt, jacksmelt, northern anchovy) accounted for more than 86 percent of all samples collected. The next most abundant prey (> 7% of total) were various surfperch species (*Embiotocidae*).

Based on the above information, the diet of adult female California least terns is comprised solely of small fish from various species. Several of these species (northern anchovy, topsmelt, jacksmelt, California killifish) appear to account for the majority of prey items taken by both courting and nesting terns, including those birds that forage in estuarine and tidal waters. In addition, data indicate that the majority of fish captured by breeding terns are small (5 cm or less) and all are young-of-year (Atwood and Kelly, 1984). According to the *Trophic Level and Exposure Analyses for Selected Piscivorous Birds and Mammals* (Vol. III) (U.S. Environmental Protection Agency, 1995b), these prey species are generally considered trophic level 3. Even juvenile fishes from this group (*e.g.*, topsmelt, northern anchovy) are listed as trophic level 3 by this reference.

It is important to note that all of these forage fish species exhibit some amount of omnivory, feeding to varying degrees on primary producers and detritus. Juvenile northern anchovies generally consume small crustaceans and other zooplankton, although algae and other phytoplankton may constitute a substantial portion of their diet (Wang,1986). Anchovies can be filter-feeding or biting planktivores, indicating the ability to selectively prey on individual organisms (California Department of Fish and Game, 2001). Similarly, the diet of the California killifish consists primarily of benthic and planktonic invertebrates, with juveniles more likely than adults to feed on terrestrial insects and zooplankton (Moyle, 2002). West and Zedler (2000) examined gut contents of adult killifish and reported algae and detritus as minor dietary items. Nonetheless, both anchovy and killifish appear to feed primarily on trophic level 2 organisms.

In contrast to the anchovy and killifish, the feeding habits of the other two primary tern prey fish (topsmelt and jacksmelt) indicate a greater dietary dependence on trophic level 1 food. Wang (1986) listed the major food items for juvenile jacksmelt as algae, detritus, and small crustaceans. In addition, amphipods were described as a common food item. The same reference (Wang, 1986) states that juvenile topsmelt feed on crustaceans, diatoms, algae, detritus, chironomids, and amphipods. The California Department of Fish and Game (2001) states that topsmelt inhabiting intertidal areas consume algae and fly larvae, as well as crustaceans. Moyle (2002) points out that the diet of small topsmelt (4.9 - 5.6 cm) in one estuary consisted primarily of diatoms and filamentous algae (50% by volume), and detritus (29%), with chironomid midge larvae and amphipods comprising an additional 20 percent.

While all of these forage fish may incorporate some amount of primary producers and detritus in their diets, none can be considered exclusively trophic level 2 consumers. California least terns are not species-specific predators; therefore, their overall dietary composition will vary depending on the relative abundance of suitable prey species. At any given time or location, it is impossible to predict whether prey fish are primarily consuming plant material or the trophic level 2 organisms that feed on plant material. In order to adequately evaluate the full potential

impact of the methylmercury TRC on the endangered California least tern, a diet of **100 percent trophic level 3 fish** is assumed.

The FMR for least terns was estimated using Nagy's allometric equation for all birds (*in* U.S. Environmental Protection Agency, 1993):

FMR (kcal/day) = 2.601 × (body weight in g)$^{0.640}$
FMR = 2.601 × 45 $^{0.640}$
FMR = 29.7 kcal/day

The FIR was then calculated using the equation:

FIR = FMR ÷ metabolizable energy from food (ME)

where ME equals the gross energy (GE) from the food type times the assimilation efficiency (AE) of the animal consuming that food. The GE of bony fishes is 1.2 kcal/g wet weight. The AE for birds consuming fish is 79%. Therefore, the ME for the least tern is 0.948 kcal/g fish.

FIR = 29.7 kcal/day ÷ 0.948 kcal/g fish

FIR for California least tern = 0.031 kg wet weight/day

IV.C. California Clapper Rail (*Rallus longirostris obsoletus*):

The California clapper rail (*R. l. obsoletus*) is the largest of the three rail subspecies considered in this evaluation, followed in descending order by the light-footed and Yuma clapper rails (U.S. Fish and Wildlife Service, 1976). In the only literature found for this particular subspecies that provided body weights, nineteen female California clapper rails from south San Francisco Bay were examined as part of a Master's Degree thesis (Albertson, 1995). Weights ranged from 300 to 400 g, with a **mean weight of 346.1 g**. This mean value was used for the calculation of a wildlife value for this subspecies.

The most comprehensive assessment of the California clapper rail diet is presented by Moffitt (1941). Stomach contents from 18 birds were examined and the food items identified and measured as a volumetric percentage. On average, animal matter accounted for approximately 85 percent of the diet, with the remainder composed of seed and hull fragments of marsh cordgrass. Over half (56.5%) of the overall diet was comprised of plaited horse mussels (*Modiolus demissus*). Spiders of the family Lycosidae (wolf spiders) accounted for 15 percent of the diet, while little macoma clams (*Macoma balthica*) (7.6%), yellow shore crabs (*Hemigrapsis oregonensis*) (3.2%), and worn-out nassa snails (*Ilyanassa obsoletus*) (2.0%) were the remaining important dietary items. Worms, insects, and carrion combined accounted for a total of 1.1 percent of the remaining diet found by Moffitt (1941) in the 18 clapper rail stomachs. The importance of crabs in the clapper rail diet was confirmed by Varoujean (1972), who observed

rails eating striped shore crabs (*Pachygrapsus crassipes*).

Although Moffitt (1941) reported that plant matter accounted for approximately 15 percent on average of the clapper rail diets, the author stated that this percentage probably represented the maximum of a vegetable diet. This conclusion was based on the fact that the birds were collected in early February, a time when animal food items would typically be at lowest abundance. However, it is important to note that this reported average for plant food (~15%) was calculated from a wide range of percentages in the 18 birds examined (0% - 58% plant food). As with other omnivorous species, the amount of any particular food item consumed at any given time may vary substantially depending on a number of factors. While clapper rails most likely do not eat a set amount of plant matter daily, it is clear from Moffitt (1941) that vegetation generally constitutes a substantial dietary item over time.

Based on Moffitt's (1941) assumption that his mid-winter gut analyses represented a maximum for vegetation in the clapper rail diet, and the knowledge that clapper rails nest during a time when animal foods would be in greater abundance (mid-March - July) (U.S. Fish and Wildlife Service, 1984), the overall rail diet for this effort is assumed to be 10 percent vegetation and 90 percent animal matter. For the purposes of this evaluation, the vegetation portion of the diet will be considered as food not contributing to the daily ingested dose of methylmercury. Although mercury is known to accumulate in aquatic plants (Gupta and Chandra, 1998; Ellis and Eslick, 1997; Breteler *et al*., 1981), the scientific literature indicates that accumulation is primarily in the roots rather than in the rhizomes or above-ground tissues (Boening, 2000; Breteler *et al*., 1981).

The primary animal foods of clapper rails according to Moffitt (1941) appear to be mussels, wolf spiders, clams, shore crabs, and snails. Mussels and clams are mainly filter-feeders on plankton, which may include zooplankton, and both are designated as trophic level 2.2 (U.S. Environmental Protection Agency, 1995b). However, phytoplankton and detritus make up the bulk of these organism's diets; therefore, mussels and clams are considered trophic level 2 for this evaluation. Although the EPA classifies snails as trophic level 2 organisms (U.S. Environmental Protection Agency, 1995b), the EPA notes that some marine forms are carnivorous. According to Morris *et al*. (1980), the species of nassa snails consumed by clapper rails are primarily herbivorous deposit feeders; however, Morris *et al*. note that at least one San Francisco Bay population is also carnivorous, preying on polychaete worms. This feeding behavior warrants the classification of trophic level 3 for nassa snails consumed by California clapper rails. The EPA views crabs as trophic level 3.3 organisms; however, this assumption was based on larger, more predatory crabs (*e.g.*, blue crabs) consuming small fish, other crabs, molluscs, and other invertebrates (U.S. Environmental Protection Agency, 1995b). The two crab species identified as food for the California clapper rail, *Hemigrapsis oregonensis* and *Pachygrapsus crassipes*, are primarily herbivorous, feeding on algae and diatoms (Morris *et al*., 1980; Roth and Brown, 1980). Therefore, it is more appropriate to classify these crab species as trophic level 2 organisms for this evaluation.

Evaluating the importance of wolf spiders in the clapper rail diet presents a unique challenge.

Spiders are generally classified as trophic level 3 organisms due to their predatory nature (U.S. Environmental Protection Agency, 1995b). Spiders are also generally regarded as terrestrial species, with limited involvement with aquatic food webs. However, wolf spiders are active hunters and those inhabiting the wetland habitats of clapper rails may be preying on trophic level 2 aquatic invertebrates. At least one species in this family, *Arctosa serii*, inhabits the sandy intertidal zone in the Gulf of California and actively preys on amphipods and ground beetles (Roth and Brown, 1980). If the wolf spiders consumed by California clapper rails exhibit the same feeding behavior, this would suggest a direct accumulation pathway, similar to the consumption of a trophic level 3 fish. However, it is unknown what effect the physiological processes involved with the capture and ingestion of spider prey (*e.g.*, venom immobilization, digestion) would have on the bioavailability of any methylmercury in that prey. In addition, although Moffitt (1941) reported wolf spiders comprising up to 73 percent of the animal matter in clapper rail stomachs, the relative importance in the overall diet may be minor. Moffitt's (1941) analyses were based on volumetric percentages, not on mass. The small amount of digestible body mass in spiders, relative to mussels, clams, crabs, and snails, suggests spiders may be an insignificant component of the overall diet and of the daily ingested dose of methylmercury.

For this evaluation, 90 percent of the California clapper rail diet is assumed to be from aquatic animal matter and 10 percent from vegetation. Based on the trophic level analyses presented above, **5 percent of the overall diet is assumed to be from trophic level 3 organisms (*i.e.*, nassa snails) and the remaining 85 percent from trophic level 2 organisms (*i.e.*, mussels, clams, and crabs)**. While these values are not static, and individual birds may consume varying percentages of each food type or additional prey items, this trophic level breakdown represents a reasonable dietary composition for California clapper rails based on the best available information.

Clapper rails may consume a wide variety of foods. Values for the gross energy content for some of these foods (*e.g.*, shell-less bivalves, shelled crabs) and the efficiency at which rails assimilate them can be found in the *Wildlife Exposure Factors Handbook* (U.S. Environmental Protection Agency, 1993). However, because rails do not consume set amounts of these food types, FIR must be estimated using one of the generic allometric equations from Nagy (2001). Out of the 17 avian categories for predicting FIRs presented by Nagy (2001), Charadriiformes is the taxonomic order most closely related to rails (Gill, 1995). In addition, the rail's feeding ecology most closely resembles that of birds in the Charadriiformes category (*i.e.*, shore birds, gulls, auks). Therefore, the FIR for California clapper rails was calculated using the following equation:

FIR (wet weight) = $1.914 \times (\text{body weight in g})^{0.769}$
FIR = $1.914 \times 346.1^{0.769}$
FIR = 171.63 g/day wet weight

FIR for California clapper rail = 0.172 kg wet weight/day

IV.D. Light-footed Clapper Rail (*Rallus longirostris levipe*):

As the light-footed clapper rail is smaller than the California clapper rail (U.S. Fish and Wildlife Service, 1976), the body weight for the California rail was not considered appropriate for this subspecies. No subspecies-specific information on body weights was found in the scientific literature. Dunning (1993) reported an average weight of 271 g for seven female clapper rails (*R. longirostris*, unidentified subspecies) from South Carolina. While an average body weight for the light-footed subspecies may be slightly more or less than the average reported by Dunning (1993), this value (**271 g**) was used in the calculation of a wildlife value in this effort.

Light-footed clapper rails occupy coastal marsh habitats, similar to the California clapper rail. The most robust documentation of the light-footed clapper rail's diet is presented by Zembal and Fancher (1988). Through direct observations of foraging and from analyses of food materials regurgitated by light-footed clapper rails, a list of prey items were identified. Observations of foraging revealed that clapper rails hunted in marsh vegetation over 90 percent of the time. During these foraging bouts, rails focused on invertebrates at the base of plants or under dried pieces of vegetation and debris. According to the observations of successful capture and swallowing, rails consumed hundreds of these invertebrates per hour. These small organisms could not be identified but appeared to be very mobile, as they would scatter rapidly when discovered by the rails. Due to the amount of time rails foraged on these organisms and the large numbers swallowed during foraging bouts, the researchers concluded that these invertebrates were important dietary items.

When not foraging in vegetation, rails would switch strategies and hunt tidal creek banks, mudflats, and open water. Rails were observed catching and swallowing various shore crabs (*i.e.*, *Pachygrapsus crassipes*, *Hemigrapsus oregonensis*) and fiddler crabs (*Uca crenulata*) from the creek banks. Both fish (*i.e.*, longjaw mudsucker - *Gillicthys mirabilis*) and ribbed horse mussels (*Ischadium demissum*) were taken from the mudflat habitats. However, observations of foraging on the mussels suggests that only portions of the animals were consumed, as the mussels would close upon first attack and rails appeared unable to reopen them. Other rails in open water were seen capturing California killifish (*Fundulus parvipinnis*) and tadpoles of the Pacific treefrog (*Hyla regila*). Scavenging on fish carcasses was also observed, although the rails may have been eating insect larvae on the carcasses.

Examination of regurgitated pellets provided additional information on clapper rail diets. The most abundant items were the remains of the shore crab species mentioned above. The next most abundant items were the remains of California horn snails (*Cerithidea californica*) and salt marsh snails (*Melampus olivaceous*). Other animal remains identified in regurgitated pellets included crayfish, beetles, isopods, and decapods. These additional items were not ranked according to abundance, although regurgitated pellets collected along a freshwater ditch were composed primarily of crayfish exoskeletons. Plant remains were rare in the regurgitated pellets, with the exception of two pellets that contained 75 elderberry seeds (representing about 25 fruits). The only other plant remains were three small unidentified seeds and several cordgrass seeds. The

researchers noted that only three clapper rails were ever observed feeding on plants, two consuming tips of pickleweed stems and one extracting and swallowing pith from broken cordgrass stems.

Light-footed clapper rails appear similar to other omnivorous birds in that a wide range of both plant and animal foods may be included in the diet, the composition of which may vary depending on any number of environmental or physiological factors. No information was provided by Zembal and Fancher (1988) regarding the percentage of specific food items in the rail diet; however, the authors offered some conclusions about the relative importance of certain organisms. Crabs and snails were considered important prey because of their large size and abundance in rail habitats. The two shore crabs and two snails identified above as prey for clapper rails are all trophic level 2 organisms, feeding on plants or detritus (Morris *et al*., 1980). Fiddler crabs feed primarily on detritus (Barnes, 1980; Kozloff, 1990); therefore, they are also considered trophic level 2 organisms. The small invertebrates consumed by clapper rails were also considered important in the diet because of the large numbers eaten and the amount of time rails spent foraging on them. Although these invertebrates could not be identified by the researchers, the small size of the animals and their tendency to cluster in large concentrations indicates that they should be classified as trophic level 2 organisms.

Zembal and Fancher (1988) did not offer any conclusions regarding the importance of other dietary items such as fish, mussels, tadpoles, and crayfish. However, they observed rails capturing fish numerous times and suggested that fish consumption may be more common than their results would indicate. The two fish species identified as prey, California killifish and longjaw mudsucker, are trophic level 3 predators (Moyle, 2002). In addition to trophic level 3 fish, crayfish were identified in pellets regurgitated by clapper rails. The EPA classifies crayfish at an intermediate trophic level (2.4), noting that crayfish are primarily herbivorous and that animal food is a minor part of the diet if vegetation is available (U.S. Environmental Protection Agency, 1995b). However, Slotton *et al*. (2000) found that signal crayfish (*Pacificasticus leniusculus*) in California can accumulate mercury to high concentrations, similar to predatory fish. While *P. leniusculus* is in a different genus than those identified in the pellets regurgitated by light-footed clapper rails, the omnivorous nature of all crayfish indicates the potential for a greater reliance on animal food than on plant material. For this evaluation, a higher intermediate trophic level (*i.e.*, 2.8) was assigned to crayfish consumed by light-footed clapper rails. Assuming 10 percent of the overall diet is crayfish, 8 percent of this contribution was assigned to trophic level 3 and 2 percent to trophic level 2 (*i.e.*, TL2.8 = 80% TL3, 20% TL2). Further assuming the trophic level 3 fish prey contributes 10 percent of the diet, a total of 18 percent of the overall diet was assigned to trophic level 3 (*i.e.*, 8% from crayfish, 10% from fish).

As noted above, plants appeared to play a minor role in the light-footed clapper rail diet, with the exception of elderberry fruits near a freshwater ditch (Zembal and Fancher, 1988). The fact that rails were only seen eating vegetation by the researchers on three occasions, despite approximately 180 hours of visual contact between March 1979 and August 1987, indicates that vegetation may be an insignificant food source, relative to the overall diet. For this reason, the

breakdown of dietary trophic level composition is based on an assumption of 100 percent animal foods.

The predominant foods of the light-footed clapper rail appear to be trophic level 2 crabs, snails, and small invertebrates. Other important foods, from a bioenergetic standpoint, include trophic level 3 fish and crayfish. Although no specific information was found regarding the percentage of each trophic level contributing to the overall diet, a reasonable assumption of **82 percent trophic level 2 and 18 percent trophic level 3** was used in the calculation of wildlife values for the light-footed clapper rail.

Although differing from the California clapper rail, in that fish and crayfish are important dietary items and vegetation appears insignificant, the similarly indefinite composition of the light-footed clapper rail's diet requires that FIR be estimated using the same allometric equation (Charadriiformes group) from Nagy (2001). For this effort, the body weight for the light-footed rail was estimated to be 271 g.

FIR (wet weight) = $1.914 \times (\text{body weight in g})^{0.769}$
FIR = $1.914 \times 271^{0.769}$
FIR = 142.2 g/day wet weight

FIR for light-footed clapper rail = 0.142 kg wet weight/day

IV.E. Yuma Clapper Rail (*Rallus longirostris yumaensis*):

The Yuma clapper rail is considered smaller than the both the California and light-footed clapper rails (U.S. Fish and Wildlife Service, 1976). However, there was no defensible way to determine a lower body weight for the Yuma rail than the one used for the light-footed rail. No subspecies-specific information on body weights was found in the scientific literature. Subsequently, the **average body weight of 271 g** reported by Dunning (1993) was used in the calculation of a wildlife value in this effort.

The Yuma clapper rail is unique from other clapper rail subspecies in that it resides and breeds in freshwater marshes (Anderson and Ohmart, 1985). Early literature on Yuma clapper rails suggested that the majority of the birds wintered in brackish marshes along the western coast of Mexico and then returned to their freshwater breeding grounds in the U.S. along the Colorado River and the Salton Sea for the spring and summer nesting period (U.S. Fish and Wildlife Service, 1976; Anderson and Ohmart, 1985). Both the California and light-footed clapper rails are considered non-migratory, although the California clapper rail is known to "wander" from its breeding grounds in fall and early winter (U.S. Fish and Wildlife Service, 1976). The Yuma clapper rails that did overwinter in freshwater habitats in the U.S. were considered a small part of the overall population (U.S. Fish and Wildlife Service, 1976; 1983). One possible explanation given for this migratory behavior was that it was in response to reduced food resources in the winter months (Anderson and Ohmart, 1985). However, radio telemetry work conducted

between February 1985 and December 1987 revealed that at least 70 percent of the population along the lower Colorado River remains resident (Eddleman, 1989). Therefore, the dietary information for birds residing in freshwater marshes is assumed on a year-round basis.

Comprehensive dietary information was presented by Ohmart and Tomlinson (1977), who examined stomach contents from 11 Yuma clapper rails collected from California and Arizona. Four birds from the Colorado River Delta in Mexico were also examined. Crayfish (*Procambarus* spp. and *Oropectes* spp.) were by far the most dominant prey items in the nine birds collected from along the Colorado River, averaging 95 percent by volume (range: 80-100%) of the stomach contents. Other food items included various insects, spiders, and molluscs. A small mammal bone was found in one stomach and plant seeds in another. Of the two birds collected from the confluence of the Gila and Colorado Rivers, one stomach contained an introduced freshwater clam (*Corbicula* sp.) (98%) and the other contained isopods (97%). The remaining food items in these two stomachs were unidentified insect parts. The birds collected in Mexico showed a more diverse food assemblage, with the predominant foods being water beetles (56%) and unidentified fish (32%). Fish do not appear to be important dietary items outside of the river delta habitats. A small amount of vegetative matter was also found in these birds, although plant matter appears to play an insubstantial role in the diet for all birds.

The trophic level dietary composition for Yuma clapper rails is based on 100 percent animal foods. It is clear that Yuma clapper rails residing along the Colorado River rely heavily on various freshwater crayfish. While it was once thought that these crayfish became dormant during the winter months, precipitating migratory behavior in the rails, evidence indicates that crayfish are present year-round in at least some locations and reproduce in autumn and early winter (Eddleman, 1989). As noted above in the analysis for light-footed clapper rails, crayfish are considered trophic level 2.8 organisms for determining the dietary composition. However, it is unlikely that Yuma clapper rails feed exclusively on crayfish, based on evidence that the birds supplement their diets with other foods ranging from terrestrial and aquatic insects to molluscs, depending on location and availability. Some of these supplemental food items may be aquatic (*e.g.*, isopods, damselfly nymphs, molluscs) or removed from the aquatic ecosystem (*e.g.*, grasshoppers, weevils, ground beetles). Assuming a reasonable high volume diet of 90 percent crayfish, 72 percent of this contribution can be assigned to trophic level 3 and 18 percent to trophic level 2 (*i.e.*, TL2.8 = 80% TL3, 20% TL2). Based on the dietary assessment provided by Ohmart and Tomlinson (1977), the diet for the Yuma clapper rail can therefore be assumed as **72 percent trophic level 3 organisms (from crayfish), 23 percent trophic level 2 organisms (from crayfish and other TL2 foods), and 5 percent non-aquatic organisms**.

The FIR for Yuma clapper rails was estimated using the same allometric equation (Charadriiformes group) from Nagy (2001). For this effort, the body weights for all three clapper rail subspecies were estimated to be equal (271 g). Therefore, the FIR calculation for the Yuma clapper rail will be identical to the one for the California and light-footed clapper rails.

FIR (wet weight) = 1.914 × (body weight in g)$^{0.769}$
FIR = 1.914 × 271$^{0.769}$
FIR = 142.2 g/day wet weight

FIR for Yuma clapper rail = 0.142 kg wet weight/day

IV.F. Western Snowy Plover (*Charadrius alexandrinus nivosus*):

Snowy plovers are small shorebirds weighing from 34 - 58 g, ranging in length from 15 - 17 cm (U.S. Fish and Wildlife Service, 2001). Dunning (1993) reports a mean weight of 41.4 g from 38 specimens of *Charadrius alexandrinus* (unknown gender) from California, with a range from 37 - 49 g. No information was found indicating gender-specific differences in weight. Therefore, **a weight of 41 g** was used in the calculation of wildlife values for western snowy plovers.

The snowy plover diet consists primarily of aquatic and terrestrial invertebrates (Page *et al*., 1995), with little quantitative information about specific food habits (U.S. Fish and Wildlife Service, 2001). A wide variety of food items are reported for coastal birds: mole crabs, crabs, polychaetes, amphipods, tanaidaceans, flies, beetles, clams, and ostracods (Page *et al*., 1995). Plovers on beaches forage above and below the mean high-tide line, gathering invertebrates from the sand surface, kelp, foredune vegetation, and marine mammal carcasses (Page *et al*., 1995). Flies, beetles, moths, and lepidopteran caterpillars were taken by birds at San Francisco Bay salt-evaporation ponds (Page *et al*., 1995). Plovers in California have been observed pecking small flying insects from mid-air (U.S. Fish and Wildlife Service, 2001), and are known to charge with open mouth into aggregations of adult flies (Page *et al*., 1995).

Tucker and Powell (1999) examined snowy plover fecal samples from a southern California coastal breeding site. Results indicated that the primary prey were terrestrial insect families (*i.e.*, various flies and beetles), although mole crab and nassa snail parts were also identified. Insect larvae were found in 25 percent of the fecal samples. The authors concluded that their results were consistent with findings from other snowy plover diet studies in that the major prey items are flies and beetles. However, the authors noted that polychaete worms are digested too completely to be identified by their technique, and stated that these worms may be important prey items.

Although it appears that snowy plovers mainly feed on non-aquatic insects, of both larval and adult forms, at least some aquatic organisms are included in the diet. These aquatic prey (mole crabs, nassa snails, polychaete worms, amphipods, ostracods, clams, tanaidaceans) can all be classified as trophic level 2 organisms based on their diets (U.S. Environmental Protection Agency, 1995b; Morris *et al*., 1980). For this evaluation, an assumption was made that **trophic level 2 organisms constituted 25 percent** of the overall snowy plover diet. The remaining portion of the diet **(75%) was assumed not to be significantly contributing to the daily ingested dose of methylmercury**. Additional research into the possible relationship between methylmercury in an aquatic system and its bioavailability to terrestrial insects may remove some

of the uncertainty in this assumption.

Due to the wide variety of potential prey items and the subsequent variability in gross energy content and assimilation efficiencies, the FIR for snowy plovers was determined using Nagy's (2001) allometric equation for Charadriiformes (shore birds, gulls, auks):

FIR (wet weight) = $1.914 \times (\text{body weight in g})^{0.769}$
FIR = $1.914 \times 41^{0.769}$
FIR = 33.3 g/day wet weight

FIR for western snowy plover = 0.033 kg wet weight/day

IV.G. Bald Eagle (*Haliaeetus leucocephalus*):

The bald eagle was a representative species used for the derivation of wildlife criteria in the aforementioned GLI (U.S. Environmental Protection Agency, 1995c). For that effort, the bald eagle body weight used in criteria calculations (4.6 kg) was based on the mean of average male and female eagle body weights, although it was noted that female eagles are approximately 20 percent heavier than males. As the avian reference dose for methylmercury is based on adverse reproductive effects manifested by laying females, it is more appropriate to use average female body weights in the calculation of wildlife values.

In the GLI, the EPA presented an average body weight of 5.2 kg for female bald eagles. This value was based on the weights of 37 birds, taken from Snyder and Wiley (1976). Dunning (1993) presented an average female body weight of 5.35 kg, also based on the weights of 37 birds, taken from Palmer (1988). Taking both values into consideration, a **body weight of 5.25 kg** was used in the calculation of wildlife values for this evaluation.

The bald eagle diet has been extensively studied throughout the country. Although generally known as a piscivorous species, bald eagles are opportunistic predators and carrion scavengers (Buehler, 2000). Various birds, mammals, reptiles, amphibians, and crustaceans may serve as additional bald eagle prey (Buehler, 2000). As explained in the introduction to this section, FIRs can be most accurately estimated for an animal consuming different food types (*e.g.*, fish and birds) when there is information about the metabolic energy available from these foods and a reliable estimate of the amount of each food type consumed daily (*e.g.*, 75% fish, 25% birds). Information presented in the Wildlife Exposure Factors Handbook (U.S. Environmental Protection Agency, 1993) regarding the metabolizable energy available from various prey types and the ability of bald eagles to assimilate this energy allows for the use of this method to estimate daily food requirements. However, attempting to quantify a specific dietary composition for bald eagles is more difficult than for other species with a narrower range of prey types, and is further confounded by the fact that food preferences may vary both geographically and temporally.

An additional difficulty in calculating a general FIR for deriving the WV for bald eagles arises because the trophic level composition of the diet can also vary substantially between seasons, locations, or individuals. Calculating the FIR based solely on the percentage of various food types in the diet may not result in a WV representative of the greatest risk from methylmercury in the diet. For example, the daily FIR for an eagle with a diet of 95 percent fish / 5 percent birds will be greater than the FIR for an eagle with a diet of 80 percent fish / 20 percent birds (*i.e.*, less energy available from fish prey requires a greater amount consumed to satisfy bald eagle's free-living metabolic rate). The higher FIR, in turn, results in a lower WV, which may seem the most desirable outcome of this methodology. However, if the bulk of the 95/5 diet consists of trophic level 2 fish and terrestrial birds, the methylmercury concentration in the eagle's overall diet will remain substantially below the WV, regardless of the trophic level approach used. By contrast, the higher WV calculated from the 80/20 diet may be substantially exceeded by either trophic level approach if the diet consists primarily of trophic level 4 fish and piscivorous birds.

In this example, using the dietary composition resulting in the lowest WV as a surrogate for all eagles would give the misleading impression that all eagles may be protected (false negative) by the TRC, while using the higher WV would indicate that all eagles may be at risk from the TRC (false positive). However, the goal of this analysis is to evaluate the protectiveness of the two trophic level approaches, using data for birds with the greatest potential for methylmercury exposure through their diet. Therefore, the FIR used to calculate the WV must be based on the most reliable bald eagle diet with the highest combined percentage of trophic level 4 fish and aquatic-dependent avian prey, and the lowest percentage of terrestrial prey (*i.e.*, no connection to methylmercury in the aquatic environment).

The feeding ecology of avian prey of bald eagles is critical for this analysis because prey birds that consume aquatic biota represent an additional exposure pathway for bald eagles, as methylmercury in fish and aquatic invertebrates is biomagnified as it moves through successively higher trophic level organisms. The biomagnification of methylmercury through piscivorous avian prey was factored into the GLI effort, as data showed piscivorous herring gulls (*Larus argentatus*) were an important dietary component (5.6% of the dietary biomass on average) of Lake Superior bald eagles (U.S. Environmental Protection Agency, 1995d). The study used to determine the bald eagle diet for the GLI effort (Kozie and Anderson, 1991) also found various waterfowl in eagle prey remains. These waterfowl species were not considered piscivorous, yet for some, trophic level 2 aquatic biota can constitute a substantial part of their diet. These waterfowl were not included in the GLI estimate of methylmercury exposure, as the bulk of the bird prey component was comprised of herring gulls. However, in areas where bald eagles consume large numbers of these aquatic-dependent birds, the biomagnification of methylmercury from trophic level 2 organisms into waterfowl tissues may contribute substantially to the bald eagle's daily ingestion of methylmercury.

Several efforts to develop protective mercury criteria (*e.g.*, U.S. Environmental Protection Agency, 1997a; Buchanan *et al*., 2001; California Regional Water Quality Control Board - Central Valley Region, 2001) have used the dietary composition developed in the GLI (U.S.

Environmental Protection Agency, 1995c). Using information on bald eagles nesting on islands and along the shore of Lake Superior in Wisconsin (*from* Kozie and Anderson, 1991), and adjustment factors to estimate the relative number of birds and fish delivered to a nest based on the prey remains found under the nest, the EPA determined that 92 percent of the dietary biomass was comprised of fish and 8 percent comprised of birds or mammals. The adjustment factor was developed to account for the inherent error in estimating a dietary composition based solely on the analysis of prey remains. The Kozie and Anderson (1991) study used to determine bald eagle diets reported that fish comprised 50 percent and birds comprised 48.4 percent of the nest site prey remains. However, direct observations of three nests during part of the study period revealed that fish constituted 97 percent of the captured prey. To address this discrepancy, the EPA's adjustment factors (*i.e.*, - the ratios between the number of each prey type found in nest remains and the number of each prey type observed in nest deliveries during the same period) were applied to the prey remain data for all nest sites in the study. This allowed for an estimate of the total number of birds and fish consumed by bald eagles. Then, using standard body weights for the bird and fish species identified, the percentage of biomass for each food type was calculated.

Using this dietary composition of 92 percent fish and 8 percent birds, along with information about the energetic needs of adult eagles and their ability to assimilate the caloric content of these food types, the GLI presented estimates of the amount of each food type ingested daily: 0.464 kg fish and 0.040 kg birds/mammals (U.S. Environmental Protection Agency, 1995c). The fish component of the overall diet was further broken down as 74 percent trophic level 3 (0.371 kg) and 18 percent trophic level 4 (0.0928 kg), based on data indicating the average trophic level for the fish component of Lake Superior bald eagles is 3.2 (*i.e.*, 80% TL3, 20% TL4). The remaining bird/mammal component of the overall diet was delineated as 5.6 percent piscivorous herring gulls (0.0283 kg) and 2.4 percent non-piscivorous other food (0.0121 kg). Although the GLI breakdown of the bald eagle diet has been used as a default composition in subsequent wildlife criteria efforts, studies of bald eagle diets from other parts of the country reveal a wide range of possible composition preferences. Several of these studies are summarized below.

A study of bald eagles in a desert riparian habitat in central Arizona found that fish comprised 77 percent of the total prey remains found under nests (Haywood and Ohmart, 1986). Mammals accounted for an additional 12 percent, birds 11 percent, and reptiles or amphibians 0.6 percent. The same study compared the findings from prey remains with direct observations of prey capture (73% fish, 5% mammals, 1% birds, 4% reptiles or amphibians, and 17% unidentifiable) and found only a minimal difference in percent composition.

By contrast, bald eagles nesting at various sites along the coast of Washington displayed a stronger dietary preference for birds, which accounted for 53 percent of the total prey remains (N = 1198) found under nests in three different regions (Knight *et al.*, 1990). Fish comprised 34 percent of the total remains, with mammals (9%) and invertebrates (4%) making up the rest. There were composition differences between the three sites evaluated, but in each case, birds accounted for the majority of food. Birds comprised 78 percent of all prey remains at Olympic

Peninsula nest sites, but down to 48 percent at San Juan Island sites. The researchers also compared their findings from collected prey remains with direct observations of prey delivery (*N* = 47) and concluded that birds were over-represented in prey collections beneath nests and fish were over-represented in observations of prey carried to nests. The high incidence of bird prey remains (53%) during the observation period is in contrast to the frequency of observations in which birds were delivered to the nest (8%). The frequency of observed fish deliveries was high (92%), but was much lower in prey remain collections (44%) during the observation period. Birds may be over-represented in nest collections due to a greater persistence than fish remains in the environment, while over-representation of fish in observations may be due to the relative ease of identification (Mersmann *et al*., 1992; Knight *et al*., 1990). However, this study indicates that birds are important prey for coastal bald eagles.

Dietary habits of resident bald eagles from three nesting areas in southcentral Oregon were studied between 1979 and 1983 (Frenzel, 1984). Nest site prey remain collections and direct observations of 16 eagles fitted with radio transmitters were the methods used. The three study areas were Upper Klamath Lake, outer Klamath Basin, and the Cascade Lakes region. Discrepancies between prey remain collections and observations of predation were also found in this study. At the Upper Klamath Lake site, fish comprised only 25 percent of the prey remains but accounted for 62 percent of the observed prey taken during the breeding season. The amount of fish observed taken at this site increased to 69 percent during the post-breeding season, but then dropped to less than 20 percent in fall and winter. Birds became the dominant food during these seasons, accounting for over 82 percent of the observed prey taken. Mammals were observed taken throughout the breeding and post-breeding seasons, but were not observed during the fall and winter. At Wickiup Reservoir in the Cascade Lakes study area, fish accounted for 100 percent of the observed prey taken during the breeding and post-breeding seasons. The same study looked at the diets of wintering-only bald eagles in the Klamath Basin. For these eagles, wintering and staging waterfowl were the primary food source, supplemented with some mammal prey. No fish remains were found in bald eagle castings from communal roosts, and no foraging attempts on fish were observed through the study.

In addition to the above studies, Volume III of *Trophic Level and Exposure Analyses for Selected Piscivorous Birds and Mammals* (U.S. Environmental Protection Agency, 1995b), presented summaries of bald eagle dietary habit studies throughout the U.S. and British Columbia, along with estimated prey trophic levels. The diets presented in these summaries confirm the wide variability of prey types inherent with an opportunistic forager like the bald eagle. While none of the studies described provided one definitive diet composition preferred by bald eagles, they show that fish are generally the predominant food item during the spring and summer breeding seasons. Birds are second in importance, followed by mammals.

As mentioned previously, the dietary composition developed for the bald eagle in the GLI has been used in various places for the derivation of avian wildlife criteria. However, this dietary composition was specifically determined for the aquatic ecosystem of the Great Lakes and may not be an appropriate default for other parts of the country. Califomia supports both wintering

and resident bald eagles, with a broad array of suitable foraging habitats. Because of this variety, eagle diets in California likely span a wide range of possible food types and trophic level combinations. It is not possible in the scope of this analysis to determine all the potential bald eagle diets in California and evaluate them with regard to the trophic level approaches for the methylmercury criterion.

Instead, a weighted risk approach was taken to determine the appropriate eagle diet for calculation of wildlife values. The goal of this approach was to establish a diet based on the highest trophic level composition reasonably likely to occur, from the predominant habitat type characteristic of California's breeding bald eagles. The primary breeding habitats are mountain and foothill forests and woodlands close to reservoirs, lakes, and rivers (California Department of Fish and Game, 2000). Wintering bald eagles can be found in these same habitats throughout the State, but also forage in a variety of different habitats, such as rangelands and coastal wetlands. Basing the diet on the main habitat of resident breeding birds rather than on some other localized habitat used by non-resident birds is a more appropriate method for evaluating potential adverse reproductive effects from the methylmercury criterion, as it is impossible to predict maternal body burdens of methylmercury once wintering eagles reach their breeding grounds outside of California.

Bald eagles are known to nest in several locations and habitat types dispersed throughout California, including in the central and southern Sierra Nevada range, the central coast range, inland southern California, and on Santa Catalina Island. However, most breeding territories are in the northern part of the State (California Department of Fish and Game, 2000). The results of a 1977-1978 study of 95 bald eagle nest sites revealed that 91 percent of the nesting territories were located in five northern counties (Lehman, 1979). A large majority of these nests (87%) were within one mile of a waterbody, and 70 percent of the nests were associated with reservoirs. Two studies of foraging ecology in these characteristic northern California breeding habitats provided detailed assessments of the trophic level composition of bald eagle diets.

Through collection of nest site prey remains, direct observations of foraging eagles, and time-lapse photography of nest activity, the dietary composition was estimated for bald eagles nesting along a hydrologically-regulated section of northern California's Pit River (Hunt *et al*., 1992). The study area encompassed 24.5 km of reservoirs and 45.8 km of flowing, regulated river. The study took place over a period of two years, with results indicating that fish comprised approximately 87 percent of the total prey items, while birds (9%) and mammals (4%) comprised the remainder. Based on estimates of edible biomass determined from the prey remains around eight nests, the biomass comprised of fish ranged from 43.8 to 92.6 percent. For all nesting eagle pairs, one fish species (Sacramento sucker - *Catostomus occidentalis*) was the dominant prey; however, eagles at one reservoir (Lower Britton) foraged on a greater percentage of cyprinid fish (*e.g.*, hardhead, tui chub, Sacramento pikeminnow) than the other study regions. While trophic levels for various species of *Catostomus* range from 2 to 3 (U.S. Environmental Protection Agency, 1995b), the food of Sacramento suckers can be dominated by algae, detritus, or invertebrates, depending on the size of the fish, location, or time of year (Moyle, 2002). The next

two most important fish species in all study areas were the hardhead (*Mylopharodon conocephalus*) and Sacramento pikeminnow (*Ptychocheilus grandis*). These fish should be classified as trophic level 3 and 4, respectively, based on their diets (Moyle, 2002).

A variety of avian species were identified in the prey remains collected in this study, amounting to 102 individual birds. In terms of edible biomass, the percentage of the diet comprised of birds ranged from 4.9 to 46.3 percent among the eight nests sampled. While the bird species composition or estimated biomass of birds consumed were not presented for each individual study nest, 18 (17.6%) of the total 102 birds identified were piscivorous species. Based on the overall percentage of all birds in the eagle diets (9%), piscivorous birds accounted for roughly 1.6 percent of the total eagle diet (*i.e.*, - 0.09 × 0.176 × 100 = 1.58%).

While this study (Hunt *et al*., 1992) presents estimates of the percent biomass for each food type at each study site, including a breakdown for individual fish species, the estimates were based solely on an analysis of prey remains. The prey remains analysis conducted in this study was quite rigorous, in that individual fish scales were included in the collections and used to determine total numbers of fish prey. Other studies of bald eagle diets (*e.g.*, Kozie and Anderson, 1991) relied solely on samples of bones and feathers collected from nest sites. However, in a subset of the entire Hunt *et al*. (1992) study, diets were analyzed for three nests using a comparison of prey remains with time-lapse photographic observations of prey delivered to the nests. The number of fish delivered to the nests during this period ($N = 117$) was almost twice the number estimated from prey remains during the same period ($N = 64$). The biomass estimated from photographic observations of fish prey (55.1 kg) was also substantially greater than the estimate from prey remains (37.6 kg). The authors suggested that some remains may have been dropped or taken from the nests and that other prey items may have been entirely consumed. Further confounding the analysis, the authors reported that a total of 236 prey deliveries were recorded by the time-lapse cameras, yet only the 117 fish deliveries were presented in the journal article. If the 119 unidentified prey deliveries were birds or mammals, this suggests that fish only accounted for 49.5 percent of the diet during the observation period. Although these discrepancies make it difficult to assign a general dietary composition from this study, the author's comparison of prey remains data and photographic observations indicated that larger fish species were not over-represented in prey remains because of larger and more persistent bones, and smaller fish were not under-represented in prey remains because of softer, less persistent bones.

In an expansion of the previous work, prey remains from 56 eagle nesting territories in three major drainage basins (Sacramento-San Joaquin, Lahontan, Klamath) were collected between 1983 and 1992 (Jackman *et al*., 1999). The total study area comprised numerous rivers, lakes, and reservoirs. Over 80 percent of studied nesting territories were near reservoirs, with the remainder on natural lakes. Riverine habitats were also available as foraging sites for all nesting eagles. Prey remains were collected from in and below nests, sometimes during the late nestling stage but primarily after the young had fledged. Sample collections included bones, fur, feathers, and fine nest lining, the latter containing fish scales and fine bones. The authors acknowledged

that the dietary analysis was biased in that it was based exclusively on prey remains (*i.e.*, no comparison of remains with prey deliveries). However, as demonstrated in the earlier Pit River study, the authors noted that their inclusion of fish scale analysis from the nest lining samples helped to mitigate the potential over- or under-representation of certain fish types. In addition, fish scales may have a greater environmental persistence at nest sites than fish bones, which are typically used in prey remain analyses. Although it is commonly suggested that birds and mammals may be over-represented in dietary studies due to a greater environmental persistence of their prey remains compared with fish remains (*i.e.*, feathers vs. bones), the inclusion of fish scales in the dietary analysis may also help to mitigate this potential bias.

From the 56 nesting territories sampled in this study, 2,351 individual prey items were identified. Fish accounted for over 70 percent of both overall prey numbers and total estimated biomass (1,637 kg). The mean standard lengths of the most commonly taken fish were over 30 cm, with the exception of tui chub (28 cm) and brown bullhead (24 cm). Birds contributed approximately 22 percent and mammals less than 6 percent to total prey numbers and biomass. Western pond turtles and crayfish were the only other prey items identified, and contributed insignificant amounts to the overall diet (<1%). The prey composition varied substantially between 19 waterway study groups, with fish accounting for greater than 50 percent of prey numbers and biomass at most locations. However, birds and mammals were the predominant prey at several individual locations isolated from large rivers. Overall, 20 species of fishes, 41 species of birds, and 15 species of mammals were identified from prey remains.

Of the 20 fish species identified (71.2% of total biomass in overall bald eagle diet), the four primary prey species were brown bullhead (*Ameiurus nebulosus*), Sacramento sucker (*Catostomus occidentalis*), common carp (*Cyprinus carpio*), and tui chub (*Gila bicolor*). The majority of the 20 fish species identified should be classified as trophic level 3 consumers based on their diets of trophic level 2 organisms (Moyle, 2002). However, at the body sizes estimated from the prey remain analysis and the dietary habits presented in Moyle (2002), several fish species identified should be classified as trophic level 4 piscivores: Sacramento pikeminnow (*Ptychocheilus grandis*), rainbow trout (*Onchorhyncus mykiss*), largemouth bass (*Micropterus salmoides*), and Sacramento perch (*Archoplites interruptus*). In addition to the identified fish species, numerous other fish remains could only be identified to family: Centrarchidae, Ictaluridae, Cyprinidae, Salmonidae, and Catostomidae. Of these, it can be assumed that the fish prey identified as Salmonidae should be classified as trophic level 4 organisms.

With the exception of largemouth bass, the majority of the Centrarchid prey remains could not be identified to species, although bass (*Micropterus* spp.), smallmouth bass (*Micropterus dolomieui*), sunfish (*Lepomis* spp.), and bluegill (*Lepomis macrochirus*) were noted in the general Centrarchid grouping. It was impossible to assign a single trophic level to the general Centrarchidae dietary contribution, as large bass should be considered trophic level 4 fish and smaller sunfish and bluegills should be considered trophic level 3 fish (Moyle, 2002). Therefore, an intermediate trophic level (*i.e.*, 3.5) was assigned to the non-specific Centrarchidae contribution to the bald eagle diet. This resulted in 50 percent of the "Other sunfish

(Centrarchidae)" grouping assigned to each of trophic level 3 and 4 (*i.e.*, TL3.5 = 50% TL3, 50% TL4).

The two Ictalurids identified in the study [brown bullhead and channel catfish (*Ictalurus punctatus*)] are opportunistic omnivores, consuming whatever prey they can locate. Benthic invertebrates often constitute the majority of the diet for smaller Ictalurids; however, as bullheads and catfish increase in size, small trophic level 3 fish can become the predominant prey item (Moyle, 2002; U.S. Environmental Protection Agency, 1995b). The fish lengths determined from Ictalurid prey remains in this study ranged from 12.9 - 35.6 cm for brown bullhead and 25.1 - 55.1 cm for channel catfish, suggesting that an intermediate trophic level of 3.5 be assigned to all Ictalurids eaten by bald eagles. As with the non-specific Centrarchids, 50 percent of the Ictalurid biomass contribution to the bald eagle diet, whether identified to species or family, was assigned to each of trophic levels 3 and 4.

With the exception of the Sacramento pikeminnow, Cyprinid minnows in California should be considered trophic level 3 (Moyle, 2002). Therefore, the dietary contribution from fish prey grouped under "Unidentified minnows (Cyprinidae)" was assigned as trophic level 3 for this effort. All fish prey under the "Unidentified suckers (Catostomidae)" grouping were assigned as trophic level 3.

Using the intermediate trophic level breakdown for Centrarchids and Ictalurids, together with the other trophic level 4 fish identified from the prey remains, indicates that 12.7 percent of the overall estimated biomass in the entire study area was comprised of trophic level 4 fish. The remainder of the overall fish component to the biomass (58.5%) is classified as trophic level 3.

Of the 41 bird species identified (22.8% of total biomass in overall bald eagle diet), the two most commonly seen in prey remains were American coot (*Fulica americana*) and mallard (*Anas platyrhynchos*), representing 4.2 and 3.2 percent, respectively, of the total estimated biomass. Several of the species identified are exclusively terrestrial (*e.g.*, mountain quail); however, the majority are dependent on the aquatic ecosystem. Several of these aquatic-dependent species are primarily piscivorous: western grebe (*Aechmophorus occidentalis*), gull (*Larus* spp.), pied-billed grebe (*Podilymbus podiceps*), and common merganser (*Mergus merganser*). These piscivorous birds accounted for approximately 5 percent of the total estimated biomass of the bald eagle diet. Eagles also consumed waterfowl (*e.g.*, *Anas* spp., diving ducks, coots) that depend to varying degrees on prey that are considered trophic level 2 organisms (*e.g.*, aquatic invertebrates and zooplankton). These birds contributed approximately 13 percent (including the 4.2% and 3.2% represented by American coots and mallards) to the total estimated biomass in the overall bald eagle diet.

Based on the dietary analysis presented by Jackman *et al*. (1999), and the trophic level assessment provided above, a generic composition for the bald eagle diet can be estimated as 6 percent mammals, 71.2 percent fish (58.5% TL3, 12.7% TL4) and 22.8 percent birds (13.2% TL2 consumers, 4.8% TL3 consumers, 4.8% non-aquatic consumers). These figures represent an

average dietary composition for all bald eagles in the study area. However, the study also presented dietary composition results from 19 separate sub-areas, described as waterway territory groups. The data from these sub-areas do not provide the level of taxonomic detail regarding prey species as was presented for the entire study area, but they do reveal that substantial differences exist between nesting territories in the relative contribution of birds, mammals, and trophic level 4 fish to the bald eagle diet. Trophic level 4 fish constituted over 35 percent of the dietary biomass in several of the sub-areas, while at three different sub-areas, birds contributed over 60 percent of the dietary biomass. At one sub-area, birds and mammals accounted for 70.6 and 24.7 percent, respectively, of the dietary biomass.

The dietary compositions for each sub-area were presented in percent biomass of major prey groups (*i.e.*, fish, birds, mammals), with the fish group further divided into seven categories (*e.g.*, trout, suckers, sunfish). This sub-area breakdown illustrates the broad range of dietary compositions possible in these characteristic bald eagle habitats, and allowed for an estimation of a bald eagle diet with the greatest potential for methylmercury exposure (*i.e.*, the highest percentage of TL4 fish and aquatic-dependent birds, with the lowest percentage of terrestrial prey). Because the data were only presented in terms of major prey groups and broad fish categories, the degree of certainty in estimating specific trophic level diets varied with each sub-area. For example, fish represented by the "Minnow" category could be considered trophic level 3 (*e.g.*, Sacramento blackfish) or trophic level 4 (*e.g.*, Sacramento pikeminnow). Similarly, the general "Bird" category could include any combination of aquatic-dependent and/or terrestrial species. Jackman *et al*. (1999) provided a level of species-specific detail for each sub-area that allowed for a reasonable determination of the trophic composition of each fish category; however, sub-area specific detail for bird prey was lacking. By evaluating the estimated biomass contribution of each bird species for the entire study area, a general percentage breakdown of the three bird types (*i.e.*, TL2 consumers, TL3 consumers, non-aquatic consumers) could be determined and applied to the overall bird contribution to each sub-area. For the entire study area, birds that consume aquatic invertebrates (TL2 consumers) accounted for approximately 58 percent, piscivorous birds (TL3 consumers) accounted for approximately 21 percent, and terrestrial birds (non-aquatic consumers) accounted for 21 percent of the total avian prey biomass. Using this breakdown, the relative contribution of birds in the diet for each sub-area could be delineated. For example, if the percentage biomass of birds for a particular sub-area was reported as 25 percent, the relative contribution of each bird type was delineated as 14.5 percent TL2 consumers (25 × 0.58), 5.25 percent TL3 consumers (25 × 0.21), and 5.25 percent non-aquatic consumers (25 × 0.21).

The data for all 19 sub-areas were analyzed to identify the bald eagle diet with the greatest potential exposure to methylmercury. Prey remains from one eagle pair foraging at the inflow of the North Fork Feather River to the Oroville Reservoir indicated that fish and birds comprised 83 and 17 percent, respectively, of the total dietary biomass. **The fish component of this total was comprised of both trophic level 4 (39%) and trophic level 3 (44%) species. The avian component of this total was comprised of TL2-consuming birds (10%), TL3-consuming birds (3.5%), and non-aquatic consuming birds (3.5%)**. This diet represented the highest

combined percentage of trophic level 4 fish and aquatic-dependent birds from the entire study area.

The bald eagle FIR based on this diet (83% fish / 17% birds) was calculated using the methodology in the aforementioned *Technical Support Document for Wildlife Criteria* (U.S. Environmental Protection Agency, 1995c), wherein the animal's free-living metabolic rate (FMR) is divided by the metabolizable energy (ME) from the animal's prey. The FMR was determined by Nagy's (1987) allometric equation relating FMR for birds to body weight:

FMR (kcal/day) = 2.601 × body weight (g)$^{0.640}$
FMR = 2.601 × 5250$^{0.640}$
FMR = 625 kcal/day

According to the *Wildlife Exposure Factors Handbook* (U.S. Environmental Protection Agency, 1993), metabolizable energy equals the gross energy (GE) of the food in kcal/g wet weight times the assimilation efficiency (AE) of the consumer. The Handbook gives a GE value of 1.2 kcal/g for bony fishes, while bird GEs are given as either 1.9 (passerines, gulls, terns) or 2.0 (mallard). Although the majority of avian prey species identified in the Jackman *et al*. (1999) study are more closely related to mallards than to the other bird types, the lower value was used in this analysis because the GE for mallards was for consumption of flesh only. The AEs for eagles consuming birds and fish are given as 78 and 79 percent, respectively.

ME_{fish} = 1.2 kcal/g × 0.79 = **0.948 kcal/g fish**

ME_{birds} = 1.9 kcal/g × 0.78 = **1.482 kcal/g birds**

Following the process in the TSD, if:

Y = grams of birds consumed, and
4.88Y = grams of fish consumed (*i.e.*, 83% fish ÷ 17% birds = 4.88)

then the FIR for each food can be determined by the equation:

FMR = [Y(g) × 1.482(kcal/g birds] + [4.88Y(g) × 0.948 kcal/g fish]

625 kcal/day = 1.482Y + 4.626Y

625 kcal/day = 6.108Y

Y = 102 g birds consumed/day

4.88Y = 498 g fish consumed/day

The total FIR for bald eagles becomes:

FIR = [102 g birds + 498 g fish]/day
FIR = 600 g wet weight/day

FIR for bald eagle = 0.600 kg wet weight/day

V. SPECIES-SPECIFIC WILDLIFE VALUES

Species-specific input parameters, using the RfD generated with a UF_A of 1, and the resulting WVs are presented in Table 2. Table 3 provides WVs using the RfD generated with a UF_A of 3. Wildlife Values were calculated using Equation 6, described previously:

$$\mathbf{WV} = \frac{\mathbf{RfD} \times \mathbf{BW}}{\sum \mathbf{FIR_i}}$$

Table 2. Wildlife Values for Methylmercury Calculated Using Reference Dose Generated with an Interspecies Uncertainty Factor (UF_A) of 1

Species	RfD (mg/kg/day)	Body Weight (kg)	FIR (kg/day)	WV (mg/kg diet)
Southern sea otter	0.018	19.8	6.5	0.055
California least tern	0.021	0.045	0.031	0.030
California clapper rail	0.021	0.346	0.172	0.042
Light-footed clapper rail	0.021	0.271	0.142	0.040
Yuma clapper rail	0.021	0.271	0.142	0.040
Western snowy plover	0.021	0.041	0.033	0.026
Bald eagle	0.021	5.25	0.600	0.184

Table 3. Wildlife Values for Methylmercury Calculated Using Reference Dose Generated with an Interspecies Uncertainty Factor (UF_A) of 3

Species	Alternate RfD (mg/kg/day)	Body Weight (kg)	FIR (kg/day)	WV (mg/kg diet)
California clapper rail	0.007	0.346	0.172	0.014
Light-footed clapper rail	0.007	0.271	0.142	0.013
Yuma clapper rail	0.007	0.271	0.142	0.013
Western snowy plover	0.007	0.041	0.033	0.009

VI. BIOMAGNIFICATION INTO AVIAN PREY OF BALD EAGLES

The next step in the approach was to evaluate the protectiveness of the TRC under each trophic level approach. To do this required the trophic level breakouts (*i.e.*, %TL2, %TL3, %TL4) for the diet of each species of concern, the trophic level concentrations determined in each TRC evaluation approach, and Equation 1:

DC = (%TL2 × FDTL2) + (%TL3 × FDTL3) + (%TL4 × FDTL4)

However, additional information was required to perform this evaluation for the bald eagle. As mentioned previously, bald eagles may consume substantial numbers of birds that feed from the aquatic environment. These aquatic-dependent species may be omnivorous (*i.e.*, - feed to varying degrees on plant matter and trophic level 2 biota) or primarily piscivorous. The biomagnification of methylmercury into these prey birds represents a potentially important additional exposure for bald eagles that must be factored into the estimate of a daily ingested dose. For the GLI effort (U.S. Environmental Protection Agency, 1995d), bald eagle consumption of piscivorous herring gulls (*Larus argentatus*) was included in the criteria derivation because herring gulls in the Great Lakes feed primarily on trophic level 3 fish. The EPA applied a biomagnification factor (BMF) of 10 in the calculation of wildlife criteria to account for the biomagnification from these trophic level 3 fish into herring gull tissues. In effect, the BMF is analogous to a food chain multiplier (FCM) because it represents the amount of methylmercury transfer between a prey organism (TL3 fish) and its predator (piscivorous bird). Although the GLI effort did not consider biomagnification into omnivorous waterfowl, the contribution of methylmercury from this pathway should also be included in the risk assessment

for bald eagles. In order to include the consumption of piscivorous and omnivorous birds in the evaluation for bald eagles, additional terms must be incorporated into Equation 1:

$$DC = (\%TL2 \times FDTL2) + (\%TL3 \times FDTL3) + (\%TL4 \times FDTL4) + (\%OB \times FDOB) + (\%PB \times FDPB)$$

%OB - percent of omnivorous birds (TL2-consumers) in diet
FDOB - methylmercury concentration in omnivorous bird prey
%PB - percent of piscivorous birds in diet
FDPB - methylmercury concentration in piscivorous bird prey

As the two trophic level approaches presented in this evaluation are based only on estimated methylmercury concentrations in aquatic organisms, the terms FDOB and FDPB need to incorporate the biomagnification of methylmercury from the aquatic trophic levels into the tissues of birds consumed by bald eagles. In effect:

FDOB = FDTL2 (concentration in TL2 organisms) × **MOB** (*i.e.*, some BMF value representing biomagnification into omnivorous bird prey)

FDPB = FDTL3 (concentration in TL3 organisms) × **MPB** (*i.e.*, some BMF value representing biomagnification into piscivorous bird prey)

VI.A. Biomagnification Factor for Trophic Level 3 Fish to Piscivorous Bird Prey: **MPB**

The BMF of 10 used in the GLI to represent the biomagnification from trophic level 3 fish into herring gulls was arrived at from data indicating that tissue mercury concentrations in piscivorous birds tends to be from 3 to 12 times higher than the tissue mercury concentrations in the fish that the birds feed on (U.S. Environmental Protection Agency, 1995d). An analysis of the three studies used for the EPA's determination (Vermeer *et al*., 1973; Norheim and Froslie, 1978; and Wren *et al*, 1983) is provided below.

Vermeer *et al*. (1973) examined total mercury residues in herring gull eggs and in breast muscle from 83 ducks (six species) from Clay Lake in western Ontario. Only four of the 83 ducks were adults, the rest being flightless ducklings or immature birds. Many of the immature birds were also flightless. Breast muscle samples from five of the collected birds were also analyzed for methylmercury content. The authors concluded that elevated total mercury residues in herring gull eggs did not affect reproductive success, but no information was provided about methylmercury in herring gull tissues or the gull's prey. No conclusions about BMF values can be drawn from the herring gull portion of this study.

In addition to the duck breast muscle samples, food items were collected from the esophagi and stomachs of three of the duck species and analyzed for total mercury concentrations. These food items included yellow perch (*Perca flavescens*) and shiners (*Notropis* sp.) consumed by common mergansers (*Mergus merganser*), and a variety of aquatic invertebrates consumed by common goldeneyes (*Bucephala clangula*) and hooded mergansers (*Lophodytes cucullatus*). Breast

muscle sampled from the five individual ducks was analyzed for methylmercury, which accounted for 69-99 percent of total mercury concentrations. However, the food items from the three mentioned duck species were analyzed for total mercury, making direct assessments of methylmercury biomagnification difficult. While it is commonly accepted that the majority of mercury in fish muscle is methylmercury, it is unclear whether the same holds true for the various molluscs, crayfish, insects, and annelids found as food items in these ducks. In addition, the information regarding biomagnification from these non-fish prey items into duck tissues would have had limited value for the estimation of a BMF to herring gulls for the GLI.

Ten yellow perch collected from esophagi and stomachs of common mergansers during this study averaged 2.7 mg/kg (range 1.6 - 3.6) total mercury. Common merganser breast muscle was not analyzed for methylmercury, but a mean concentration of 6.79 mg/kg (range 4.4 - 13.1) total mercury was reported from 17 analyzed birds. Assuming the relative proportion of mercury to methylmercury is similar in fish tissue and duck breast muscle, an average methylmercury BMF for these birds would be 2.5. An important consideration in evaluating this BMF, however, is that the birds sampled were either ducklings or sub-adults. If the birds were in a stage of substantial feather growth, much of the ingested methylmercury could have been shunted into the feathers instead of muscle tissue (Elbert, 1996; Wiener *et al*., 2002). Body burdens of methylmercury in adult female muscle tissue prior to egg laying may have been substantially greater than the values reported for ducklings and sub-adults.

In the work of Norheim and Froslie (1978), the degree of methylation and organ mercury distribution in several raptorial species in Norway was examined. While this study provided data on methylmercury concentrations in various raptor tissues and evidence of demethylation in raptor organs, prey items were not evaluated. Because of this data gap, no conclusions can be drawn regarding the biomagnification of methylmercury from the diet into tissues of the raptors examined.

Wren *et al*. (1983) examined the bioaccumulation and biomagnification of 21 naturally occurring elements into abiotic and biotic components in an undisturbed Precambrian Shield lake in Ontario. Among the biotic samples were 5 herring gulls, 20 rainbow smelt (*Osmerus mordax*), and 20 bluntnose minnows (*Pimephales notatus*), although it is not clear from the report whether all 20 of the minnows were analyzed. Breast muscle samples from the herring gulls and dorso-lateral muscle samples from the fish were analyzed for mercury. It appears from the report that analysis was for total mercury; however, as has been discussed previously, mercury in fish and avian muscle tissues is primarily methylmercury. This allows for a reasonable estimation of a methylmercury BMF. Average mercury concentration in herring gull breast muscle was 1.7 mg/kg (range 0.66 - 4.0). Average concentration in bluntnose minnow muscle was 0.12 mg/kg (range 0.05 - 0.26), and in rainbow smelt the average concentration was 0.32 mg/kg (range 0.15 - 0.67). The mean length of collected rainbow smelt and bluntnose minnows was 17.3 and 7.4 cm, respectively.

The authors of this study (Wren *et al*., 1983) offered no indication of what the sampled herring gulls preyed upon, except to say that the gulls would "...generally feed on small fish which contain relatively low Hg levels." Herring gulls in the lower Great Lakes were reported to feed primarily on alewife and smelt, with females feeding more on the smaller smelt (mean length: 9 cm) and males feeding more on alewife (mean length: 16 cm) (U.S. Environmental Protection Agency, 1995c). If female herring gulls on the Wren *et al*. (1983) study lake preyed primarily on the smaller bluntnose minnows, a BMF of 14.2 can be calculated (*i.e.*, 1.7 mg/kg in gull breast muscle divided by 0.12 mg/kg in minnow muscle). However, if rainbow smelt are the primary prey, a BMF of 5.3 is calculated (*i.e.*, 1.7 mg/kg divided by 0.32 mg/kg). Taking the average of these two values results in a BMF just under 10, the BMF used by the EPA in the GLI effort.

There has been a great deal of research over the past several decades examining the relationship between dietary mercury concentrations and the resultant concentrations in avian tissues. Controlled laboratory feeding studies, as well as field studies examining mercury concentrations in bird tissues and in the organisms the birds generally feed on, can provide data with which BMFs can be calculated. However, these studies typically are designed to evaluate mercury concentrations in individual tissues such as the liver, kidney, feathers, blood, or brain. While these types of data, and the information they generate regarding biomagnification, are extremely valuable in understanding the toxicokinetics and toxicodynamics of mercury in the exposed bird, they are of limited value for determining BMFs from food into a "whole body" concentration. Whole body concentrations are needed when evaluating the consumption of exposed birds by a predator such as the bald eagle. Ideally, all edible tissues of a dosed bird would be analyzed to provide the averaged methylmercury concentration for the entire bird. Then, knowing the methylmercury concentration in the food, the most accurate BMF for the consumer can be calculated.

Lacking studies where all edible tissues of an exposed bird are analyzed, the most appropriate BMF when considering consumption of the exposed bird by a bald eagle should be based on the relationship between concentrations in the muscle of the test bird and the concentrations in its food. Muscle tissue represents the majority of edible matter in a consumed bird; the pectoralis major and supracoracoideus muscles of the breast by themselves account for between one-fifth and one-third of body weight in flying birds (Proctor and Lynch, 1993). Therefore, methylmercury concentrations in muscle should serve as the best surrogate for whole body concentrations. Muscle tissue concentrations may underestimate the actual whole body concentration, as methylmercury levels in other tissues may be substantially higher; however, the relatively small contribution of these other tissues to the overall edible mass should help to minimize these differences.

As described, two of the studies used to determine a BMF in the GLI effort for trophic level 3 fish to piscivorous birds examined muscle tissues in the target birds. While these studies provide some information regarding mercury biomagnification into piscivorous birds that could be consumed by bald eagles, there was sufficient uncertainty in their extrapolation of BMFs to warrant further analysis for this current effort. An attempt was made to find data directly

connecting methylmercury concentrations in documented food items to methylmercury concentrations in the muscle tissue of adult piscivorous birds.

The work done by Henny *et al*. (2002), previously discussed in Section IV.C (Determination of Test Doses), provided an assessment of mercury in the food and tissues of three piscivorous birds nesting along the lower Carson River in Nevada. Various tissues from both adult and juvenile double-crested cormorants (DCC), black-crowned night-herons (BCNH), and snowy egrets (SE) were analyzed, including methylmercury concentrations in stomach contents. Based on stomach content analyses, it was determined that mean total mercury concentrations in the diets of the three species in 1998 were 0.515 mg/kg (BCNH), 0.905 mg/kg (SE), and 1.44 mg/kg (DCC). Methylmercury accounted for most of the mercury detected, with mean concentrations of 0.48 mg/kg (BCNH), 0.775 mg/kg (SE), and 1.18 mg/kg (DCC).

In 1998, total mercury was measured in liver, kidney, brain, blood, and feathers of all three species examined. Using these concentrations and the data for total mercury in stomach contents, it is possible to calculate total mercury BMFs for each of these specific tissues. However, these values do not allow for an estimate of whole body methylmercury concentrations for two reasons: 1) mercury found in the liver and kidney samples was predominantly inorganic due to postabsorptive demethylation, and 2) the relative contribution of the analyzed tissues to the total edible biomass of each bird is small compared to the contribution of muscle tissue. Although no muscle tissue from any of the bird species was analyzed in this study, it was possible to estimate muscle methylmercury concentrations based on an assumed relationship in piscivorous birds between muscle and brain tissue concentrations. Once muscle methylmercury concentrations were estimated for the birds in the Henny *et al*. (2002) study, a methylmercury BMF from food into a whole body concentration could be calculated.

Additional analyses in the Henny *et al*. (2002) study on a small number of BCNH egg, feather, blood, and brain samples confirmed that mercury residues in these types of avian tissues are essentially 100 percent methylmercury. Brain tissue concentrations were selected to establish the relationship with muscle tissue for several reasons: 1.) no egg concentration values were reported, 2.) feathers were only collected from nestling/fledgling birds, 3.) no studies were found in the scientific literature in which both avian blood and muscle tissue were analyzed for mercury, and 4.) scientific studies examining mercury in avian muscle tissues most commonly include liver, kidney, and brain samples in the analyses.

In reviewing the scientific literature for studies reporting tissue mercury concentrations in piscivorous birds, work done by Elbert (1996) and Elbert and Anderson (1998) with western and Clarke's grebes (*Aechomorphorus occidentalis* and *Aechomorphorus clarkii*) in California provided the most useful data for establishing a brain / muscle relationship. Twenty-three adult birds were collected from three California lakes in 1992, with liver, kidney, breast muscle, and brain tissues analyzed for total mercury. All three lakes are representative of the characteristic habitat used for determining the bald eagle diet used in this analysis; however, one of the three (Clear Lake) is known to be impaired by mercury contamination. Of the other two study sites,

Eagle Lake is relatively pristine, while Tule Lake has previously had problems with organochlorine compounds in the eggs of nesting western grebes (Elbert and Anderson, 1998). Neither of these two lakes are known to have elevated mercury concentrations.

For all birds sampled from the three Elbert and Anderson (1998) study lakes, mean muscle and brain mercury concentrations were 0.79 and 0.22 mg/kg, respectively. These results suggest breast muscle mercury concentrations in piscivorous birds are approximately 3.6 times the concentrations found in brain tissues. Examining the data from each lake, however, reveals variations in this ratio. Mean muscle and brain mercury concentrations in birds at Tule Lake were 0.46 and 0.16 mg/kg, respectively, resulting in a ratio of approximately 2.9. At Eagle Lake, the values for muscle and brain were 0.43 and 0.13 mg/kg, resulting in a ratio of 3.3. Mercury concentrations in birds at Clear Lake were substantially higher, with 1.06 and 0.28 mg/kg in muscle and brain tissue, respectively. These data suggest breast muscle mercury concentrations in piscivorous birds at a mercury contaminated site are approximately 3.8 times the concentrations found in brain tissue.

Because the birds examined in the study by Henny *et al*. (2002) were also sampled from mercury contaminated sites, the mean mercury concentrations reported for brain tissues were multiplied by 3.8 to estimate the concentrations expected in breast muscle. Estimated muscle concentrations for the three species are: BCNH - 6.61 mg/kg (brain = 1.74), SE - 8.74 mg/kg (brain = 2.30), DCC - 42.79 mg/kg (brain = 11.26). Taking the estimated muscle concentrations and dividing by mean methylmercury concentrations in the stomach contents for each species provides BMF values.

BCNH:	6.61 mg/kg in muscle ÷ 0.48 mg/kg in food = **13.77**
SE:	8.74 mg/kg in muscle ÷ 0.775 in food = **11.27**
DCC:	42.79 mg/kg in muscle ÷ 1.18 mg/kg in food = **36.26**

The BMFs estimated for night-herons and egrets are similar in magnitude to the value used for the EPA's GLI effort, while the estimated BMF for the double crested cormorant is more than three times the GLI value. One possible reason for this disparity may be the degree of piscivory exhibited by cormorants compared with the other two species. Henny *et al*. (2002) reported that the stomachs of all the cormorants sampled contained only fish, whereas the contents of the night-heron and egret stomachs varied from 100 percent fish to 100 percent aquatic insects. Based on the percentage volume of stomach items for these two species, the average diet for night-herons and egrets was approximately 34 and 49 percent fish, respectively. It is possible that methylmercury biomagnification from fish into avian muscle tissue is substantially greater for those bird species that are almost exclusively piscivorous, such as the double-crested cormorant and belted kingfisher (*Ceryle alcyon*).

While the remains of both double-crested cormorants and belted kingfishers were found at the nest sites examined in the study used to develop the bald eagle diet for this effort (Jackman *et al*., 1999), their contribution to the overall prey biomass was minimal. Therefore, the BMFs

estimated for black-crowned night-herons and snowy egrets served as the more appropriate surrogates for developing the MPB value for this evaluation.

Averaging the estimated BMFs for the black-crowned night-heron and snowy egrets results in an **MPB** value of **12.5**, used in this evaluation for the bald eagle.

VI.B. Biomagnification for Trophic Level 2 Organisms to Omnivorous Bird Prey: **MOB**

The majority of research on methylmercury and its biomagnification through the aquatic food chain into avian species has focused on piscivorous birds, as the consumption of fish (*i.e.*, higher trophic level biota) represents a pathway with the greatest potential exposure. A review of the scientific literature revealed little that was useful in developing a standardized biomagnification factor for omnivorous waterfowl. However, some data were examined that allowed estimation of a reasonable BMF for this effort.

The Vermeer *et al*. (1973) study discussed in the previous section examined mercury levels in the breast muscle of several species of piscivorous and omnivorous waterfowl, as well as in the stomach contents from individuals of three of these species. Breast muscle samples from 21 common goldeneyes (*Bucephala clangula*), an omnivorous species, showed a mean total mercury concentration of 7.80 mg/kg (range: 0.9 - 19.4). Two individual goldeneyes were further sampled to compare total mercury to methylmercury levels. In these two samples, methylmercury accounted for 73 and 77 percent of the total mercury values. Applying a value of 75 percent methylmercury to the mean total concentration of 7.80 mg/kg results in a mean methylmercury value of 5.85 mg/kg.

Food items from the esophagi and stomachs from seven of the collected goldeneyes confirmed the predominantly invertebrate diet of this species. These food items were analyzed for total mercury; however, the results were reported in a manner that prevents calculation of a precise average concentration. Average total mercury concentrations in the various food items (*e.g.*, bivalves, aquatic insect nymphs, crayfish) ranged from 0.30 to 7.1 mg/kg. Based on the reported values, the average total mercury concentration in the goldeneye diet is approximately 2 mg/kg. As previously noted, making direct assessments of methylmercury biomagnification from this concentration is difficult because it is unknown what percentage of the total mercury in the various invertebrates is methylmercury. In a recent review of mercury ecotoxicology (Wiener *et al*., 2002), the authors point out that the percentage of total mercury present as methylmercury in aquatic invertebrates can vary substantially. Examples of this variation include methylmercury ranging from 9 to 82 percent of total in aquatic insects from northern Wisconsin lakes, and from 20 to 95 percent of total in benthic aquatic insects (detritivores and predatory dragonflies, respectively) from hydroelectric reservoirs in northern Quebec.

With these wide variations possible, the approximate total mercury concentration of 2.0 mg/kg in the goldeneye diet from the Vermeer *et al*. (1973) study could translate into methylmercury

concentrations of 0.18 mg/kg (9% of total) to 1.9 mg/kg (95% of total). Biomagnification factors for the transfer from prey items into goldeneye breast muscle could therefore range from 32.5 (5.85 mg/kg ÷ 0.18 mg/kg) to 3.08 (5.85 mg/kg ÷ 1.9 mg/kg). The true value is likely toward the lower end of the range, as many of the invertebrate prey identified were themselves predatory, possibly resulting in a higher percentage of mercury in the methylated form. However, as discussed previously, an important consideration in evaluating biomagnification from these data is that the birds sampled were either ducklings or sub-adults. If the birds were in a stage of intense feather growth, much of the ingested methylmercury could have been shunted into the feathers instead of muscle tissue (Elbert, 1996; Wiener *et al*., 2002). In addition, body burdens of methylmercury in adult female muscle tissue prior to egg laying may have been substantially greater than the values reported for ducklings and sub-adults.

In an expansion on the previous study, Fimreite (1974) examined 184 piscivorous and omnivorous waterfowl specimens from five different lakes in the same locale of northwestern Ontario. Liver, breast muscle, and stomach contents from twelve of these birds, including three common goldeneyes representing predominantly invertebrate feeders, were analyzed for total and methylmercury. Invertebrates from the three goldeneye stomachs were not identified; however, the contents of each bird were analyzed separately. Methylmercury concentrations in these stomach contents were reported as 0.09, 0.19, and 0.36 mg/kg. These values represented 100, 56, and 47 percent, respectively, of total mercury concentrations. The corresponding breast muscle samples contained 0.11, 0.23, and 0.51 mg/kg methylmercury. For each bird, the reported values indicate biomagnification from diet into breast muscle is only slightly greater than 1 (~ 1.2 - 1.4).

Although life stage was not reported, the three birds sampled were most likely adults. In a separate component of this study, breast muscle and liver from 12 adult and 3 duckling goldeneyes were analyzed for methylmercury. Results showed that mean methylmercury concentrations in duckling breast muscle (7.10 mg/kg) were substantially higher than in adult breast muscle (0.76 mg/kg). While the data suggest biomagnification from food into adult goldeneye breast muscle is low, the timing of sample collection may have masked a greater level of biomagnification prior to the study than indicated from the results. Birds for this study were collected during the periods 20 July - 5 August 1970 and 20 June - 28 July 1971. These periods coincide with the periods of greatest postnuptial molt of goldeneyes in central Ontario, as well as the late stages of duckling growth (Eadie *et al*., 1995). It is possible that adult body burdens of methylmercury were being depurated into replacement feathers, while the young may have finished producing their adult plumage and were no longer eliminating ingested methylmercury through this pathway. Biomagnification into muscle tissue during non-molt periods or after cessation of juvenile feather growth may be substantially greater. If these late stage ducklings were consuming invertebrates with the same methylmercury concentrations as observed in adult stomach contents, biomagnification factors from food into breast muscle could range from approximately 20 to 80 (*e.g.*, 7.10 mg/kg ÷ 0.9 mg/kg = 78.8).

Depuration of methylmercury into growing feathers, excretion in the feces, and deposition into eggs are the principal means of mercury elimination in adult female birds (Wiener *et al*., 2002).

For many of the omnivorous waterfowl species that would be consumed by California bald eagles, molting and egg laying would occur in the spring and summer on northern breeding grounds outside of California. Such was the case with the common goldeneyes in both of the above studies (Vermeer *et al*., 1973; Fimreite, 1974). Although neither study was designed to determine biomagnification factors, the data they generated could considerably underestimate the extent of biomagnification in California birds.

In order to minimize this potential underestimation, an attempt was made to find data for omnivorous birds in California waters. Eared grebes (*Podiceps nigricollis*) and samples of their invertebrate prey were collected from Eagle Lake, California (Eagles-Smith *et al*., in prep.). Eagle Lake, a relatively pristine body not known to have substantial mercury contamination, is the same location where Elbert and Anderson (1998) examined western and Clarke's grebes. This is a breeding area for eared grebes, while their wintering habitats are Pacific coastal regions, southwestern United States, Baja California, and Mexico (Cullen *et al*., 1999).

In the Eagle Lake work, six adult (3 male, 3 female) and three juvenile birds were collected between August and September of 2000. All adults had completed breeding, and were flightless at the time of collection (*i.e.*, both primary and body feather molt). As with the previous two studies discussed, feather replacement during this molt cycle could be an important elimination pathway for the bird's methylmercury body burden. Breast muscle from each bird was sampled and analyzed for total mercury. Concentrations ranged from 0.031 to 0.104 mg/kg (converted from dry weight using 71.5% moisture), with an average of 0.069 mg/kg.

Eared grebes are known to feed predominantly on brine shrimp and brine flies at fall staging areas prior to their winter migration (Cullen *et al*., 1999). However, their diet at freshwater breeding lakes consists mainly of caddisfly and mayfly larvae (~50%), amphipods (~20%), water beetles (~20%), aquatic snails (~10%), and an occasional fish (Eagles-Smith *et al*., in prep.). Approximately 50 invertebrate samples were collected from Eagle Lake, from locations where grebes were taken, and analyzed for total mercury after being sorted into general taxonomic groups. Based on the general dietary composition presented above, the analytical results were combined in a weighted average approach to provide an overall mercury concentration for the integrated eared grebe diet. The average total mercury concentration for this integrated diet was 0.02 mg/kg dry weight. Using a general value of 75 percent moisture for these aquatic invertebrates results in a wet weight concentration of 0.005 mg/kg total mercury.

Neither the grebe muscle nor invertebrate samples were analyzed for methylmercury. Applying the same value of 75 percent observed in common goldeneyes from the Vermeer *et al*. (1973) study to represent the ratio of total mercury to methylmercury, the average methylmercury concentration in the eared grebe breast muscle was 0.052 mg/kg. As discussed previously, the methylmercury percentage in aquatic invertebrates can vary considerably, depending on factors such as the organism's trophic position. For the invertebrates sampled in the Eagle Lake study, it was estimated that methylmercury accounted for approximately 60 - 70 percent of total mercury (Eagles-Smith *et al*., in prep). Of the two primary grebe prey items, only the caddisfly larvae are

considered omnivorous, occupying a higher trophic position, while mayfly larvae are strictly herbivorous (Kozloff, 1990). The amphipods and naucorids consumed by grebes may also exhibit varying degrees of omnivory. These higher trophic level prey, combined with the occasional fish, allow for a reasonable justification for using the higher value of 70 percent methylmercury in invertebrates. This results in an average methylmercury concentration in the grebe's invertebrate diet of 0.0035 mg/kg.

Dividing the average grebe breast muscle concentration (0.052 mg/kg) by the average integrated invertebrate diet concentration (0.0035 mg/kg) results in a biomagnification factor for methylmercury of slightly less than 15 (14.86). Considering these data were generated from a time when a substantial amount of the grebe's methylmercury body burden may have been shunted into replacement feathers, non-molt biomagnification may be substantially greater. These data demonstrate that methylmercury biomagnification in omnivorous waterfowl can be substantially higher than previous studies would indicate.

Assigning an omnivorous waterfowl biomagnification factor for this effort was complicated by numerous factors, including the fact that the various species consumed by bald eagles can exhibit widely varying degrees of omnivory. The eared grebe feeds exclusively on animal matter while other species, such as the American coot (*Fulica americana*), Northern pintail (*Anas acuta*), or American wigeon (*Anas americana*), rely on animal foods to a much lesser extent (Brisbin and Mowbray, 2002; Mowbray, 1999; Austin and Miller, 1995). For every eagle prey bird like the eared grebe having a biomagnification factor of 15 or greater, there may be another exhibiting biomagnification at less than a factor of five. The processes of molting and egg production also contribute to the difficulty in estimating muscle concentrations at any given time of year. It would be virtually impossible to determine true field biomagnification for all omnivorous waterfowl consumed by bald eagles; however, given the information presented above, it is reasonable to assign a general biomagnification factor of 10 for that portion of the bald eagle diet consisting of omnivorous waterfowl.

An **MOB** value of **10** was used in the evaluation for the bald eagle.

VII. EVALUATION OF THE HUMAN HEALTH METHYLMERCURY CRITERION

Once these additional terms for the bald eagle were defined, the modified Equation 1 was used to evaluate the human health criterion for all species of concern.

$$DC = (\%TL2 \times FDTL2) + (\%TL3 \times FDTL3) + (\%TL4 \times FDTL4) + (\%OB \times FDOB) + (\%PB \times FDPB)$$

Inclusion of the additional terms for bald eagles did not affect the calculations for the other species evaluated in this effort, as they only resulted in zero values for those components of the equation (*i.e.*, if %OB = 0, then [%OB × FDOB] = 0). The modified Equation 1 yields the expected overall dietary concentration (DC) resulting from the amount of food eaten from each trophic level, in conjunction with the trophic level methylmercury concentrations estimated from

each of the two TRC trophic level approaches. The DC values calculated for each species could then be compared to the species-specific WV concentrations generated using reference doses, body weights, and food ingestion rates. This simple comparison showed whether either trophic level approach will result in dietary concentrations higher or lower than the protective WV. If lower, then it may be assumed that the species should not be at risk from dietary exposure to methylmercury. If higher, it could be assumed that the species would likely have a dietary exposure that may place it at risk for adverse effects from methylmercury toxicity. In these latter instances, the methodology outlined in the Average Concentration Trophic Level approach can be used to calculate the trophic level-specific methylmercury concentrations necessary to maintain the DC at or below that species' WV.

VII.A. Average Concentration Trophic Level Approach

As explained previously (see Section II.A.), applying the Average Concentration Trophic Level Approach to the TRC of 0.3 mg/kg yields the following trophic level-specific concentrations in aquatic biota:

FDTL2 = 0.029 mg/kg
FDTL3 = 0.165 mg/kg
FDTL4 = 0.66 mg/kg

For the bald eagle, the two biomagnification factors determined previously were used to estimate methylmercury concentrations in the eagle's avian prey:

FDOB = FDTL2 × MOB
FDOB = 0.029 mg/kg × 10
FDOB = 0.29 mg/kg

FDPB = FDTL3 × MPB
FDPB = 0.165 mg/kg × 12.5
FDPB = 2.06 mg/kg

Then, applying these predicted methylmercury concentrations and the trophic level dietary breakouts determined for each species of concern to the modified Equation 1 yielded the total dietary concentrations (DC) presented in Table 4.

Table 4. Predicted Dietary Concentrations (DC) of Methylmercury Under Average Concentration TL Approach

Modified Equation 1:

$$DC = (\%TL2 \times FDTL2) + (\%TL3 \times FDTL3) + (\%TL4 \times FDTL4) + (\%OB \times FDOB) + (\%PB \times FDPB)$$

Species	%TL2	%TL3	%TL4	%OB	%PB	%OF*	DC (mg/kg)
Southern sea otter	0.80	0.20	na	na	na	na	0.056
California least tern	na	1.00	na	na	na	na	0.165
California clapper rail	0.85	0.05	na	na	na	0.10	0.033
Light-footed clapper rail	0.82	0.18	na	na	na	na	0.053
Yuma clapper rail	0.23	0.72	na	na	na	0.05	0.125
Western snowy plover	0.25	na	na	na	na	0.75	0.007
Bald eagle	na	0.44	0.39	0.10	0.035	0.035	0.431

* - The term '%OF' (*i.e.*, other foods) represents dietary items not expected to significantly contribute dietary methylmercury, and is presented in the table only to provide the full dietary composition assessment for each species. These %OF items include plants, terrestrial insects, or avian prey not dependent on aquatic biota. The term was not included in the equation to determine DC values because the assumed absence of significant methylmercury in these food items would only result in a zero value for that component of the equation, thus having no effect on the final DC value:

[%OF × FDOF (methylmercury concentration in other foods)]
[%OF × 0] = 0

The DC values from Table 4., representing the methylmercury concentration in the overall diet of the species resulting from the trophic level-specific concentrations generated by the Average Concentration Trophic Level Approach, were directly compared with the species-specific WVs (Table 5). These comparisons allowed for the presentation of the DC value as a percentage of the corresponding WV, which provided a measure of the protectiveness afforded by the TRC under this approach.

Table 5. Ratio of DC Values to WVs Under Average Concentration TL Approach

Species	DC Values	WVs*	Ratio (DC/WV)
Southern sea otter	0.056	0.055	102%
California least tern	0.165	0.030	550%
California clapper rail	0.033	0.042 (0.014)	79% (236%)
Light-footed clapper rail	0.053	0.040 (0.013)	133% (408%)
Yuma clapper rail	0.125	0.040 (0.013)	313% (962%)
Western snowy plover	0.007	0.026 (0.009)	27% (77%)
Bald eagle	0.431	0.184	234%

* - Values in parentheses represent the WVs generated from the alternative RfD for clapper rails and snowy plover generated using the UF_A of 3, and the subsequent relationships to the DC values.

Wildlife values for the California least tern, light-footed clapper rail, Yuma clapper rail, and bald eagle would be significantly exceeded if their prey contained methylmercury concentrations allowed under the Average Concentration Trophic Level Approach. Wildlife values determined for all three clapper rail subspecies using the alternative RfD would be exceeded under this approach. The WV for the southern sea otter appears as though it would not be significantly exceeded under this approach, while the DC for the western snowy plover would remain well below the WV regardless of the RfD used.

VII.B. Highest Trophic Level Approach

As explained previously (see Section II.B.), applying the Highest Trophic Level Approach to the TRC of 0.3 mg/kg yields the following trophic level-specific concentrations:

FDTL2 = 0.013 mg/kg
FDTL3 = 0.075 mg/kg
FDTL4 = 0.3 mg/kg

For the bald eagle, the two biomagnification factors determined previously were used to estimate methylmercury concentrations in the eagle's avian prey:

FDOB = FDTL2 × MOB
FDOB = 0.013 mg/kg × 10
FDOB = 0.13 mg/kg

FDPB = FDTL3 × MPB
FDPB = 0.075 mg/kg × 12.5
FDPB = 0.94 mg/kg

Then, applying these predicted methylmercury concentrations and the trophic level dietary breakouts determined for each species of concern to the modified Equation 1 yielded the total dietary concentrations (DC) presented in Table 6.

Table 6. Predicted Dietary Concentrations (DC) of Methylmercury Under Highest TL Approach

Modified Equation 1:

$$DC = (\%TL2 \times FDTL2) + (\%TL3 \times FDTL3) + (\%TL4 \times FDTL4) + (\%OB \times FDOB) + (\%PB \times FDPB)$$

Species	%TL2	%TL3	%TL4	%OB	%PB	%OF*	DC (mg/kg)
Southern sea otter	0.80	0.20	na	na	na	na	0.025
California least tern	na	1.00	na	na	na	na	0.075
California clapper rail	0.85	0.05	na	na	na	0.10	0.015
Light-footed clapper rail	0.82	0.18	na	na	na	na	0.024
Yuma clapper rail	0.23	0.72	na	na	na	0.05	0.057
Western snowy plover	0.25	na	na	na	na	0.75	0.003
Bald eagle	na	0.44	0.39	0.10	0.035	0.035	0.196

* - The term '%OF' (*i.e.*, other foods) represents dietary items not expected to significantly contribute dietary methylmercury, and is presented in the table only to provide the full dietary composition assessment for each species. These %OF items include plants, terrestrial insects, or avian prey not dependent on aquatic biota. The term was not included in the equation to determine DC values because the assumed absence of significant methylmercury in these food items would only result in a zero value for that component of the equation, thus having no effect on the final DC value:

[%OF × FDOF (methylmercury concentration in other foods)]
[%OF × 0] = 0

The DC values from Table 6., representing the methylmercury concentration in the overall diet of the species resulting from the trophic level-specific concentrations generated by the Highest Trophic Level Approach, were directly compared with the species-specific WVs (Table 7). These comparisons allowed for the presentation of the DC value as a percentage of the corresponding WV, which provided a measure of the protectiveness afforded by the TRC under this approach.

Table 7. Ratio of DC Values to WVs Under Highest TL Approach

Species	DC Values	WV Values*	Ratio (DC/WV)
Southern sea otter	0.025	0.055	45%
California least tern	0.075	0.030	250%
California clapper rail	0.015	0.042 (0.014)	36% (107%)
Light-footed clapper rail	0.024	0.040 (0.013)	60% (185%)
Yuma clapper rail	0.057	0.040 (0.013)	143% (438%)
Western snowy plover	0.003	0.026 (0.009)	12% (33%)
Bald eagle	0.196	0.184	107%

* - Values in parentheses represent the WVs generated from using the alternative RfD for clapper rails and snowy plover generated using the UF_A of 3, and the subsequent relationships to the DC values.

Wildlife values for the California least tern and Yuma clapper rail would be substantially exceeded if their prey contained methylmercury concentrations allowed under the Highest Trophic Level Approach. The bald eagle WV would only be slightly exceeded by this approach. Using the alternative RfD, the WV for the light-footed and Yuma clapper rails would be substantially exceeded under this approach, while the WV for the California clapper rail would only be slightly exceeded. The DC for the western snowy plover would remain substantially below the WV regardless of the RfD used.

VIII. EVALUATION RESULTS

VIII.A. Southern Sea Otter

The southern sea otter was federally listed as threatened in 1977 (42 Federal Register 2965). Critical habitat for the species has not been designated. A revised recovery plan was published in 2003 (U.S. Fish and Wildlife Service, 2003).

Life History: Generally, the home ranges of southern sea otters consist of several heavily used areas with travel corridors between them. Animals often remain in an area for a long period of time and then suddenly move long distances; these movements can occur at any time of the year. Male southern sea otters have larger home ranges and are less sedentary than females. Juvenile males move further from natal groups than do juvenile females, likely due to territorial and aggressive behavior exhibited toward juvenile males by older males. Most male southern sea otters leave the central portion of the range and travel to its ends during the pupping season, which occurs primarily in the winter and spring (Riedman and Estes, 1990). Southern sea otters mate and pup throughout the year. A peak period of pupping occurs from January to March, and a secondary pupping season occurs in late summer and early fall. Parental care is provided solely by the female. Because of their ability to eat large quantities of marine invertebrates, sea otters play an extremely important role in the nearshore marine community.

Historic and Current Range: Southern sea otters once ranged from the central coast of Baja California north to at least northern California, although they may have ranged as far north as Prince William Sound in Alaska (Riedman and Estes, 1990; Wilson *et al*., 1991). Prior to being protected from hunting for their pelts in 1911, southern sea otters were reduced to only a remnant colony near Bixby Creek along the Big Sur coast in California. Since 1911, the species has expanded north and south from the Bixby Creek colony. Currently, the range of the southern sea otter extends from about Half Moon Bay to Point Conception, with a small translocated colony at San Nicolas Island in southern California.

Rangewide Trends and Current Threats: Historically, the number of southern sea otters was probably between 16,000 and 20,000 (California Department of Fish and Game, 1976). By the end of the 19th century, the sea otter had been hunted nearly to extinction throughout its range. Southern sea otters along the central coast of California experienced a general recovering trend, increasing from as few as 50 animals in 1911 to an estimated 1,789 in 1976. Limitations on set-net fisheries imposed by the California Department of Fish and Game contributed to population increases in the late 1970s and early 1980s (Estes, 1990). Population counts declined from 1995 through 1999 but have since stabilized or increased. During the spring of 2003, a total of 2,505 sea otters were counted.

Current threats to the southern sea otter include disease, exposure to environmental contaminants, intentional take (shooting), and entanglement in fishing gear. Oil spills, which could occur at any time, threaten the southern sea otter with catastrophic decimation or localized

extinction (U.S. Fish and Wildlife Service, 2003).

Evaluation Results: Although the southern sea otter is at risk of exposure to methylmercury from the aquatic organisms in its diet, the analyses performed under each Trophic Level Approach indicate that the EPA's human health TRC (0.3 mg/kg) is not likely to result in a dietary exposure that would place sea otters at risk from methylmercury toxicity (see Tables 5 & 7). Due to the preponderance of trophic level 2 organisms in the otter's diet, neither the Average Concentration nor Highest Trophic Level Approach would result in dietary concentration (DC) values significantly above the calculated Wildlife Value (WV). The DC value generated from the otter's dietary composition and the trophic level methylmercury concentrations determined in the Average Concentration TL Approach is essentially the same as the calculated WV (DC - 0.056 mg/kg, WV - 0.055 mg/kg). The DC value generated in the Highest TL Approach is substantially below the WV (DC - 0.025 mg/kg, WV - 0.055 mg/kg).

VIII.B. California Least Tern

The California least tern was federally listed as endangered in 1970 (35 Federal Register 16047). A detailed account of the taxonomy, ecology, and biology of the California least tern is presented in the approved Recovery Plan for this species (U.S. Fish and Wildlife Service, 1985a).

Life History: California least terns are migratory. They arrive in California in April to breed and depart to wintering areas in Central and South America by the end of September. Little is known about least tern wintering areas. While in California, least tern adults court, mate, and select nest sites; lay, incubate, and hatch eggs; and raise young to fledging prior to departing from the breeding site.

After their eggs hatch, breeding adults catch and deliver small fish to the flightless young. The adults shift their foraging strategy when chicks hatch in order to obtain the very small sized fish suitable for nestlings (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000). The young begin to fly at about 20 days of age, but continue to be fed and are taught how to feed by their parents for some time after fledging. Most foraging activity is conducted within a couple miles of the colony (Atwood and Minsky, 1983). After fledging, the young terns do not become fully proficient at capturing fish until after they migrate from the breeding grounds.

Historic and Current Range: The California least tern continues to occupy nesting sites distributed throughout its historic range. The historic breeding range extended along the Pacific Coast from Moss Landing, Monterey County, California, to San Jose del Cabo, southern Baja California, Mexico (American Ornithologists Union, 1957; Dawson, 1924; Grinnell, 1928; Grinnell and Miller, 1944). However, least terns were nesting several miles north of Moss Landing at the mouth of the Pajaro River, Santa Cruz County, California, at least from 1939 (W.E. Unglish, Western Foundation of Vertebrate Zoology egg collection) to 1954 (Pray, 1954); and although nesting at San Francisco Bay was not confirmed until 1967 (Chandik and Baldridge, 1967), numerous spring and summer records for the area suggest nesting may have

occurred previously (Allen, 1934; Chase and Paxton, 1965; Grinnell and Wythe, 1927; Sibley, 1952). Since 1970, nesting sites have been documented in California from San Francisco Bay to the Tijuana River at the Mexican Border; and in Baja California from Ensenada to San Jose del Cabo at the tip of the peninsula.

Rangewide Trends and Current Threats: There are no reliable estimates describing the historic numbers of California least terns along the Pacific Coast (U.S. Fish and Wildlife Service, 1985a). Early accounts describe the existence of substantial colonies along the southern and central California coast (Bent, 1921), including a colony of about 600 breeding pairs along a 3-mile stretch of beach in San Diego County (Shepardson, 1909). At the time of its Federal listing as endangered in 1970, the total U.S. population of the California least tern was estimated to be 600 breeding pairs (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000). The dramatic decline in breeding least terns has been attributed to the degradation or loss of breeding sites, colonies, and foraging areas, which resulted from human development and disturbance, and pollution (U.S. Fish and Wildlife Service, 1985a).

The current U.S. population of the California least tern is grouped into 5 geographically discrete clusters, which support multiple active and historic breeding sites. These clusters include: (1) San Diego County, (2) Los Angeles/Orange Counties, (3) Ventura County, (4) San Luis Obispo/Santa Barbara Counties, and (5) San Francisco Bay area. Since its listing, the statewide population of the least tern has reached an estimated 4,009 breeding pairs in 1997 (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000). Despite this dramatic increase in breeding pairs, statewide monitoring has revealed threats to the least tern which emphasize the importance of demography to the least tern's survival and recovery.

California least terns were once common along the central and southern California coast. The decline of the California least tern is attributed to prolonged and widespread destruction and degradation of nesting and foraging habitats, and increasing human disturbance to breeding colonies. Conflicting uses of southern and central California beaches during the California least tern nesting season have led to isolated colony sites that are extremely vulnerable to predation from native, feral, and exotic species, overwash by high tides, and vandalism and harassment by beach users. Control of predators constitutes one of the most crucial needs at California least tern nesting sites.

Evaluation Results: In contrast to the evaluation results for the southern sea otter, applying the TRC under either of the trophic level approaches examined here is likely to result in a dietary exposure that may place California least terns at risk for adverse effects from methylmercury toxicity. Due to the tern's relatively small body size and its exclusively piscivorous diet, the WV (0.030 mg/kg) would be significantly exceeded by the DC values generated from the trophic level concentrations under each TL approach. In the case of the Highest TL Approach, the trophic level concentrations would result in a DC value (0.075 mg/kg) 250 percent of the tern's WV (see Table 7). The trophic level concentrations under the Average Concentration TL Approach would result in an even greater DC value (0.165 mg/kg), 550 percent of the WV (see Table 5). While

the extent of any potential adverse effects from either DC value cannot be quantified, the degree of WV exceedance under each TL approach suggests a high probability that dietary methylmercury exposure from the TRC could reach a level at which adverse effects to least terns may be expected. Based on the analyses performed in this effort, methylmercury concentrations in TL3 fish, the tern's sole prey base, would have to be substantially lower than the TL3 concentrations expected under each TL approach in order to maintain dietary exposure at the protective WV for California least terns.

VIII.C. California Clapper Rail

The clapper rail was federally listed as endangered in 1970 (35 Federal Register 16047). A detailed account of the taxonomy, ecology, and biology of the clapper rail can be found in the approved Recovery Plan for this species (U.S. Fish and Wildlife Service, 1984).

Life History: Clapper rails are non-migratory residents of San Francisco Bay tidal marshes. Research in a north San Francisco Bay marsh concluded that the clapper rail breeding season, including pair bonding and nest construction, may begin as early as February (Evens and Page, 1983). Field observations in south San Francisco Bay marshes suggest that pair formation also occurs in February in some areas (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000). The clapper rail breeding season has two nesting peaks, one between mid-April and early-May and another between late-June and early-July. Harvey (1988) and Foerster *et al.* (1990) reported mean clutch sizes of 7.27 and 7.47 for clapper rails, respectively. The end of the breeding season is typically defined as the end of August, which corresponds with the time when eggs laid during renesting attempts have hatched and young are mobile.

Historic and Current Range: Of the 193,800 acres of tidal marsh that bordered San Francisco Bay in 1850, about 30,100 acres currently remain (Dedrick, 1993). This represents an 84 percent reduction from historical conditions. Furthermore, a number of factors influencing remaining tidal marshes limit their habitat values for clapper rails. Much of the east San Francisco Bay shoreline from San Leandro to Calaveras Point has undergone erosion, resulting in a potential loss of local clapper rail populations. In addition, an estimated 600 acres of former salt marsh along Coyote Creek, Alviso Slough, and Guadalupe Slough, had been converted to fresh- and brackish-water vegetation marshes due to freshwater discharge from south San Francisco Bay wastewater facilities. Converted marshes are of lower quality for clapper rails.

The suitability of many marshes for clapper rails is further limited, and in some cases precluded, by their small size, fragmentation, and lack of tidal channel systems and other micro-habitat features. These limitations render much of the remaining tidal marsh acreage unsuitable or of low value for the species. In addition, tidal amplitudes are much greater in the south Bay than in San Pablo or Suisun bays (Atwater *et al.*, 1979). Consequently, many tidal marshes are completely submerged during high tides and lack sufficient escape habitat, likely resulting in nesting failures and high rates of predation. The reductions in carrying capacity in existing marshes necessitate the restoration of larger tracts of habitat to maintain stable populations.

Several years ago, the clapper rail population was estimated to be approximately 500 to 600 individuals in the southern portion of San Francisco Bay, while a conservative estimate of the north San Francisco Bay population, including Suisun Bay, was 195 to 282 pairs (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000). Historic populations at Humboldt Bay, Elkhorn Slough, and Morro Bay are now extinct; therefore, the 30,100 acres of tidal marsh remaining in San Francisco Bay represent the current distribution of this subspecies.

Rangewide Trends and Current Threats: As described above, the clapper rail's initial decline resulted from habitat loss and degradation, and reduction in range. Throughout San Francisco Bay, the remaining clapper rail population is besieged by a suite of mammalian and avian predators. At least 12 native and 3 non-native predator species are known to prey on various life stages of the clapper rail (Albertson, 1995). Artificially high local populations of native predators, especially raccoons, result as development occurs in the habitat of these predators around the Bay margins (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000). Encroaching development not only displaces lower order predators from their natural habitat, but also adversely affects higher order predators, such as coyotes, which would normally limit population levels of lower order native and non-native predators, especially red foxes (Albertson, 1995).

Hunting intensity and efficiency by raptors on clapper rails also is increased by electric power transmission lines, which criss-cross tidal marshes and provide otherwise-limited hunting perches (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000). Non-native Norway rats (*Rattus norvegicus*) long have been known to be effective predators of clapper rail nests (DeGroot, 1927; Harvey, 1988; Foerster *et al.*, 1990). Placement of shoreline riprap favors rat populations, which results in greater predation pressure on clapper rails in certain marshes. These predation impacts are exacerbated by a reduction in high marsh and natural high tide cover in marshes.

The proliferation of non-native red foxes into tidal marshes of the south San Francisco Bay since 1986 has had a profound effect on clapper rail populations. As a result of the rapid decline and almost complete elimination of rail populations in certain marshes, the San Francisco Bay National Wildlife Refuge implemented a predator management plan in 1991 (Foerster and Takekawa, 1991) with an ultimate goal of increasing rail population levels and nesting success through management of red fox predation. This program has proven successful in increasing the overall south San Francisco Bay populations from an all-time low; however, it has been difficult to effectively conduct predator management over such a large area as the south San Francisco Bay, especially with the many constraints associated with conducting the work in urban environments (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000).

Predator management for clapper rails is not being regularly practiced in the north San Francisco Bay, and rail populations in this area remain susceptible to red fox predation. Red fox activity has been documented west of the Petaluma River and along Dutchman Slough at Cullinan Ranch. Along Wildcat Creek near Richmond, where recent red fox activity has been observed,

the rail population level in one tidal marsh area has declined considerably since 1987, even though limited red fox management was performed in 1992 and 1993 (U.S. Fish and Wildlife Service and National Marine Fisheries Service, 2000).

In addition to habitat loss and predation pressures, pollutants in the aquatic environment appear to be a continuing threat to California clapper rail populations. Schwarzbach *et al*. (in press) examined factors affecting clapper rail reproductive success in San Francisco Bay, including predation, flooding, and contaminant exposure. Both predation and contaminants appeared to contribute to observations of low hatching success and overall fecundity for clapper rail nests in six intertidal salt marshes in the Bay. Egg hatchability was depressed in all marshes, with observations of deformities, embryo hemorrhaging, and embryo malpositions. Failed-to-hatch eggs contained various levels of trace element and organochlorine contaminants, with mercury at elevated concentrations in at least some eggs from all six marshes. The researchers stated that mercury appeared to consistently be the contaminant most likely to produce the low hatchability observed in all marshes sampled.

Evaluation Results: As explained previously in this document, the analyses for all three rail subspecies and the western snowy plover included evaluations using two WVs, based on RfDs generated from different interspecies uncertainty factors (UF_A). The WV calculated for the California clapper rail with the UF_A of 1 is 0.042 mg/kg. Comparing this WV with the expected DC values from the trophic level concentrations under both the Average Concentration TL Approach (DC - 0.033 mg/kg) and the Highest TL Approach (DC - 0.015 mg/kg) indicate that the TRC is not likely to result in dietary exposure that would place California clapper rails at risk for adverse effects from methylmercury toxicity, as both DC values are substantially below the WV (see tables 5 & 7).

However, the WV calculated with the UF_A of 3 (0.014 mg/kg) produces different results. The DC value from the Average Concentration TL Approach (0.033 mg/kg) is 236 percent of this WV, indicating that dietary exposure in California clapper rails may place them at risk under this TL approach. The DC value from the Highest TL Approach (0.015 mg/kg) is only slightly above the WV. The small differential (<10%) between the two is well within reasonable bounds, recognizing the various uncertainties and assumptions inherent in this methodology, to conclude that dietary exposure resulting from applying the TRC under the Highest TL Approach should not place California clapper rails at risk for adverse effects from methylmercury toxicity.

The question of which UF_A is the most appropriate to represent the clapper rail's sensitivity relative to mallard ducks, the species used in establishing the avian test dose (Heinz, 1979), cannot yet be definitively answered. However, data collected in the last decade on California clapper rails in the San Francisco Bay region allows for a parallel evaluation of the protectiveness afforded by the two WV values and the UF_As on which they were based.

Schwarzbach *et al*. (in press) collected failed-to-hatch clapper rail eggs from various marshes around San Francisco Bay in 1991-1992 (south Bay) and 1998-1999 (north Bay). The eggs were

analyzed for a number of pollutants, including mercury. Mean egg total mercury concentrations were then calculated for both south Bay eggs (0.54 mg/kg fresh wet weight, range: 0.17 - 2.52) and north Bay eggs (0.36 mg/kg fww, range: 0.11 - 0.87). A subset of collected rail eggs was analyzed for methylmercury, with results demonstrating that methylmercury was on average 95 percent of the total mercury found. South and north Bay means could then be adjusted to 0.513 and 0.342 mg/kg methylmercury, respectively. The south Bay average is equivalent to the avian 'lowest observed adverse effects concentration' (LOAEC) seen in pheasants (Fimreite, 1971).

In a corollary investigation (Schwarzbach *et al.*, 1996), clapper rail prey organisms (*i.e.*, snails, crabs, mussels) were collected in 1992 and 1994 from the same Bay marshes used in rail egg collections. The prey collections from 1992 were analyzed for total mercury, while those from1994 were analyzed for methylmercury. Only the south Bay marsh collections included all three prey organisms. The mean methylmercury concentration for all prey organisms in the south Bay, assuming 75 percent moisture, was 0.036 mg/kg (range: 0.0357 - 0.0363). This value is lower than the WV (0.042 mg/kg) calculated to be protective of clapper rails using the UF_A of 1.

These data allowed the calculation of a diet-to-egg transfer factor for California clapper rails in south San Francisco Bay. Taking the mean rail egg concentration of 0.513 mg/kg divided by the mean prey concentration of 0.036 mg/kg results in a methylmercury diet-to-egg transfer factor of 14.25. Multiplying the WV (0.042 mg/kg) generated with the UF_A of 1 by the diet-to-egg transfer factor of 14.25 results in an estimated methylmercury concentration in the egg of 0.598 mg/kg, higher than what is presently found in south Bay rail eggs. Multiplying the alternate WV (0.014 mg/kg) generated with the UF_A of 3 results in an estimated methylmercury concentration in the egg of 0.199 mg/kg. Based on the egg injection work discussed previously (Heinz, pers. comm., 2003) and assessments of the rail's current reproductive status (Schwarzbach *et al.*, in press), it has been estimated that a value of 0.2 mg/kg fww methylmercury in rail eggs would be a reasonable and appropriate 'no observed adverse effects concentration' (NOAEC) (Schwarzbach, pers. comm., 2003).

Although these data are limited in that collecting failed-to-hatch eggs does not represent a random sample analysis of methylmercury concentrations, they did provide parallel support that a UF_A of 3 is necessary to determine an appropriately protective RfD (0.007 mg/kg bw/day), and subsequent WV (0.014 mg/kg), for the California clapper rail. Given this additional validation of the higher UF_A, it can then be concluded that applying the TRC only under the Highest TL Approach is necessary to maintain dietary exposure at the protective WV for California clapper rails.

VIII.D. Light-footed Clapper Rail

The light-footed clapper rail was federally listed as endangered on October 13, 1970 (35 Federal Register 16047) and state listed as endangered in California on June 27, 1971. The original recovery plan for this species was approved in July 1979 and a revision was published on June 24, 1985 (U.S. Fish and Wildlife Service, 1985b). Critical habitat has not been designated for

this species.

Life History: Rails use coastal salt marshes, lagoons, and their maritime environs (Zembal, 1989). The birds nest in the lower littoral zone of coastal salt marshes where dense stands of cordgrass (*Spartina foliosa*) are present. They also build nests in pickleweed (*Salicornia virginica*) (Massey *et al*., 1984). Rails have also been known to reside and nest in freshwater marshes, although this is not common (Thelander and Crabtree, 1994). They require shallow water and mudflats for foraging, with adjacent higher vegetation for cover during high water (Zeiner *et al*., 1990). Rails forage in all parts of the saltmarsh, concentrating their efforts in the lower marsh when the tide is out, and moving into the higher marsh as the tide advances (Zembal *et al*., 1989).

The pair bond in rails endures throughout the season, and often from year to year. Nesting usually begins in March and late nests have usually hatched by August. Nests are placed to avoid flooding by tides, yet in cover dense enough to be hidden from predators and to support the relatively large nest (Storey *et al*., 1988). Females lay approximately 4-8 eggs, which hatch in 18-27 days (U.S. Fish and Wildlife Service, 1985b). Both parents care for the young; while one forages, the other adult broods the chicks (U.S. Fish and Wildlife Service, 1985b). By the age of two days, chicks will accompany adults on foraging trips; however, adults have been observed feeding fully grown chicks of at least six weeks of age within 25 meters of their incubation nest (U.S. Fish and Wildlife Service, 1985b).

Very limited evidence exists for inter-marsh movements by rails, and this subspecies is resident in its home marsh except under unusual circumstances (Zembal, 1989). Within marsh movements are also confined and generally no greater than 400 meters (Zembal, 1989). Minimum home range sizes for nine rails that were studied using radio telemetry at Upper Newport Bay varied from approximately 0.3 to 1.7 hectares, with larger areas and daily movements by first year birds attempting to claim their first breeding territories (Zembal, 1989). Despite the lack of direct evidence for inter-marsh movement by rails, at least four sites where rails appeared to be extirpated for six or more years were subsequently re-occupied, indicating likely inter-marsh re-colonization (Zembal and Hoffman, 2001).

Historic and Current Range: The rail currently inhabits coastal marshes from the Carpinteria Marsh in Santa Barbara County, California, to Bahia de San Quintin, Baja California, Mexico (Zembal, 1989; Zembal *et al*., 1998). It is believed that most salt marshes along the coastline at one time supported clapper rails (Grinnell *et al*., 1918), but recent census data indicate that less than 50 percent of the coastal wetlands in California are currently occupied (Zembal *et al*., 1998).

Rangewide Trends and Current Threats: The first rail census in southern California was conducted in 1972-73, and the population was estimated at about 500 pairs (Wilbur, 1974). Annual surveys conducted from 1980 to 2001 showed an erratic trend in the population, with a peak estimate of 325 pairs in 1996 (Zembal and Hoffman, 2001). The most recent population census in 2001 found 217 pairs (Zembal and Hoffman, 2001). The three largest sub-populations

(at Newport Bay, Tijuana Estuary, and Seal Beach National Wildlife Refuge) comprised 86 percent of the breeding rails in southern California in 2001 (Zembal and Hoffman, 2001). Many smaller rail sub-populations are under threat of extirpation, but with appropriate management could become nuclei for recovery (U.S. Fish and Wildlife Service, 1985b). The number of marshes inhabited by breeding rails in coastal southern California has fluctuated widely since population censuses began in 1980. The number of occupied marshes declined from 19 marshes in 1984 to 8 in 1989, but increased to 16 occupied marshes in 1997 (Zembal *et al*., 1998).

Habitat loss at several major estuaries in southern California approaches ninety-nine percent (U.S. Fish and Wildlife Service, 1985b). Although salt-marsh habitat loss, degradation, and fragmentation are the leading threats to rails, they are also threatened by disturbance, diseases, contaminants, and predation by non-native red foxes (Thelander and Crabtree, 1994). Rails may also be hit by vehicles in marshes adjacent to or bisected by roads (Zembal *et al*., 1989).

Evaluation Results: As with the California clapper rail, two WVs were calculated for the light-footed clapper rail, based on UF_As of 1 or 3. However, due to the light-footed rail's smaller body weight, WVs are slightly less than those for the California rail. The UF_A of 1 resulted in a WV of 0.040 mg/kg, while the UF_A of 3 yielded a WV of 0.013 mg/kg.

Based on the light-footed rail's diet, which has a greater percentage of trophic level 3 organisms than in the California rail's diet, the trophic level concentrations expected under the Average Concentration TL Approach would produce a DC value of 0.053 mg/kg. This value is more than 400 percent of the lower WV (0.013 mg/kg). The Highest TL Approach produces a DC value of 0.024 mg/kg, 185 percent of the same WV. Both levels of WV exceedance demonstrate that, if 3 is the appropriate UF_A to determine a protective RfD and WV (0.013 mg/kg) for the light-footed clapper rail, the TRC under either TL approach is likely to result in dietary exposure that may place this subspecies at risk for adverse effects from methylmercury toxicity.

No information was found regarding diet-to-egg relationships for this subspecies, so no parallel assessment could be made regarding the appropriateness of 3 as the UF_A. Although it is reasonable to assume that both the light-footed and California clapper rails would be similarly sensitive to methylmercury, it is possible that the light-footed rail is better adapted to detoxify ingested methylmercury because of its more piscivorous diet (see Section III,D: Determination of Reference Dose). If so, then it may be more appropriate to consider the light-footed rail as an obligate piscivore, using the RfD and subsequent WV (0.040 mg/kg) generated with the UF_A of 1.

Comparison of the DC values expected from both TL approaches with the higher WV (0.040 mg/kg) produces variable results. The DC value from the Average Concentration TL Approach (0.053 mg/kg) is more than 130 percent of this WV, indicating dietary exposure is still likely to place these rails at risk of adverse effects from methylmercury toxicity. In contrast, the DC value from the Highest TL Approach (0.024 mg/kg) is only 60 percent of this higher WV, indicating a dietary exposure not likely to place light-footed rails at risk from the TRC.

Regardless of which UF_A (1 or 3) and subsequent WV (0.040 or 0.013) are used in the analysis, the trophic level concentrations expected under the Average Concentration TL Approach would result in a DC value substantially greater than either WV. Dietary exposure under this TL approach may place light-footed clapper rails at risk for adverse effects from methylmercury toxicity. However, comparison of the DC value expected from the Highest TL Approach with the two WVs results in conflicting conclusions. Assuming the UF_A of 1 is appropriate, the analysis suggests that applying the TRC under the Highest TL Approach would be sufficient to maintain dietary exposure at or below the corresponding protective WV (0.040 mg/kg). If the UF_A of 3 is the more appropriate value, then the TRC under this TL approach would result in a dietary exposure above the corresponding WV (0.013 mg/kg). Given the various uncertainties and assumptions used in these analyses (*e.g.*, dietary composition, food chain multipliers), the only conclusion that can be drawn at this point is that, of the two TL approaches evaluated, the Highest TL Approach poses less risk of a dietary exposure that could place light-footed clapper rails at risk for adverse effects from methylmercury toxicity. Further research must be conducted to verify whether the trophic level concentrations expected under the Highest TL Approach are sufficient or need to be lower to ensure adequate protection for the light-footed rail.

VIII.E. Yuma Clapper Rail

The Yuma clapper rail was federally listed as endangered on March 11, 1967 (32 Federal Register 4001). The Yuma Clapper Rail Recovery Plan, approved in 1983, provides background information on the species and identifies new or ongoing tasks necessary to achieve recovery of this species (U.S. Fish and Wildlife Service, 1983). The State of California added the bird to its list of rare wildlife in May of 1971 and later listed it as threatened on February 22, 1978.

Life History: Yuma clapper rail habitat is characterized by cattail (*Typha*), bulrush (*Scirpus*), or tule stands, and shallow, slow-moving water near high ground. Cattail and bulrush stands are often dissected by narrow channels of flowing water that may be covered by downed vegetation. These open channels are important for foraging. Rails commonly use areas with low stem densities and little residual vegetation. They are also found in the ecotone between emergent vegetation and higher ground, such as the shoreline, channel edge, or hummocks in a marsh. In studies conducted along the lower Colorado River, rails were found to use areas far from a vegetative edge during early winter (Conway *et al*., 1993). The depth of water used by clapper rails also varied with season, with shallower water used during the breeding season, and water of moderate depth used during the winter. Although clapper rails are often found in larger stands of vegetation, they have also been found to use patches of habitat within agricultural drains (Bennett and Ohmart, 1978).

The Yuma clapper rail begins breeding activities in February, with egg-laying from March to July in marshes along the Colorado River from the Nevada/California border south to the Colorado River Delta region in Mexico. Chicks generally fledge by mid-September (Eddleman and Conway, 1994). It builds its nest on a raised platform of vegetation concealed in dense marsh vegetation (Patten *et al*., in press). Males may build multiple nests, and the female chooses one

for egg-laying. Alternate nests are used as platforms for loafing, preening, and as brood platforms, but may also be useful for incubation if predators or high water disturb the primary nest (Eddleman and Conway, 1994). This subspecies is partially migratory, with many birds wintering in brackish marshes along the Gulf of California but some remain on their breeding grounds throughout the year (U.S. Bureau of Land Management, 2001). Yuma clapper rails are found around the Salton Sea, and in agricultural drains and canals that support marsh vegetation (i.e., cattail, giant bulrush, alkali bulrush, and common reed). This subspecies breeds only in the lower Colorado River Valley and in the Salton Sink, the latter area holding about 40 percent of the United States population (Setmire *et al*., 1990). The breeding site for the largest population of the Yuma clapper rail in the United States is at the Wister unit of the California Department of Fish and Game (CDFG) Imperial Wildlife Area, near the Salton Sea. The sea's elevation is important to the Yuma clapper rail (U.S. Department of the Interior, 1998) as clapper rails use shallow freshwater habitat that has formed at the mouths of many of the inflows to the Salton Sea. Yuma clapper rails avoid deeper water because it increases juvenile mortality (California Department of Fish and Game, 1990).

Historic and Current Range: The Yuma clapper rail occurs primarily in the lower Colorado River Valley in California, Arizona, and Mexico, and is a fairly common summer resident from Topock south to Yuma in the U.S. and at the Colorado River Delta in Mexico. There are also populations of this subspecies at the Salton Sea in California, and along the Gila and Salt Rivers to Picacho Reservoir and Blue Point in central Arizona (Rosenberg *et al*., 1991). In recent years, individual clapper rails have been heard at Laughlin Bay and Las Vegas Wash in southern Nevada (Nevada Division of Wildlife, 1998). Population centers for this subspecies include Imperial Wildlife Management Area (Wister Unit), Sonny Bono Salton Sea National Wildlife Refuge (NWR), Imperial NWR, Cibola NWR, Mittry Lake, West Pond, Bill Williams Delta, Topock Gorge, and Topock Marsh.

In California this species nests along the lower Colorado River, in wetlands along the Coachella Canal, the Imperial Valley, the upper end of the Salton Sea at the Whitewater River delta, and Salt Creek (NatureServe, 2001). Hydroelectric dams along the Colorado River have apparently increased the amount of marsh habitat, and population numbers of the Yuma clapper rail may have increased expanding the range northward in response to the increase in available habitat (U.S. Bureau of Land Management, 2001). Also, habitat was expanded through the creation of the Salton Sea in the early 1900s.

Rangewide Trends and Current Threats: The U.S. Fish and Wildlife Service (1983) estimated a total of 1,700 to 2,000 individuals throughout the range of the subspecies. Between 1990 and 1999, call counts conducted throughout the subspecies range in the U.S. have recorded 600 to 1,000 individuals. In 1985, Anderson and Ohmart (1985) estimated a population size of 750 birds along the Colorado River north of the international boundary. A substantial population of Yuma clapper rails exists in the Colorado River Delta in Mexico. Eddleman (1989) estimated that 450 to 970 rails inhabited this area in 1987. Piest and Campoy (1998) reported a total of 240 birds responding to taped calls in the Cienega de Santa Clara region of the Delta. These counts

are only estimates of the minimum number of birds present. The population is probably higher than these counts show, since up to 40 percent of the birds may not respond in call surveys (Piest and Campoy, 1998). Based on the call count surveys, the population of Yuma clapper rails in the U.S. appears stable (U.S. Fish and Wildlife Service, unpublished data). The range of the Yuma clapper rail has been expanding over the past 25 years, and the population may be increasing (Ohmart and Smith, 1973; Monson and Phillips, 1981; Rosenberg *et al*., 1991; McKernan and Braden, 1999). A recent genetic analysis showed that this subspecies is outbred; population numbers of the Yuma clapper rail have not become low enough to reduce genetic diversity (U.S. Bureau of Land Management, 2001).

The Yuma clapper rail apparently expanded its range in the early 1900's in response to changes in the vegetation along the Colorado River. Damming and associated changes in hydrology induced vegetation changes in some areas that favored rails. At the same time, damming and diversion of the Colorado River reduced the amount of water flowing into the Colorado River Delta, and reduced the availability of rail habitats in the Delta. Approximately two-thirds of the formerly extensive marshlands of the Delta disappeared following completion of Hoover Dam (Sykes, 1937).

Yuma clapper rail habitat has been further affected by channelization, fill, dredging projects, bank stabilization, and water management practices along the Colorado River. Rail habitat has also been adversely affected by the spread of salt cedar (*Tamarisk ramosissima*). Salt cedar consumes an unusually high amount of water, which results in reduced wetland areas for vegetation preferred by the rail.

Many of the currently occupied breeding sites in the United States are on State and Federal lands that are protected and managed for wildlife (U.S. Fish and Wildlife Service, 1983). However, adequate water supplies are needed to assure the long-term availability of this habitat. Wintering areas and needs are not well known and require further study before habitat preservation needs can be determined. Many of the Mexican breeding sites are located in the Rio Colorado Delta area and require adequate flows in the lower Colorado River for long-term use by Yuma clapper rails. The population of Yuma clapper rails at the Cienega de Santa Clara is threatened by the loss of the source of water that maintains the wetland habitat.
Other threats to the Yuma clapper rail include mosquito abatement activities, agricultural activities, development, and the displacement of native habitats by exotic vegetation (California Department of Fish and Game, 1991).

Evaluation Results: The two WVs (0.013 and 0.040 mg/kg) calculated for the Yuma clapper rail are the same as those used for the light-footed clapper rail. However, due to the Yuma rail's reliance on higher trophic level organisms for its diet, the DC values expected with each TL approach are substantially higher than those expected for either the light-footed or California clapper rails.

The WV for the Yuma rail calculated using the UF_A of 3 is 0.013 mg/kg. The DC value expected from trophic level concentrations under the Highest TL Approach is 0.057 mg/kg, more than 430 percent of the WV (see Table 7). The DC value from the Average Concentration TL Approach is 0.125 mg/kg, almost 1000 percent of the WV (see Table 5). Clearly, if 3 is the appropriate UF_A to determine a protective RfD and WV for the Yuma clapper rail, the TRC under either TL approach is likely to result in dietary exposure that may place this subspecies at risk for adverse effects from methylmercury toxicity.

The WV calculated using the UF_A of 1 is 0.040 mg/kg. This WV (0.040 mg/kg) is substantially closer than the previous WV to the DC value of 0.125 mg/kg expected from the Average Concentration TL Approach, but this DC is still more than 300 percent of this higher WV (see Table 5). This higher WV is even closer to the DC value of 0.057 mg/kg expected from the Highest TL Approach (see Table 7); however, a DC value exceeding the WV by more than 40 percent is still likely to result in a dietary exposure that may place Yuma rails at risk for adverse effects from methylmercury toxicity. Based on these comparisons, both TL approaches would still be insufficient to maintain dietary exposure in this subspecies at or below the calculated WVs.

VIII.F. Western Snowy Plover

The Pacific coast population of the western snowy plover was federally listed as threatened on March 5, 1993 (58 Federal Register 12864) and critical habitat was designated on December 7, 1999 (64 Federal Register 68508). A draft recovery plan for the species has been completed (U.S. Fish and Wildlife Service, 2001).

Life History: Western snowy plovers prefer coastal beaches that are relatively free from human disturbance and predation. Sand spits, dune-backed beaches, beaches at creek and river mouths, and salt pans at lagoons and estuaries are the preferred habitats for nesting. The attributes considered essential to the conservation of the coastal population of the western snowy plover can be found in the final ruling for the designation of critical habitat (64 Federal Register 68508). The primary constituent elements for the western snowy plover are those habitat components that are essential for the primary biological needs of foraging, nesting, rearing of young, roosting, and dispersal, or the capacity to develop those habitat components. The primary constituent elements of critical habitat for the species are provided by intertidal beaches (between mean low water and mean high tide), associated dune systems, and river estuaries. Important components of the beach/dune/estuarine ecosystem include surf-cast kelp, sparsely vegetated foredunes, interdunal flats, spits, washover areas, blowouts, intertidal flats, salt flats, and flat rocky outcrops. Several of these components (sparse vegetation, salt flats) are mimicked in artificial habitat types used less commonly by western snowy plovers (*i.e.*, dredge spoil sites and salt ponds and adjoining levees).

The breeding season for western snowy plovers extends from March to late September, with birds at more southerly locations breeding earlier. Most nesting occurs on unvegetated or

moderately vegetated, dune-backed beaches and sand spits. Other less common nesting habitats include salt pans, dredge spoils, and salt pond levees. Nest site fidelity is common, and mated birds from the previous breeding season frequently reunite. Nest sites are scrapes in the substrate, in which females lay eggs (typically three but up to six). Both sexes incubate eggs, with the female tending to incubate during the day and the male at night (Warriner *et al*., 1986). Snowy plovers often renest if eggs are lost. Hatching lasts from early April through mid-August, with chicks fledging approximately one month after hatching. Adult plovers tend chicks while feeding, often using distraction displays to lure predators and people away from chicks. Females generally desert both mates and broods by the sixth day after hatching, and thereafter the chicks are typically accompanied by only the male. While males rear broods, females obtain new mates and initiate new nests (Page *et al*., 1995)

Historic and Current Range: The Pacific coast population of the western snowy plover breeds primarily on coastal beaches from southern Washington to southern Baja California, Mexico. Historically, western snowy plovers bred or wintered at 157 locations on the Pacific coast, including 133 sites in California. Larger numbers of birds are found in southern and central California, in Monterey Bay (estimated 200 to 250 breeding adults), Morro Bay (estimated 85 to 93 breeding adults), Pismo Beach to Point Sal (estimated 130 to 246 breeding adults), Vandenberg Air Force Base (estimated 130 to 240 breeding adults), and the Oxnard Lowland (estimated 69 to105 breeding adults).

During the non-breeding season western snowy plovers may remain at breeding sites or may migrate to other locations. Most winter south of Bodega Bay, California. Many birds from the interior population winter on the central and southern coast of California.

Rangewide Trends and Current Threats: Historical records indicate that nesting western snowy plovers were once more widely distributed in coastal Washington, Oregon and California than they are currently. Only 1,200 to 1,900 adult western snowy plovers remain on the Pacific coast of the United States (Page *et al*., 1991). In 1995, approximately 1,000 western snowy plovers occurred in coastal California. Historically, western snowy plovers bred at 53 coastal locations in California prior to 1970. Only eight sites continue to support 78 percent of the remaining California coastal breeding population. These are San Francisco Bay, Monterey Bay, Morro Bay, the Callendar-Mussel Rock dunes area, the Point Sal to Point Conception area (Vandenberg Air Force Base), the Oxnard lowland, Santa Rosa Island, and San Nicolas Island (Page *et al*., 1991).

The Pacific coast population of the western snowy plover has experienced widespread loss of nesting habitat and reduced reproductive success at many nesting locations due to urban development and the encroachment of European beachgrass (*Ammophila arenaria*). Human activities such as walking, jogging, unleashed pets, horseback riding, and off-road vehicles can destroy the western snowy plover's cryptic nests and chicks. These activities can also hinder foraging behavior, cause separation of adults and their chicks, and flush adults off nests and away from chicks, thereby interfering with essential incubation and chick-rearing behaviors. Predation by coyotes, foxes, skunks, ravens, gulls, and raptors has been identified as a major factor limiting

western snowy plover reproductive success at many Pacific coast sites.

Evaluation Results: Compared to the other species considered in this evaluation, the western snowy plover is unique in that little of its overall diet is comprised of aquatic organisms. Although the species lives and nests along coastal and estuarine river beaches, the scientific literature indicates that the bulk of the plover diet comes from larval and adult terrestrial insects (primarily flies and beetles). Due to this dietary characteristic, all the analyses performed in this effort indicate that the TRC should not result in a dietary exposure that would place snowy plovers at risk for adverse effects from methylmercury toxicity (see Tables 5 & 7). Dietary concentration values expected from both of the TL approaches should remain substantially below the plover's calculated WV (0.026 mg/kg). Even when using the alternative reference dose (RfD) generated with the interspecies uncertainty factor (UF_A) of 3, expected DC values remain well below the corresponding lower WV (0.009 mg/kg).

These results must be interpreted with some caution, however, as recent research suggests plovers may be at risk from a unique dietary methylmercury exposure pathway not previously considered in toxicity assessments. Hothem and Powell (2000) collected 68 abandoned or inviable snowy plover eggs from five sites in southern California between 1994 and 1996. Twenty-three of these eggs were analyzed for metals and trace elements. Total mean mercury concentrations in these eggs ranged from 0.078 to 0.19 mg/kg. These values are substantially below accepted lowest observed adverse effects concentrations (LOAEC) for avian eggs, and the authors concluded that concentrations of mercury and other environmental contaminants were not sufficiently elevated in the study eggs to be contributing to population declines. However, snowy plover eggs collected in 2000 from Point Reyes National Seashore in northern California revealed highly elevated mercury concentrations (U.S. Fish and Wildlife Service, unpublished data). Nine failed-to-hatch eggs and two abandoned eggs were collected and analyzed for total mercury. Dry weight concentrations ranged from 0.9 to 12.48 mg/kg, with a mean of 2.56 mg/kg. Adjusted for percent moisture at the time of analysis and moisture loss from the time of laying, the mean fresh wet weight (fww) concentration in the failed and abandoned eggs was reported as 1.07 and 0.27 mg/kg, respectively, with a mean of 0.92 mg/kg for all 11 eggs. The maximum concentration detected from the failed eggs (12.48 mg/kg dry weight) adjusted to 3.1 mg/kg fww. This value is nearly as high as the highest concentration yet detected (3.3 mg/kg fww) in eggs of Fortser's terns, an exclusively piscivorous species, collected from the south San Francisco Bay area (Schwarzbach and Adelsbach, 2002). Mean and maximum concentrations in the failed eggs were substantially above accepted avian egg LOAECs [0.5 mg/kg (Fimreite, 1971); ~0.8 mg/kg (Heinz, 1979)], possibly high enough to account for egg failure through direct toxic effects to plover embryos.

The U.S. Fish and Wildlife Service investigators observed an order of magnitude variation in egg mercury concentrations between the different nests sampled along Point Reyes National Seashore in 2000, with no apparent spatial gradients. As mercury in eggs is thought to closely reflect recent dietary uptake (Walsh, 1990), the Point Reyes data indicated to the investigators that the degree of variation observed reflected a highly heterogenous source of dietary mercury. There

are no known mercury inputs to the coastal beaches used by breeding plovers; however, the investigators noted that an inoperative mercury mine continues to discharge mercury-laden sediments into Tomales Bay, east of the Point Reyes peninsula. Although breeding plovers likely do not forage in Tomales Bay, the investigators suggested that marine mammals foraging in this water body may serve as a mercury pathway into the plover diet. Marine pinnipeds are known to accumulate mercury, usually exhibiting the highest reported tissue concentrations among non-human mammals (Eisler, 2000). As snowy plovers are known to feed on insect larvae that develop on marine mammal carcasses (Page *et al*., 1995), the Point Reyes investigators hypothesized that the elevated plover egg mercury concentrations they observed were the result of localized consumption of invertebrates from pinniped carcasses washed ashore into plover breeding territories. This hypothesis is supported by the fact that at least four marine pinnipeds washed ashore at Point Reyes National Seashore during the 2000 plover breeding season, including a harbor seal carcass that was allowed to decompose on site near the plover nest with the maximum observed egg mercury concentration (Ruhlen and Abbott, 2000).

More work is needed to confirm whether plovers may be exposed to mercury via marine mammal carcasses, and it is not currently possible to incorporate this potential exposure pathway into the methodology developed for this evaluation. To do so would require an analysis of mercury biomagnification from pinniped prey items into the insect larvae developing on pinniped carcasses, information currently unavailable. Even if the hypothesis is confirmed, the mercury levels in Tomales Bay prey biota may already be substantially elevated above the trophic level concentrations expected under the human health TRC, due to the historic and ongoing mercury inputs from the upstream mine. As noted above, the analyses performed for this effort indicate that dietary exposure in snowy plovers should not place them at risk from methylmercury toxicity by either of the TL approaches described. However, given the uncertainties surrounding the potential marine mammal pathway and the plover's sensitive conservation status, applying the Highest TL approach to the TRC would provide the most reasonable assurance of protection.

VIII.G. Bald Eagle

The bald eagle was listed as federally endangered in 1978 (43 Federal Register 6230). The Pacific Bald Eagle Recovery Plan was released in 1986 for the recovery and maintenance of bald eagle populations in the 7-state Pacific recovery region (Idaho, Nevada, California, Oregon, Washington, Montana, and Wyoming) (U.S. Fish and Wildlife Service, 1986). In recent years, the status of bald eagle populations has improved throughout the United States. The bald eagle was downlisted from endangered to threatened on July 12, 1995, throughout the lower 48 states (60 Federal Register 36000). A proposed rule to remove the species from the list of endangered and threatened wildlife was made on July 6, 1999 (64 Federal Register 36454) but this rule has not been finalized. Critical habitat has not been designated for this species. In addition to the Endangered Species Act, the bald eagle is protected under the Migratory Bird Treaty Act of 1918, as amended (16 U.S.C. §§703-712) and the Bald Eagle Protection Act of 1940, as amended (16 U.S.C. §§668-668d).

Life History: The species is long-lived, and individuals do not reach sexual maturity until four or five years of age. Breeding generally occurs February to July (Zeiner *et al*., 1990) but breeding can be initiated as early as January via courtship, pair bonding, and territory establishment. The breeding season normally ends approximately August 31 when the fledglings have begun to disperse from the immediate nest site. One to three eggs are laid in a stick platform nest 50 to 200 feet above the ground and usually below the tree crown (Zeiner *et al*., 1990). Incubation may begin in late February to mid-March, with the nestling period extending to as late as the end of June. From June thru August, the chicks remain restricted to the nest until they are able to move around within their environment.

Nesting territories are normally associated with lakes, reservoirs, rivers, or large streams and are usually within two miles from water bodies that support an adequate food supply (Lehman, 1979; U.S. Fish and Wildlife Service, 1986). Most nesting territories in California occur from 1000 to 6000 feet elevation, but nesting can occur from near sea level to over 7000 feet (Jurek, 1988). The majority of nests in California are located in ponderosa pine and mixed-conifer stands and nest trees are most often ponderosa pine (*Pinus ponderosa*) (Jurek, 1988). Other site characteristics, such as relative tree height, tree diameter, species, position on the surrounding topography, distance from water, and distance from disturbance, also appear to influence nest site selection (Lehman *et al*., 1980; Anthony and Isaacs, 1981). Bald eagles often construct up to five nests within a territory and alternate between them from year to year (U.S. Fish and Wildlife Service, 1986). Nests are often reused and eagles will add new material to a nest each year (DeGraaf *et al*., 1991). Lehman (1979) found that 73 percent of nest sites surveyed were within one-half mile of a waterbody, 87 percent within 1 mile, and 100 percent within 2 miles.

Isolation from disturbances is an important feature of bald eagle wintering habitat. Wintering habitat is associated with open bodies of water, with some of the largest wintering bald eagle populations in the Klamath Basin (Detrich, 1981, 1982). Smaller concentrations of wintering birds are found at most of the larger lakes and man-made reservoirs in the mountainous interior of the northern half of the state and at scattered reservoirs in central and southwestern California. Some of California's breeding birds winter near their nesting territories.

Historic and Current Range: The bald eagle once nested throughout much of North America near coasts, rivers, lakes, and wetlands. The species experienced population declines throughout most of its range, including California, due to exposure to environmental contaminants, habitat loss and degradation, shooting, and other disturbances (Detrich, 1981; Stalmaster *et al*., 1985; U.S. Fish and Wildlife Service, 1986). The species' status has improved since the initial listing under the Endangered Species Act.

The bald eagle continues to be found throughout much of North America and breeds or winters throughout California, except in the desert areas (Zeiner *et al*., 1990; DeGraaf *et al*., 1991). In California, most breeding occurs in Butte, Lake, Lassen, Modoc, Plumas, Shasta, Siskiyou, and Trinity Counties (Zeiner *et al*., 1990). California's breeding population is resident year-long in most areas as the climate is relatively mild (Jurek, 1988). Between mid-October and December,

migratory bald eagles arrive in California from areas north and northeast of the state. The wintering populations remain in California through March or early April.

Rangewide Trends and Current Threats: Though the construction of dams has limited the range of anadromous fish, an important historic bald eagle prey base, reservoir construction and the stocking of fish in reservoirs in the west have provided bald eagles with habitat for population expansion (Detrich, 1981; U.S. Fish and Wildlife Service, 1986). The Califomia bald eagle nesting population has increased in recent years from under 30 occupied territories in 1977 to 151 occupied territories in 1999 (Jurek, 2000). Based upon annual wintering and breeding bird survey data, it is estimated that between 100-300 bald eagles winter on National Forests in the Sierra Nevada, and at least 151-180 pairs remain year-round to breed (U.S. Forest Service, 2000). Most of the breeding population is found in the northern third of the state, primarily on public lands. Seventy percent of nests surveyed in 1979 were located near reservoirs (Lehman, 1979) and this trend has continued, with population increases occurring at several reservoirs since the time of that study.

The Bald Eagle Recovery Plan identifies reasons for the decline of the bald eagle, and states that habitat loss is the most important long-term threat to bald eagle populations. Other threats to the bald eagle include recreational development and human activities affecting the suitability of breeding, wintering, and foraging areas. Bald eagles are susceptible to disturbance by human activity during the breeding season, especially during egg laying and incubation, and such disturbances can lead to nest desertion or disruption of breeding attempts (U.S. Fish and Wildlife Service, 1986). Types of disturbance include recreational activities, fluctuating fish populations and availability of roost trees as a result of reservoir level fluctuations, wild fire, fragmentation of habitat, home sites, campgrounds, mines, timber harvest, and roads. Human activities are more likely to disturb bald eagles when located near roosting, foraging, and nesting areas (Stalmaster and Kaiser, 1998; Stalmaster *et al*., 1985; U.S. Fish and Wildlife Service, 1986).

Evaluation Results: For this effort, a weighted risk approach was taken to determine the appropriate eagle diet for calculation of wildlife values, based on the highest trophic level composition reasonably likely to occur, from the predominant habitat type characteristic of California's breeding bald eagles. In effect, this diet represented the greatest potential for dietary methylmercury exposure in bald eagles. Although alternate diets with higher trophic level compositions could be hypothesized, the diet for this effort was determined using a robust dataset for breeding California eagles.

Results of the analyses performed indicate that applying the human health TRC under the Average Concentration TL Approach is likely to result in dietary exposure that may place bald eagles at risk for adverse effects from methylmercury toxicity. The eagle's dietary concentration (DC) of methylmercury expected from the trophic level concentrations under this approach would be more than 230 percent of the eagle's calculated WV (DC - 0.431 mg/kg, WV - 0.184 mg/kg) (see Table 5). While the extent of any potential adverse effects from this DC cannot be quantified, the degree of WV exceedance suggests a high probability that dietary methylmercury

exposure from the TRC could reach a level at which adverse effects to bald eagles may be expected.

In contrast, the DC expected from the concentrations under the Highest TL Approach (DC - 0.196 mg/kg) would be less than 10 percent above the eagle's WV (see Table 7). Given the small differential between the two values, and a recognition of the various uncertainties and assumptions (*e.g.*, LOAEL-to-NOAEL extrapolation, allometric-derived FIR) inherent in the methodology, it is reasonable to conclude that dietary exposure resulting from applying the TRC under the Highest TL Approach should not place bald eagles at risk for adverse effects from methylmercury toxicity.

IX. EVALUATION RESULTS SUMMARY

IX.A. Average Concentration Trophic Level Approach

Based on the analyses conducted for this evaluation, applying the TRC with the estimated trophic level methylmercury concentrations under the Average Concentration TL Approach may be sufficiently protective for only two of the seven species considered: southern sea otter and Western snowy plover. The five other species examined (California least tern; California, light-footed, and Yuma clapper rails; bald eagle) would likely have dietary exposures under this approach that may place them at risk for adverse effects from methylmercury toxicity. The California clapper rail would not have been considered at risk under this approach if the WV generated with the UF_A of 1 was appropriate to represent the rail's sensitivity to methylmercury toxicity, relative to mallard ducks. However, the parallel evaluation discussed previously demonstrated that the WV generated with the UF_A of 3 was more appropriate for this subspecies, resulting in the conclusion that California clapper rails would also likely have dietary exposures that may place them at risk under this TL approach.

IX.B. Highest Trophic Level Approach

This approach, with its lower estimated trophic level methylmercury concentrations, would provide a greater degree of protection than the prior alternative. Applying the TRC under the Highest TL Approach should be sufficiently protective for four of the seven species considered: southern sea otter, California clapper rail, Western snowy plover, and bald eagle. At this time, no conclusion can be drawn regarding the light-footed clapper rail. If this subspecies' sensitivity to methylmercury is the same as the California clapper rail (*i.e.*, the alternative WV generated with the UF_A of 3 is appropriate), and the analysis of its dietary composition is correct, the light-footed rail would likely have dietary exposures under this approach that may place them at risk. However, if other biological characteristics (*e.g.*, a greater ability to detoxify ingested methylmercury, lower diet-to-egg transfer efficiency) indicate the WV generated with the UF_A of 1 is more appropriate for the light-footed rail, the evaluation results suggest this TL approach should be sufficiently protective for this subspecies. Further research is required to definitively answer these questions. The evaluation for the Yuma clapper rail, regardless of the WV used in

the analysis, indicates this subspecies would likely have a dietary exposure under this approach that may place it at risk for adverse effects from methylmercury toxicity. The same questions surrounding relative sensitivity apply to this subspecies, and research should be initiated to answer these questions and determine appropriate trophic level methylmercury concentrations to provide sufficient protection against toxicity. Finally, although methylmercury concentrations for all three trophic levels are expected to be substantially lower under this approach, the estimated trophic level 3 concentration of 0.075 mg/kg would still not be low enough to remove the potential risk of adverse effects from dietary methylmercury exposure for the California least tern. Because of the tern's small body size and its diet of exclusively trophic level 3 fish, this species may be at an elevated risk from methylmercury toxicity.

X. CONSIDERATION OF OTHER TAXONOMIC GROUPS

As explained previously in this document, the evaluation of the TRC's potential to adversely affect federally listed species in California was conducted with the assumption that upper trophic level wildlife species (*i.e.*, piscivorous or omnivorous birds and mammals) would have the greatest inherent risk from methylmercury exposure, due to methylmercury's propensity to bioaccumulate and biomagnify as it moves upward through aquatic food chains. However, there are numerous other listed species in California to consider (see Appendix) which may be adversely affected by the methylmercury TRC. Once the TRC's protectiveness was evaluated for the upper trophic level birds and mammals, the scientific literature was reviewed to assess whether the methylmercury concentrations expected under each TL approach may be protective for the remaining taxonomic groups.

X.A. Fish

The methodology employed for birds and mammals in this effort was based on an assessment of potential toxicity through ingestion of methylmercury-contaminated fish, shellfish, and other aquatic organisms. For fish, assessment of risk from the TRC was based solely on the potential for adverse effects associated with the tissue methylmercury concentrations expected under each of the TL approaches. It should be noted, however, that muscle tissue-bound concentrations represent the amount of methylmercury sequestered from dietary input over a fish's lifetime. It is possible that levels of circulatory methylmercury, reflective of current dietary exposure, may be responsible for any adverse effects. This possibility is due to the fact that re-mobilization of muscle-bound methylmercury may be negligible unless a reduction in available food necessitates catabolic utilization of muscle-bound proteins. However, until further work on circulatory methylmercury is conducted, muscle tissue concentrations remain the most appropriate indicator for evaluating the impact of the TRC on fish.

A great deal of research has been conducted over the years on the bioaccumulation of mercury by fish, providing data on fish tissue mercury concentrations associated with both overt and subtle toxicological effects (see reviews by: Wiener and Spry, 1996; Jarvinen and Ankley, 1999; Eisler,

2000; Wiener *et al*., 2002). Both Wiener *et al*. (2002) and Eisler (2000) examined the relationships between body burden and toxicological significance in several fish species. All of the overt effects concentrations presented were approximately an order of magnitude above even the highest concentration expected in trophic level 4 fish (0.66 mg/kg) when applying the TRC under the Average Concentration TL Approach.

Wiener *et al*. (2002) stated that, because of the high neurotoxicity of methylmercury, exposure levels causing more subtle adverse behavioral effects are likely much lower than those that would result in overt toxicity. These sublethal neurotoxic effects can impair the ability of fish to locate, capture, and ingest prey and to avoid predators. Unfortunately, studies that demonstrate these effects are generally based on waterborne concentrations of mercury, with few providing data on subsequent fish tissue levels.

Fjeld *et al*. (1998) demonstrated long-term impairment in feeding behavior of grayling (*Thymallus thymallus*) that had been exposed as eggs to waterborne methylmercuric chloride. The 3 year old grayling that exhibited impairment developed from yolk-fry with mercury concentrations as low as 0.27 mg/kg. The yolk-fry concentration of 0.27 mg/kg resulted from eggs in the treatment group exposed to 0.8 ug/L methylmercuric chloride, much higher than environmentally realistic waterborne levels. Compared to the control group, 3 year old fish from the 0.8 ug/L treatment group exhibited a 15 percent reduction in feeding efficiency and a 49 percent reduction in competitive feeding ability.

Based on limited data indicating that mercury concentrations in embryos of methylmercury-exposed brook trout are approximately 20 percent of that in the maternal axial muscle tissue, Fjeld *et al*. (1998) calculated that their lowest observed adverse effects concentration (LOAEC) for grayling yolk-fry (0.27 mg/kg) would translate to a maternal muscle tissue concentration of 1.35 mg/kg. This is double the concentration expected in trophic level 4 fish (0.66 mg/kg) under the Average Concentration TL Approach. Extrapolating a maternal muscle methylmercury concentration from a waterborne-induced embryolarval concentration is tenuous for two reasons: the outermost membrane of fish eggs may retard the uptake of both inorganic and methylmercury from the water column, and maternally-derived egg concentrations may be more associated with dietary intake during egg formation rather than existing muscle-bound concentrations (Latif *et al*, 2001; Hammerschmidt *et al*., 1999). However, Hammerschmidt *et al*. (1999) sampled wild yellow perch (*Perca flavescens*) from four seepage lakes in northern Wisconsin and found that the concentration of total mercury in eggs ranged from 20 to 5 percent of the concentration in the maternal carcass. Using this range of concentration ratios, the embryolarval LOAEC of 0.27 mg/kg could translate to maternal muscle tissue concentrations from 1.35 mg/kg (5:1 adult-egg ratio) to 5.4 mg/kg (20:1 adult-egg ratio).

These data suggest that the adult fish tissue concentrations expected under either trophic level approach would result in egg and embryolarval concentrations substantially below the LOAEC (0.27 mg/kg) reported for grayling. How far below the LOAEC depends on the trophic level approach used and assumptions regarding the adult-egg concentration ratio. By using

conservative assumptions (*i.e.*, 5:1 adult-egg ratio), the tissue concentration expected for trophic level 4 fish (0.66 mg/kg) under the Average Concentration Trophic Level Approach would result in an egg concentration of 0.132 mg/kg, approximately half the grayling LOAEC. Applying the same adult-egg concentration ratio to the tissue concentration expected for trophic level 4 fish (0.3 mg/kg) under the Highest Trophic Level Approach would result in an egg concentration of 0.06 mg/kg, approximately one-fifth the grayling LOAEC. While Fjeld *et al*. (1998) made no conclusions regarding a NOAEC (no observed adverse effects concentration) in their experiment, they did not observe any feeding behavior impairment in their lowest dose treatment group. This treatment group was exposed to a waterborne methylmercury concentration of 0.16 ug/L, and the resulting yolk-fry had a mercury concentration of 0.09 mg/kg wet weight. Although it can be determined with some certainty that the egg mercury concentration (0.06 mg/kg) estimated from the trophic level 4 fish concentration under the Highest Trophic Level Approach would not result in feeding behavior impairments in grayling, the same cannot be said for the egg mercury concentration (0.132 mg/kg) estimated with the Average Concentration Trophic Level Approach. The relative magnitude of effects seen at the 0.27 mg/kg LOAEC for grayling yolk-fry (*i.e.*, 49% reduction in competitive feeding ability) suggests the potential for adverse effects may not be completely removed even when eggs have mercury concentrations around 0.132 mg/kg.

In a more recent study, Webber and Haines (2003) examined the potential for behavioral alterations in fish with environmentally realistic tissue methylmercury concentrations. They concluded that alterations in predator-avoidance behaviors in golden shiners (*Notemigonus crysoleucas*) with environmentally realistic tissue methylmercury concentrations (0.536 mg/kg) may increase vulnerability to predation. Golden shiners should be considered trophic level 3 fish, due to their natural diet of zooplankton and aquatic insects (Moyle, 2002). The effects concentration of 0.536 mg/kg is well above the concentrations expected for trophic level 3 fish under either of the TL approaches evaluated here (0.165 mg/kg - Average Concentration Trophic Level Approach; 0.075 mg/kg - Highest Trophic Level Approach). These data suggest that alterations in predator-avoidance behaviors would not be expected in trophic level 3 fish if the TRC is applied under either approach. Although these data do not allow for any definitive conclusions regarding adult trophic level 4 fish, the possibility that a tissue concentration of 0.536 mg/kg could result in adverse behavioral effects suggests that the more conservative trophic level concentrations expected from the Highest Trophic Level Approach may be warranted in order to ensure adequate protection for federally listed fish species.

In addition to the potential for sublethal neurotoxic effects, Wiener and Spry (1996) concluded that reduced reproductive success in wild fish populations is the most plausible adverse effect expected from environmentally realistic concentrations. They noted that methylmercury can impair reproduction by affecting gonadal development or spawning success in adult fish, or by reducing egg hatching success and embryolarval health and survival. Mercury concentrations affecting both hatching success and embryolarval health are directly linked to the adult female body burden (circulatory and/or muscle-bound concentrations), as the majority of mercury in developing eggs is methylmercury derived through maternal transfer (Wiener *et al*., 2002). However, only a small fraction of the total muscle-bound methylmercury is transferred to the egg

mass and eliminated during spawning (Wiener *et al*., 2002; Hammerschmidt *et al*., 1999). Several key studies on mercury and reproductive endpoints are discussed below.

Birge *et al*. (1979) describe the results of two experiments involving embryolarval stage rainbow trout (*Salmo gairdneri*) exposed to waterborne inorganic mercury. In one study, trout eggs exposed to approximately 100 ng/L exhibited reduced survival after four days, with 100 percent mortality after eight days (at approximately 200 - 300 ng/L). After days four and seven of the experiment, mercury content of the eggs was approximately 0.068 and 0.097 mg/kg, respectively. In a second study, trout eggs were placed in aquaria with mercury-enriched sediment and clean water. There was a 28 percent reduction in hatching success and a 49 percent reduction in 10-day survival with a sediment mercury concentration of approximately 1.05 mg/kg. In this treatment group, mercury in the water column was approximately 150 ng/L, and tissues from the hatched larvae contained approximately 0.041 mg/kg.

Both of the above experiments demonstrated substantial adverse effects at low embryolarval inorganic mercury concentrations. If the adult-egg concentration ratios from the previous discussion on grayling (Fjeld *et al*., 1998) were applied to these inorganic mercury concentrations in embryolarval rainbow trout (*e.g.*, 0.04 mg/kg larval concentration and 5:1 adult-egg ratio), adult muscle tissue concentrations as low as 0.2 mg/kg could be associated with severe reproductive effects. However, the adult-egg ratios are based on maternal transfer of accumulated mercury, which is predominantly methylmercury in both the adult tissue and the developing eggs (Wiener *et al*., 2002). The mechanisms of mercury bioaccumulation and maternal transfer prevent a reliable extrapolation of adult fish tissue methylmercury concentrations from concentrations of inorganic mercury in eggs or larvae. In addition, the waterborne concentrations of inorganic mercury (100 - 150 ng/L) used to achieve the observed effects concentrations in embryolarval rainbow trout are substantially above all but the most highly polluted natural waters (Wiener and Spry, 1996). These high waterborne concentrations necessary to see adverse effects in eggs may be due to the apparent ability of the outermost membrane on fertilized fish eggs to retard the uptake of both inorganic and methylmercury from the surrounding water column into the developing embryo (Hammerschmidt *et al*., 1999). In order to accurately assess adult fish muscle tissue levels associated with embryolarval effects, the effects should be related to maternally-derived methylmercury concentrations.

Matta *et al*. (2001) examined the effects of dietary methylmercury on reproduction and survival in three generations of mummichogs (*Fundulus heteroclitus*). Treatment groups were fed methylmercuric chloride-contaminated fish food until four target tissue concentrations were reached (0.2, 0.5, 1.0, and 11.0 mg/kg). Although adverse reproductive effects were observed in this study, they were only manifested in F_1 generation offspring of the treatment group containing tissue methylmercury concentrations of 11 and 12 mg/kg in males and females, respectively. These values are substantially higher than any of the trophic level concentrations expected with the TRC. Of greater importance from this study are the data indicating a significant increase in male mortality in the 0.5 mg/kg tissue concentration treatment group. Survival was somewhat reduced in the 0.2 mg/kg treatment group, but not significantly. However, the almost 50 percent

reduction in the 0.5 mg/kg group indicates significant mortality may occur at concentrations between 0.2 and 0.5 mg/kg. The mummichog is a trophic level 3 fish from the eastern seaboard, similar to the California killifish (*Fundulus parvipinnis*). Although the tissue concentrations associated with increased male mortality from this study (0.2 - 0.5 mg/kg) are considerably higher than the TL3 concentration (0.075 mg/kg) expected by applying the TRC under the Highest Trophic Level Approach, they are close to the TL3 concentration (0.165 mg/kg) expected under the Average Concentration Trophic Level Approach.

The influence of mercury exposure on more subtle reproductive parameters in natural settings was examined by Friedmann *et al*. (1996a). Two indices of gonadal function, gonadosomatic index (GSI) and gonadal sex steroid levels, were measured in northern pike (*Esox lucius*) collected from Lake Champlain, New York and Vermont, in 1994. Northern pike were selected because they are trophic level 4 fish, with a greater degree of mercury bioaccumulation than lower trophic level fish. The GSI was determined by the ratio of gonadal weight to total body weight. The mean total mercury concentration in muscle from the 14 fish sampled was 0.325 mg/kg (range: 0.117 - 0.623 mg/kg). The means for males (n = 7) and females (n = 7) were 0.347 and 0.303 mg/kg, respectively. The researchers found no significant correlation between mercury content, GSI, and gonadal sex steroids, suggesting that mercury exposure in natural settings might not exert as dramatic an effect on teleost fish reproduction as indicated by earlier laboratory findings. However, the researchers raised the possibility that the mercury levels they observed might have a more subtle influence on reproductive physiology which could be detected given a larger sample size.

To evaluate this possibility, the same researchers (Friedmann *et al*., 1996b) conducted a dietary methylmercury feeding experiment with juvenile walleye (*Stizstedion vitreum*). After six months of dietary exposure, fish in the low- and high-mercury diet groups had mean total mercury tissue concentrations of 0.254 and 2.37 mg/kg, respectively. The results for the low-mercury diet group are most relevant to this TRC analysis, as the mercury concentration in the test fish (0.254 mg/kg) is of the same magnitude as the concentrations expected in trophic level 4 fish under either trophic level approach. No significant differences from controls were seen in this low-mercury group for growth and mortality rates. The mean GSIs of male and female fish from both dietary groups were lower than in fish from the control group, but the differences were not statistically significant in the analysis of variance (ANOVA). However, when combining data from the two dietary groups, the mean GSI of male fish fed either mercury-contaminated diet was significantly lower than in males fed the control diet. Also, male fish in both groups exhibited varying degrees of testicular atrophy, greater in the high-mercury group. Mean GSIs for female fish in either treatment group were not significantly different from controls. Levels of plasma cortisol, which is important for stress response and immune function in teleost fish, were significantly lower in low-mercury fish than in control group fish. The above findings suggested to the authors that methylmercury at environmentally realistic fish tissue levels (0.254 mg/kg) may adversely affect reproductive success by impairing testicular development in young teleost fish and may reduce juvenile survival by impairing immune function.

However, in another study examining growth and reproductive endpoints in wild populations of mercury-contaminated fish, Friedmann *et al*. (2002) presented conflicting conclusions. Fifty-two male largemouth bass (*Micropterus salmoides*) were collected from three New Jersey water bodies of varying mercury contamination. Mean total mercury concentrations in muscle tissue were 0.30 mg/kg (Assunpink Lake), 1.23 mg/kg (Manasquan Reservoir), and 5.42 mg/kg (Atlantic City Reservoir). No significant differences between the three lakes were found for body weight, length, condition factor, or GSI. Also, no significant relationship was found between muscle mercury content and adrenocortical function, indicated by interrenal nuclear diameter and serum cortisol levels following stress. Liver somatic index (LSI) was significantly lower in fish from the Atlantic City Reservoir compared to the other two lakes, but this reduction could not be definitively correlated with mercury concentrations. The elevated mercury levels in fish from the Atlantic City Reservoir may have altered androgen profiles, as evidenced by greater levels of serum 11-ketotestosterone, but no cause-effect relationship could be established. Based on the above findings, the authors concluded that elevated mercury levels in fish (*i.e.*, as high as 5.42 mg/kg) do not substantially decrease indicators of general and reproductive health (*i.e.*, GSI). This finding is in contrast to the previous dietary mercury study with juvenile walleye which indicated that an even lower muscle concentration (2.37 mg/kg) was associated with impaired gonadal development (Friedmann *et al*., 1996b). As an explanation for this apparent discrepancy, Friedmann *et al*. (2002) pointed to findings that wild fish populations exposed to toxicants in their environment can develop adaptations that allow them to live in more polluted sites than are predicted with laboratory models. In further support of this explanation, the authors cite the observation by Friedmann *et al*. (1996a) that a correlation between muscle mercury content and reduced GSI did not exist in Lake Champlain northern pike.

Latif *et al*. (2001) collected female walleye during two successive spawning seasons from one mercury-contaminated lake and two relatively pristine lakes in Canada. Mean total mercury concentrations in muscle tissue, in mg/kg, were 0.182 (Lake Winnipeg), 0.194 (Lake Manitoba), and 2.701 (Clay Lake). Mean methylmercury concentrations in eggs (mg/kg), converted from reported dry weight concentrations assuming an 85 percent moisture content, were approximately 0.001 (Lake Manitoba), 0.002 (Lake Winnipeg), and 0.148 (Clay Lake). In addition to any maternally transferred methylmercury, eggs and subsequent larvae were then exposed to varying concentrations of waterborne methylmercury. The experimental results demonstrated a significant decline in hatching success and embryonic heart rate with increasing exposures of waterborne methylmercury, for all three lake stocks. However, after statistically adjusting for waterborne methylmercury effects, the maternally transferred methylmercury in eggs was not significantly correlated with either hatching success or embryonic heart rate. The authors noted that hatching success in eggs from Clay Lake females declined with increasing egg methylmercury concentrations, although the trend was not significant, and suggested that a larger sample size may reveal statistically significant declines. For the purposes of this evaluation, the data from this study indicate that fish tissue methylmercury concentrations in trophic level 4 fish (0.182, 0.194 mg/kg) similar to those expected with the TRC should not result in maternally deposited egg concentrations associated with reduced hatching success.

The effects of dietary methylmercury on multiple reproductive endpoints was also examined by Hammerschmidt *et al*. (2002). Using fathead minnows (*Pimephales promelas*), the researchers measured gonadal development of males and females, spawning success, days to spawning, reproductive effort of females, developmental success of embryos, hatching success of embryos, survival of larvae, and growth of larvae. No reductions in growth or survival were seen in adult fish from any of the treatment groups, regardless of the tissue concentrations. Developmental and hatching success of embryos were not measurably affected by mercury concentrations in either the diets or bodies of parental fish. Similarly, larval survival and growth were not correlated with dietary or tissue methylmercury concentrations. However, in one of the treatment groups, female fish fed the same diet during Phases 1 and 2 (continuous exposure) exhibited reduced gonadal development (based on GSI) with increasing body burden mercury concentrations. No threshold for this effect was presented, but the whole body tissue concentration from the low dose group was approximately 0.68 mg/kg in females (converted from reported dry weights assuming 80% moisture in whole body). The reduced GSI in these fish led to lower egg production (average daily number of eggs laid per gram of female carcass) with increasing mercury concentrations in the adult tissues. Fish fed the same diet during Phases 1 and 2 also exhibited reduced spawning success compared to fish fed the control diet. Male and female fish fed the low dose diet showed an average tissue concentration of 0.625 mg/kg, and had a spawning success rate of only 46 percent. Fish fed the control diet had an average tissue concentration of 0.08 mg/kg, and had a spawning success rate of 75 percent. In fish fed the continuous exposure diets, the number of days to spawning increased with increasing tissue mercury concentrations. In females, days to spawning was also inversely related to gonadal development.

The tissue concentrations in fish fed the low dose diet (average 0.625 mg/kg) during Phases 1 and 2 were substantially above the levels expected for trophic level 3 fish when applying the TRC under either trophic level approach. However, the 0.625 mg/kg average value is similar to the concentration expected in trophic level 4 fish (0.66 mg/kg) under the Average Concentration Trophic Level Approach. Based on the fathead minnow findings described above, Hammerschmidt *et al*. (2002) concluded that methylmercury decreased reproduction in adult fathead minnows at dietary concentrations realistically encountered by predatory fishes in mercury contaminated waters, with the implication that exposed fish populations could be adversely affected by this reproductive impairment.

None of the data examined for this evaluation provided definitive answers regarding the level of protection for fish afforded by the TRC. The trophic level methylmercury concentrations expected from applying the TRC under both trophic level approaches appear to be well below observed adverse effects concentrations described in the scientific literature. However, the trophic level concentrations expected under the Average Concentration Trophic Level Approach, which are higher than those under the Highest Trophic Level Approach, are much closer to these adverse effects concentrations. Although the best currently available data suggest that the TRC would be sufficiently protective of listed fish, regardless of the trophic level approach used, the increasing emphasis on examining more subtle methylmercury-induced effects may reveal even

lower tissue-based threshold effects concentrations.

X.B. Reptiles and Amphibians

Evaluating the TRC with respect to reptile and amphibian species was more problematic than the evaluation for fish, birds, and mammals. The TRC was developed as a methylmercury limit in the edible tissues of fish and shellfish. The protectiveness of the TRC could then be evaluated for fish, based on toxicity associated with various fish tissue concentrations, or for piscivorous and omnivorous birds and mammals, based on the ingestion of methylmercury contaminated organisms. An evaluation for reptiles and amphibians can be based on ingestion if the species of concern feeds primarily on aquatic organisms and if there are sufficient data to establish reference doses, food ingestion rates, and dietary composition. If these species of concern do not feed on aquatic organisms, a risk assessment based solely on toxicity endpoints associated with known tissue mercury concentrations may be performed. However, this type of assessment cannot be used to evaluate the TRC, as there is currently no reliable way to compare tissue mercury concentrations in reptiles and amphibians with the various trophic level fish tissue concentrations expected from the two approaches. Too little is presently known about mercury bioaccumulation in reptiles and amphibians to allow for any comparative risk prediction capability based on bioaccumulation in fish. The majority of the information presented below on the ecotoxicology of metals in reptiles and amphibians is from a comprehensive review by Linder and Grillitsch (2000).

No reptile mortality due to metal intoxication has ever been reported (Linder and Grillitsch, 2000); however, relevant ecotoxicological data on the effects of mercury on reptiles is severely lacking. Of the available studies, most have focused on tissue metal concentrations in free-ranging animals without reference to the ambient conditions giving rise to those concentrations. However, studies showing the highest tissue levels of mercury and other metals were associated with areas apparently having a high degree of environmental contamination. Linder and Grillitsch (2000) reported that only a few studies examined laboratory exposure to a defined dose, and none of these involved mercury. In a later review, Campbell and Campbell (2001) reviewed 20 studies examining inorganic contaminants and snakes, and found one (Hopkins *et al*., 1999) that examined effects concentrations. Unfortunately, neither the Hopkins *et al*.(1999) study nor the follow-up study examining the effects of chronic dietary exposure to trace inorganic elements (Hopkins *et al*., 2002) involved mercury. The remaining 19 studies reviewed by Campbell and Campbell (2001) only examined mercury concentrations in snake tissues, with no connection to exposure or effects. Linder and Grillitsch (2000) found that the available data indicate reptiles in general do not biomagnify metals to an extent that would correspond to their trophic level. In one study comparing whole body mercury concentrations in biota from several trophic levels, Winger *et al*. (1984) reported mercury levels corresponding to trophic level, being consistently highest in water snakes (*Natrix* spp.) and little green herons (*Butorides virescens*). However, mercury levels in the garter snake (*Thamnophis sirtalis*) were among the lowest of several vertebrate species examined, with the highest levels in piscivorous birds (Dustman *et al*, 1972). Linder and Grillitsch (2000) also reported that the available literature appears to support

the hypothesis that reptiles exhibit a generally low sensitivity to metals. However, these authors caution against drawing definitive conclusions regarding reptiles and metal contaminants, due to the almost complete absence of toxicological research under fairly defined experimental conditions, and the absence of any information on embryotoxic potential.

The dietary habits of both snakes considered in this evaluation [San Francisco garter snake (*Thamnophis sirtalis tetrataenia*) and giant garter snake (*Thamnophis gigas*)] indicate a strong dependence on aquatic ecosystems. The San Francisco garter snake is known to prey on red-legged frogs (*Rana aurora*), Pacific tree frogs (*Hyla regilla*), California newts (*Taricha torosa*), western toads (*Bufo boreas*), threespine stickleback (*Gasterosteus aculeatus*), and mosquitofish (*Gambusia affinis*) (U.S. Fish and Wildlife Service, 1985c). Known prey items of the giant garter snake include mosquitofish, common carp (*Cyprinus carpio*), Sacramento blackfish (*Orthodon microlepidouts*), and bullfrogs (*Rana catesbiana*) (U.S. Fish and Wildlife Service, 1999). It is reasonable to assume these snakes may also prey on other available fish and frog species.

These dietary habits clearly indicate that both snakes may be exposed to methylmercury through ingestion of fish and other aquatic-dependent prey. However, evaluating the effect of the TRC on these snakes based on ingestion of methylmercury contaminated prey is confounded by the lack of necessary data. Although it is possible to estimate a daily food ingestion rate for snakes from Nagy (2001) and to make assumptions regarding the trophic level composition of the diet, the existing toxicological data on snakes do not allow for determination of any reference dose. Without a scientifically determined effects concentration in snakes, no WVs can be generated. While the physiological similarities between birds and reptiles may suggest it is possible to take the avian test dose used in this effort, make certain assumptions regarding inter-taxonomic uncertainty, and then arrive at some reference dose and WVs for these snakes, any conclusions drawn from the subsequent evaluation of the TRC would be highly speculative. The combination of reptilian physiological and life history characteristics (*e.g.*, long life span, small home ranges, high trophic position, and ectothermic physiology) make such an extrapolation inappropriate (Hopkins *et al*., 2002). Nagy (2001) points out that the metabolic rate of reptiles results in daily food requirements drastically lower than both birds and mammals. A 1-kg reptile consumes only 9 percent of the amount eaten by a 1-kg bird and approximately 12 percent of the amount a 1-kg mammal requires. If snakes are no more sensitive to ingested methylmercury than are birds (*i.e.*, having the same reference dose), then the lower daily food ingestion rate resulting from the snake's metabolic needs might suggest that fish tissue methylmercury levels that are protective of birds should also be protective of snakes. Although the limited ecotoxicological data presented above may suggest that reptiles in general are less sensitive to methylmercury than other taxa, no definitive conclusions can be made regarding the protectiveness of the TRC for these species until dietary methylmercury effects concentrations can be established for snakes.

The toxicity of mercury has been studied to a much greater extent with amphibians than with reptiles. Most amphibian species have aquatic-dependent early life stages where exposure may be dominated by direct uptake of dissolved metals from water, while exposure through dietary

sources may become more predominant in the subsequent adult life stages (Linder and Grillitsch, 2000). The majority of available effects data for amphibians come from acute and chronic toxicity studies with early life stages of frogs, using waterborne concentrations of inorganic mercury (Linder and Grillitsch, 2000; U.S. Environmental Protection Agency, 1996; Birge *et al*., 1979). Lethality is the toxicological endpoint most commonly assessed in these studies, with the majority of embryo or larval LC50s (lethal concentration for 50% of test population) in the range of 10 - 100 ug/L (Linder and Grillitsch, 2000). It should be noted that several LC50s below 10 ug/L and above 100 ug/L have also been observed (Linder and Grillitsch, 2000; U.S. Environmental Protection Agency, 1996). Concentrations as low as 0.1 ug/L have resulted in up to 6 percent mortality of leopard frog (*Rana pipiens*) embryos (U.S. Environmental Protection Agency, 1996). Embryonic malformation is another commonly measured endpoint in mercury toxicity studies. Waterborne mercury concentrations associated with amphibian embryo malformations ranged from 2 - 75 ug mercuric chloride/L, with malformation rates ranging from 5 to greater than 10 percent (Birge *et al*., 1983).

Adverse effects have also been reported for amphibians exposed to methylmercuric chloride (U.S. Environmental Protection Agency, 1996). Concentrations of methylmercuric chloride between 0 - 4 ug/L resulted in an EC50 (effects concentration for 50% of test population) for embryo deformities in leopard frogs. No metamorphosis was seen in leopard frog tadpoles exposed to concentrations between 1 - 10 ug/L for 3 to 4 months. Greater than 10 percent deformity and mortality was observed in larvae of the African clawed frog (*Xenopus laevis*) exposed to 0.3 ug/L for more than 10 days.

Based on the limited data available, it appears that the early life stages of amphibians are the most sensitive to metal exposures (Linder and Grillitsch, 2000). All of the waterborne effects concentrations for mercury reported above are considerably higher than environmentally realistic levels. Although there will likely be a great deal of variation between water bodies within California, the waterborne concentrations of mercury associated with the TRC should be orders of magnitude below any of the effects concentrations described here. However, these water concentration toxicity data are insufficient to fully characterize risk from the TRC as they do not take into account dietary exposure in post-embryolarval stages or the potential for maternal transfer of bioaccumulated methylmercury into the eggs. Preliminary results from designed studies suggest that metals bioaccumulated into female amphibians may be depurated during egg development and laying (Linder and Grillitsch, 2000). This process, in combination with exposure through waterborne concentrations, could be toxicologically relevant for the embryolarval stages of amphibians.

Due to methylmercury's propensity to bioaccumulate throughout the lifetime of an animal that is dependent on the aquatic food chain, adverse effects in adult life stages may be possible from relatively low prey concentrations. Unfortunately, the effects of dietary exposure to methylmercury in later life stages of amphibians have not been adequately explored. The literature on the bioaccumulation of metals in amphibians is less developed than for reptiles, with only a few controlled experiments examining bioaccumulation from dietary sources (Linder and

Grillitsch, 2000). No data were found in the scientific literature specifically regarding mercury bioaccumulation in frogs, the only amphibian taxon considered in this evaluation of the TRC. However, the limited data on the uptake of metals by amphibians suggest that the bioaccumulation of methylmercury may be an important exposure pathway for frogs.

The single amphibian considered in this evaluation, the California red-legged frog (*Rana aurora draytonii*), feeds as an adult on both invertebrates and vertebrates. Vertebrate prey, such as the Pacific tree frog (*Hyla regilla*) and California mouse (*Peromyscus californicus*), can account for over half of the dietary biomass in large adults (U.S. Fish and Wildlife Service, 2002). It is not known how much of the frog's diet may be comprised of aquatic invertebrates, or whether small fish are ever consumed. The consumption of Pacific tree frogs may constitute an important methylmercury exposure pathway, if they are closely linked with a contaminated aquatic environment.

As discussed previously, the impact of the TRC can only be reliably evaluated for non-fish organisms if they feed on aquatic prey (*i.e.*, fish or aquatic invertebrates) and if there are sufficient data to determine an appropriate dietary test dose at which adverse effects in the organisms are observed. Although California red-legged frogs may consume substantial numbers of aquatic prey, the literature on amphibian ecotoxicology revealed no information indicating that any research has been done involving the effects of dietary exposure to mercury in amphibians (Linder and Grillitsch, 2000; U.S. Environmental Protection Agency, 1996; Birge *et al*., 1979). This lack of data eliminates the possibility of evaluating the TRC for red-legged frogs using a methylmercury ingestion approach.

The methodology used in this evaluation of the TRC is based on the assumption that upper trophic level wildlife species (*i.e.*, piscivorous and omnivorous birds and mammals) have the greatest inherent risk from exposure to methylmercury. No currently available information was found to contradict this assumption, although an increasing emphasis on ecotoxicological research with reptiles and amphibians may provide new data with which to compare these inter-taxonomic sensitivities. Consumption of aquatic organisms by the California red-legged frog and the two species of garter snakes may expose them to toxicologically relevant concentrations of methylmercury, although possibly less so than in those species (*e.g.*, piscivorous birds and mammals) with a greater daily dietary reliance on aquatic prey. The available scientific literature strongly suggests that both reptiles and amphibians can bioaccumulate methylmercury, although the degree to which this occurs has not been fully characterized. However, until the appropriate toxicological data are generated, no definitive conclusions can be drawn about the protectiveness of either TRC trophic level approach for the California red-legged frog, San Francisco garter snake, or giant garter snake.

XI. DISCUSSION

As explained previously, the objective of this effort was to evaluate whether promulgation of the EPA's human health criterion for methylmercury may affect any federally listed threatened or endangered species in California. To do this, a risk assessment methodology was developed and used to analyze the potential effect of the TRC on several of these listed species. The species selected for analysis were presumed to be at the greatest risk of dietary exposure, due to their high trophic position and/or dietary dependence on the aquatic ecosystem. The results of these analyses indicate that some of these species should be sufficiently protected against adverse effects from methylmercury toxicity, depending on the trophic level approach evaluated. For other species, the evaluation results suggest that the TRC may not be adequate to protect against adverse effects.

Risk assessments such as the one used in this effort are designed to gauge the *potential* for adverse effects. The WVs calculated in this document are assumed to represent protective dietary concentrations of methylmercury, at which no adverse effects are expected. Then, if the predicted DC value for any given species is at or below the corresponding WV, it may be concluded with reasonable confidence that adverse effects to that species are not likely to occur. In contrast, a DC value higher than the corresponding WV only results in a presumption of risk for adverse effects. This is due to the fact that WVs are derived from toxicity data for surrogate species, with various assumptions about interspecific sensitivities, dietary composition of the species of concern, and the use of uncertainty factors to estimate a dose at which no adverse effects should occur. Therefore, any presumption of risk for a species can only be definitively confirmed or dismissed by available scientific evidence that serves to remove these layers of uncertainty.

The Service's Environmental Contaminants Division believes the analyses presented in this document represent the most current state of knowledge regarding the risk to California's listed species from dietary methylmercury. The mammalian and avian test doses used in this effort, which serve as the toxicological foundation for this methodology, remain the best available benchmarks of effects concentrations for these taxonomic groups. Uncertainty factors have previously been applied to these test doses, initially for the GLI and then updated for the MSRC (U.S. Environmental Protection Agency, 1995d; 1997a, respectively), to establish reference doses for key piscivorous wildlife species at which no adverse effects would be expected. To date, no new evidence has been presented suggesting that the uncertainty factors used for this evaluation should be altered to establish higher reference doses for any of the species considered. In several cases, the dietary compositions used in species evaluations were based on limited empirical data; however, until new data are generated, these compositions remain the most reliable estimates. Finally, future controlled methylmercury dosing experiments with individuals of the species evaluated could potentially yield more accurate reference doses (*i.e.*, NOAELs); however, any such experiments are highly unlikely due to the regulatory status of these species as threatened or endangered.

For the reasons cited above, we believe the presumption of risk for certain species indicated by the results of our evaluation cannot presently be dismissed by the available scientific evidence. Those species for which the predicted DCs are significantly above the corresponding WVs (*i.e.*, >10% higher) would be considered at risk for adverse effects from methylmercury toxicity. Conclusions about the protectiveness of the TRC for each species, under both trophic level approaches evaluated, are summarized below in Table 8. These conclusions reflect the interpretation of the evaluation results by the Service's Environmental Contaminants Division only, and are not intended to represent the views of those EPA or Service scientists who helped develop the risk assessment methodology. In addition, these conclusions do not constitute the results of consultation under Section 7 of the ESA.

Table 8. Protectiveness of Tissue Residue Criterion for Seven California Species

Is the TRC Protective for...	Southern Sea Otter	Ca. Least Tern	Ca. Clapper Rail	Light-footed Clapper Rail	Yuma Clapper Rail	Western Snowy Plover	Bald Eagle
Under Average Concentration TL Approach?	Yes	**No**	Yes	**No**	**No**	Yes	**No**
- with Alternate WV Generated from UF_A of 3?	na	na	**No**	**No**	**No**	Yes	na
Under Highest TL Approach?	Yes	**No**	Yes	Yes	**No**	Yes	Yes
- with Alternate WV Generated from UF_A of 3?	na	na	Yes	**No**	**No**	Yes	na

Applying the TRC under the Average Concentration Trophic Level Approach would place five of the seven listed species at risk for adverse effects: California least tern; California, light-footed, and Yuma clapper rails; bald eagle. Only the southern sea otter and western snowy plover would be sufficiently protected under this approach. Applying the TRC under the Highest Trophic Level Approach would place two of the seven species, California least tern and Yuma clapper rail, at risk for adverse effects. The southern sea otter, California clapper rail, western snowy plover, and bald eagle should be sufficiently protected under this approach. No conclusions can be drawn at this time regarding the light-footed clapper rail, due to remaining uncertainty about this subspecies' sensitivity to methylmercury.

The two species determined to still be at risk under the Highest Trophic Level Approach are the California least tern and the Yuma clapper rail. As explained previously in this document, the methodology outlined in the Average Concentration Trophic Level Approach can be used to calculate the trophic level-specific methylmercury concentrations necessary to maintain any species' DC at or below its calculated WV. Using Equation 3 from this methodology and substituting any WV for the DC term, we can solve for the methylmercury concentration in trophic level 2 prey:

FDTL2 = WV / [(%TL2) + (%TL3 × MTL3) + (%TL4 × MTL3 × MTL4)]

Once the trophic level 2 concentration is calculated, the remaining trophic levels can be determined using our established food chain multiplier relationships:

FDTL3 = FDTL2 × MTL3
FDTL4 = FDTL3 × MTL4

Using the WVs determined for the least tern and Yuma clapper rail, along with the trophic level composition of their diets, the trophic level methylmercury concentrations required to maintain these WVs can be calculated (Table 9).

Table 9. Trophic Level Methylmercury Concentrations Calculated for California Least Tern and Yuma Clapper Rail

	California Least Tern (WV = 0.030 mg/kg)	Yuma Clapper Rail (WV generated with UF_A of 1 = 0.040 mg/kg)	Yuma Clapper Rail (WV generated with UF_A of 3 = 0.013 mg/kg)
FDTL2	0.005 mg/kg	0.009 mg/kg	0.003 mg/kg
FDTL3	0.030 mg/kg	0.053 mg/kg	0.017 mg/kg
FDTL4	0.120 mg/kg	0.210 mg/kg	0.068 mg/kg

Of the two approaches evaluated, the Highest Trophic Level Approach affords a greater degree of protection for California's listed bird and mammal species than the Average Concentration Trophic Level Approach. As stated previously, the best currently available data on mercury toxicity in fish suggest that the TRC under either approach should be sufficiently protective of all listed fish in California; however, the trophic level concentrations expected under the Average Concentration Trophic Level Approach would be much closer to observed adverse effects concentrations described in the scientific literature. Finally, although a lack of relevant data precludes any conclusions regarding the potential impact of the TRC on the reptile and amphibian species considered, the lower trophic level concentrations expected under the Highest Trophic Level Approach would afford a greater measure of protection than those expected under

the Average Concentration Trophic Level Approach. Based on the above conclusions, we believe that the TRC would not adequately protect all listed species in California; however, applying the TRC under the Highest Trophic Level Approach would reduce the number of species at risk.

Finally, it must be noted that the risk assessment methodology presented in this document was not applied to any wildlife species other than the federally listed species from the Appendix. However, other non-listed wildlife may be potentially at risk under the TRC, due to their dietary dependence on aquatic ecosystems. Using the same approach followed in this effort, regulatory agencies should be able to determine whether concentrations of methylmercury in fish tissue under the TRC may also pose a risk to these non-listed wildlife species.

XII. REFERENCES

XII.A. LITERATURE CITED

Abt Associates, Inc. 1995. Review and analysis of toxicity data to support the development of uncertainty factors for use in estimating risks of contaminant stressors to wildlife. Bethesda, Maryland. Prepared for the U.S. Environmental Protection Agency, Office of Water. Washington, DC EPA Contract No. 68-C3-0332.

Albertson, J.D. 1995. Ecology of the California clapper rail in South San Francisco Bay. M.A. Thesis, San Francisco State University.

Allen, A. 1934. The season: San Francisco region. Bird-Lore. 36:316.

American Ornithologists' Union. 1957. Check-List of North American Birds, 5th ed. American Ornithologists' Union, Washington, D.C.

Anderson, B.W. and R.D. Ohmart. 1985. Habitat use by Clapper Rails in the Lower Colorado River Valley. Condor. 87:116-126.

Anthony, R.G., and F.B. Isaacs. 1981. Characteristics of bald eagle nest sites in Oregon. J. Wildl. Manage. 53(1):148-159.

Atwater, B., S. Conrad, J. Dowden, C. Hedel, R. MacDonald, and W. Savage. 1979. History, landforms, and vegetation of the estuary's tidal marshes. *In* T.J. Conomos (ed.): San Francisco Bay, the urbanized estuary. Pacific Div., Am. Assoc. for the Adv. of Sci. 58th annual mtg., S.F. State Univ., June 12-16, 1977.

Atwood, J.L. and D.E. Minsky. 1983. Least tern foraging ecology at three major California breeding colonies. Western Birds. 14:57-72.

Atwood, J.L. and P.R. Kelly. 1984. Fish dropped on breeding colonies as indicators of least tern food habits. Wilson Bulletin. 96(1):34-47.

Austin, J.E. and M.R. Miller. 1995. Northern Pintail (*Anas acuta*). *In* The Birds of North America, No. 163 (A. Poole and F. Gill, eds.) The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C. 32 pp.

Barnes, R.D. 1980. Invertebrate Zoology 4th Ed. Saunders College Publishing, Philadelphia, PA. 1050 pp.

Barr, J.F. 1986. Population dynamics of the common loon (*Gavia immer*) associated with mercury-contaminated waters in northwestern Ontario. Canadian Wildlife Service. Occasional Paper, No. 56, 23 pp.

Bennett, W.W. and R.D. Ohmart. 1978. Habitat Requirements and Population Characteristics of the Clapper Rail (*Rallus longirostris yumanensis*) in the Imperial Valley of California. A report submitted to the Univ. of California Lawrence Livermore Laboratory.

Bent, A.C. 1921. Life histories of North American gulls and terns. U.S. Natl. Mus. Bull. 113.

Birge, W.J., J.A. Black, A.G. Westerman, and J.E. Hudson. 1979. The effects of mercury on reproduction of fish and amphibians, Chapter 23 *in* J.O. Nriagu (ed.) The Biogeochemistry of Mercury in the Environment. Elsevier Press, New York. pp. 629-655.

Birge, W.J., J.A. Black, A.G. Westerman, and B.A. Ramey. 1983. Fish and amphibian embryos: a model system for evaluating teratogenicity. Fundamentals of Applied Toxicology. 3:237-242.

Boening, D.W. 2000. Ecological effects, transport, and fate of mercury: a general review. Chemosphere. 40:1335-1351.

Bouton, S.N., P.C. Frederick, M.G. Spalding, and H. McGill. 1999. Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. Environmental Toxicology and Chemistry. 18(9):1934-1939.

Breteler, R.J., I. Valiela, and J.M. Teal. 1981. Bioavailability of mercury in several north-eastern U.S. *Spartina* ecosystems. Estuarine, Coastal, and Shelf Science. 12:155-166.

Brisbin, I.L., Jr. and T.B. Mowbray. 2002. American Coot (*Fulica americana*) and Hawaiian Coot (*Fulica alai*). *In* The Birds of North America, No. 697 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia, PA 44 pp.

Buehler, D.A. 2000. Bald Eagle (*Haliaeetus leucocephalus*). *In* The Birds of North America, No. 506 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia, PA. 40 pp.

Buchanan, G.A., D.W. Russell,, and D.A. Thomas. 2001. Derivation of New Jersey-specific wildlife values as surface water quality criteria for: PCBs, DDT, Mercury. Multi-agency report by: U.S. Fish and Wildlife Service, Pleasantville, NJ; U.S. Environmental Protection Agency, Edison, NJ; New Jersey Department of Environmental Protection, Trenton, NJ. 36 pp.

Burger, J. and M. Gochfeld. 1997. Risk, mercury levels, and birds: Relating adverse laboratory effects to field biomonitoring. Environmental Research. 75:160-172.

California Department of Fish and Game. 1976. A proposal for sea otter protection and research and request for the return of management to the State of California (DRAFT). 255 pp.+ appendices.

_____. 1990. 1989 Annual Report on the Status of California's Listed Threatened and Endangered Plants and Animals. Sacramento, California.

_____. 1991. 1990 Annual Report on the Status of California's Listed Threatened and Endangered Plants and Animals. Sacramento, California. 203pp.

_____. 2001. Bald eagles in California. California Department of Fish and Game, Habitat Conservation Planning Branch, California's Plants and Animals. Internet address: www:dfg.ca.gov/hcpb/species/.

_____. 2001. California's living marine resources: a status report. University of California Publication No. SG01-11. Internet address: www.dfg.ca.gov/mrd.

California Regional Water Quality Control Board, Central Valley Region. 2002. Clear Lake TMDL for Mercury, Final Staff Report. California Environmental Protection Agency, Regional Water Quality Control Board, Central Valley Region, Sacramento, California. 62 pp+ appendices.

Campbell, K.R. and T.S. Campbell. 2001. The accumulation and effects of environmental contaminants on snakes: a review. Environmental Monitoring and Assessment. 70:253-301.

Canadian Council of Ministers of the Environment. 2000. Canadian tissue residue guidelines for the protection of wildlife consumers of aquatic biota: Methylmercury. *In* Canadian environmental quality guidelines, 1999, Canadian Council of Ministers of the Environment, Winnipeg.

Chandik, T. and A. Baldridge. 1967. Nesting season, middle Pacific Coast region. Audubon Field Notes. 21:600-603.

Charbonneau, S.M., I.C. Munro, E.A. Nera, R.F. Willes, T. Kuiper-Goodman, F. Iverson, C.A. Moodie, D.R. Stoltz, F.A.J. Armstrong, J.F. Uthe, and H.C. Grice. 1974. Subacute toxicities of methylmercury in the adult cat. Toxic. Appl. Pharm. 27:569-581.

Chase, T. and R.O. Paxton. 1965. Middle Pacific Coast region. Audubon Field Notes. 19:574-576.

Conway, C.J., W.R. Eddleman, S.H. Anderson and L.R. Hanebury. 1993. Seasonal changes in Yuma clapper rail vocalization rate and habitat use. J. Wild. Manage. 57(2):282-290.

Cullen, S.A., J.R. Jehl Jr., and G.L. Nuechterlein. 1999. Eared Grebe (*Podiceps nigricollis*). *In* The Birds of North America, No. 433 (A. Poole and F. Gill, eds.). The Birds of North America, Inc., Philadelphia, PA 28 pp.

Dansereau, M., N. Lariviere, D. Du Tremblay, D. Belanger. 1999. Reproductive performance of two generations of female semidomesticated mink fed diets containing organic mercury contaminated freshwater fish. Arch. Environ. Contam. Toxicol. 36:221-226.

Dawson, W.L. 1924. The birds of California. South Moulton Company, San Diego, California.

Dedrick, K. 1993. San Francisco Bay tidal marshland acreages: recent and historic values. In O.T. Magoon (ed.): Proceedings of the Sixth Symposium on Coastal and Ocean Management (Coastal Zone '89). Charleston, South Carolina, July 11-14, 1989. Publ. by Am. Society of Civil Eng.

DeGraaf, R.M., V.E. Scott, R.H. Hamre, L. Ernst, and S.H. Anderson. 1991. Forest and Rangeland Birds of the United States. Natural History and Habitat Use. USDA Forest Service, Agriculture Handbook 688. 625 pp.

DeGroot, D.S. 1927. The California clapper rail: its nesting habits, enemies and habitat. Condor. 29(6): 259-270.

Detrich, P.J. 1981. Historic range of breeding bald eagles in California. Unpublished Manuscript. Redding, CA. 17 pp.

_____. 1982. Results of California winter bald eagle survey - 1982. U.S. Fish and Wildlife Service, Sacramento, CA. 16 pp.

Dunning, J. B. 1993. CRC handbook of avian body masses. CRC Press, Boca Raton, FL. 371 pp.

Dustman, E.H., L.F. Stickel, and J.B. Elder. 1972. Mercury in wild animals at Lake St. Clair, 1970, *in* R. Hartung and R.D. Dinman (eds.), Environmental Mercury Contamination. Ann Arbor, Michigan: Ann Arbor Science Publication. pp. 46-52.

Eadie, J.M., M.L. Mallory, and H.G. Limsden. 1995. Common Goldeneye (*Bucephala clangula*). *In* The Birds of North America, No. 170 (A. Poole and F. Gill, eds.). The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C. 32 pp.

Eagles-Smith, C.A., T.H. Suchanek, P.B. Moyle, and D.W. Anderson. (in prep). Bacteria to birds: mercury trophic transfer efficiency as a function of trophic complexity. In prep. for submittal to Ecosystems.

Eddleman, W.R. 1989. Biology of the Yuma Clapper Rail in the Southwestern U.S. and Northwestern Mexico. U.S. Bureau of Reclamation, IA No. 4 -AA-30-020060.

Eddleman, W.R., and C.J. Conway. 1994. Clapper Rail, Chapter 12 *in* T.C. Tacha and C.E. Braun (eds.): Migratory Shore and Upland Game Bird Management in North America. International Association of Fish and Wildlife Agencies, in cooperation with the U.S. Dept. of the Interior, Fish and Wildlife Service; ASIN: 0935868755. Allen Press, Lawrence, Kansas. 223 pp.

Eisler, R. 2000. Mercury, Chapter 5 *in* Handbook of Chemical Risk Assessment: Health Hazards to Humans, Plants, and Animals, Vol. 1: Metals. Lewis Publishers, CRC Press, Boca Raton, FL. pp. 313-391.

Elbert, R.A. 1996. Reproductive performance and mercury exposure of birds at Clear Lake, CA. MS Thesis. University of California-Davis, Davis, California, USA. 70 pp.+ appendices.

Elbert, R.A. and D.W. Anderson. 1998. Mercury levels, reproduction, and hematology in Western grebes from three California lakes, USA. Environmental Toxicology and Chemistry. 17(2):210-213.

Elliot, M.L. and W.J. Sydeman. 2002. Breeding status of the California least tern at Alameda Point (former Naval Air Station, Alameda), Alameda, California, 2001. Unpublished Report, Point Reyes Bird Observatory, Stinson Beach, California. 57 pp.

Ellis, R.W. and L. Eslick. 1997. Variation and range of mercury uptake into plants at a mercury-contaminated abandoned mine site. Bull. Environ. Contam. Toxicol. 59:763-769.

Estes, J.A. 1990. Growth and equilibrium in sea otter populations. Journal of Animal Ecology. 59:385-401.

Evens, J., and G. Page. 1983. The ecology of rail populations at Corte Madera Ecological Reserve: with recommendations for management. A report to Marin Audubon Society from Point Reyes Bird Observatory. 62 pp.

Evers, D.C., O.P. Lane, C. DeSorbo, and L. Savoy. 2002. Assessing the impacts of methylmercury on piscivorous wildlife using a wildlife criterion value based on the Common loon, 1998-2001. Report BRI 2002-08, submitted to the Maine Department of Environmental Protection. Biodiversity Research Institute, Falmouth, Maine.

Fimreite, N. 1971. Effects of dietary methylmercury on ring-necked pheasants. Can. Wildl. Serv. Occas. Pap. 9. 39pp.

Fimreite N., 1974. Mercury contamination of aquatic birds in northwestern Ontario. J. Wild. Manage. 38(1):120-131.

Fjeld, E., T.O. Haugen, and L.A. Vollestad. 1998. Permanent impairment in the feeding behavior of grayling (*Thymallus thymallus*) exposed to methylmercury during embryogenesis. The Science of the Total Environment. 213:247-254.

Foerster, K.S., and J.E. Takekawa. 1991. San Francisco Bay National Wildlife Refuge Predator Management Plan and Final Environmental Assessment. Unpublished report.

Foerster, K.S., J.E. Takekawa, and J.D. Albertson. 1990. Breeding density, nesting habitat, and predators of the California clapper rail. Final report SFBNWR-11640-90-1, prepared for San Francisco Bay National Wildlife Refuge. Newark, CA.

Frenzel, R.W. 1984. Environmental contaminants and ecology of bald eagles in southcentral Oregon. PhD Thesis, Oregon State University. 119 pp.

Friedmann, A.S., M.C. Watzin, J.C. Leiter, and T. Brinck-Johnsen. 1996a. Effects of environmental mercury on gonadal function in Lake Champlain northern pike (*Esox lucius*). Bull. Environ. Contam. Toxicol. 56:486-492.

Friedmann, A.S., M.C. Watzin, T. Brinck-Johnsen, and J.C. Leiter. 1996b. Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquatic Toxicology. 35:265-278.

Friedmann, A.S., E.K. Costain, D.L. MacLatchy, W. Stansley, and E.J. Washuta. 2002. Effect of mercury on general and reproductive health of largemouth bass (*Micropterus salmoides*) from three lakes in New Jersey. Ecotoxicology and Environmental Safety. 52:117-122.

Gill, F.B. 1995. Ornithology. W.H. Freeman and Company. 615 pp+ appendix.

Grinnell, J. 1928. A distributional summation of the ornithology of lower California. Univ. Calif. Publ. Zool. 32:1-300.

Grinnell, J., H.C. Bryant, and T.I. Storer. 1918. The game birds of California. University of California Press, Berkeley.

Grinnell, J. and M.W. Wythe. 1927. Directory of the bird-life of the San Francisco Bay region. Pacific Coast Avifauna. 18:1-160.

Grinnell, J. and A. Miller. 1944. The Distribution of the Birds of California. Pacific Coast Avifauna Number 27. Cooper Ornithological Club, Berkeley, California. Reprinted by Artemisia Press, Lee Vining, California; April 1986. 617 pp.

Gupta, M. and P. Chandra. 1998. Bioaccumulation and toxicity of mercury in rooted-submerged macrophyte *Vallisneria spiralis*. Environmental Pollution. 103:327-332.

Hammerschmidt, C.R., J.G. Wiener, B.E. Frazier, and R.G. Rada. 1999. Methylmercury content of eggs in yellow perch related to maternal exposure in four Wisconsin lakes. Environ. Sci. Technol. 33:999-1003.

Hammerschmidt, C.R., M.B. Sandheinrich, J.G. Wiener, and R.G. Rada. 2002. Effects of dietary methylmercury on reproduction of fathead minnows. Environ. Sci. Technol. 2002. 36:877-883.

Harvey, T.E. 1988. Breeding biology of the California clapper rail in south San Francisco Bay. 1988 Transactions of the Western Section of the Wildlife Society. 24:98-104.

Haywood, D.D. and R.D. Ohmart. 1986. Utilization of benthic-feeding fish by inland breeding bald eagles. Condor. 88:35-42.

Heinz G.H., 1979. Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. J. Wildl. Manage. 43(2):394-401.

Henny, C.J., E.F. Hill, D.J. Hoffman, M.G. Spalding, and R.A. Grove. 2002. Nineteenth century mercury: hazard to wading birds and cormorants of the Carson River, Nevada. Ecotoxicology. 11:213-231.

Hopkins, W.A., C.L. Rowe, and J.D. Congdon. 1999. Elevated trace element concentrations and standard metabolic rate in banded water snakes, *Nerodia fasciata*, exposed to coal combustion wastes. Environmental Toxicology and Chemistry. 18:1258-1263.

Hopkins, W.A., J.H. Roe, J.W. Snodgrass, B.P. Staub, B.P. Jackson, and J.D. Congdon. 2002. Effects of chronic dietary exposure to trace elements on banded water snakes (*Nerodia fasciata*). Environmental Toxicology and Chemistry. 21(5):906-913.

Hothem, R.L. and A.N. Powell. 2000. Contaminants in eggs of Western snowy plovers and California least terns: is there a link to population declines? Bull. Environ. Contam. Toxicol. 65:42-50.

Hunt, W.G., J.M. Jenkins, R.E. Jackman, C.G. Thelander, and A.T. Gerstell. 1992. Foraging ecology of bald eagles on a regulated river. J. Raptor Res. 26(4):243-256.

Jackman, R.E., W.G. Hunt, J.M. Jenkins, and P.J. Detrich. 1999. Prey of nesting bald eagles in northern California. Journal of Raptor Research 33(2): 87-96.

Jarvinen, A.W. and G.T. Ankley. 1999. Linkage of effects to tissue residues: development of a comprehensive database for aquatic organisms exposed to inorganic and organic chemicals. Pensacola, Florida: Society of Environmental Toxicology and Chemistry (SETAC). 364 pp.

Jurek, R.M. 1988. Five-year status report. Bald Eagle. Calif. Dept. Fish and Game. Sacramento, CA. 15 pp.

_____. 2000. Unpublished data presented by Jan Johnson, U.S. Fish and Wildlife Service, Falconiformes of Northern California: Natural History and Management Workshop. October 24-26, 2000, North Coast Inn, Arcata, CA. The Wildlife Society, California North Coast Chapter, Arcata CA.

Knight, R.L., P.J. Randolph, G.T. Allen, L.S. Young, and R.J. Wigen. 1990. Diets of nesting bald eagles, *Haliaeetus leucocephalus*, in western Washington. Canadian Field-Naturalist. 104(4):545-551.

Kozie, K.D. and R.K. Anderson. 1991. Productivity, diet, and environmental contaminants in bald eagles nesting near the Wisconsin shoreline of Lake Superior. Arch. Environ. Contam. Toxicol. 20:41-48.

Kozloff, E.N. 1990. *Invertebrates*. Saunders College Publishing, Philadelphia, PA. 843 pp.

Kvitek, R.G. and J.S. Oliver. 1988. Sea otter foraging and effects on prey populations and communities in soft-bottom environments, Chapter 3 *in* G.R. VanBlaricom and J.A. Estes (eds.), The Community Ecology of Sea Otters. Springer-Verlag, Berlin, Germany. pp. 22-45.

Latif, M.A., R.A. Bodaly, T.A. Johnston, and R.J.P. Fudge. 2001. Effects of environmental and maternally derived methylmercury on the embryonic and larval stages of walleye (*Stizostedion vitreum*). Environmental Pollution. 111:139-148.

Lehman, R.N. 1979. A survey of selected habitat features of 95 bald eagle nest sites in California. Calif. Dept. Fish and Game. Wildlife Management Branch Administrative Report No. 79-1. Sacramento, CA. 21 pp.

Lehman, D.E. Craigie, P.L. Colins, and R.S. Griffen. 1980. An analysis of habitat requirements and site selection criteria for nesting bald eagles in California. Report by Wilderness Research Institute, Arcata, CA., for U.S. Forest Service, Region 5. San Francisco, CA. 106 pp.

Linder, G. and Grillitsch. 2000. Ecotoxicology of metals, Chapter 7 *in* D.W. Sparling, G. Linder, and C.A. Bishop (eds.), Ecotoxicology of Amphibians and Reptiles. Pensacola, Florida: Society of Environmental Toxicology and Chemistry (SETAC). 904 pp.

Massey, B.W. 1974. Breeding biology of the California least tern. Proc. Linnaean Soc. 72:1-24.

Massey, B., R. Zembal, and P. Jorgensen. 1984. Nesting habitat of the light-footed clapper rail in southern California. Journal of Field Ornithology 55: 67-80.

Matta, M.B., J. Linse, C. Cairncross, L. Francendese, and R.M. Kocan. 2001. Reproductive and transgenerational effects of methylmercury or Aroclor 1268 on *Fundulus heteroclitus*. Environmental Toxicology and Chemistry. 20(2):327-335.

McKernan, R.L. and G. Braden. 1999. Status, distribution, and habitat affinities of the southwestern willow flycatcher along the LCR Year 3-1998. Submitted to U.S. Bureau of Reclamation, LCR region, Boulder City, Nevada and U.S. Fish and Wildlife Service, Carlsbad, CA.

Mersmann, T.J., D.A. Buehler, J.D. Fraser, and J.K.D. Seegar. 1992. Assessing bias in studies of bald eagle food habits. J. Wildl. Manage. 56(1):73-78.

Moffitt, J. 1941. Notes on the food of the California clapper rail. Condor 43:270-273.

Monson, G. And A. Phillips. 1981. Annotated checklist of the birds of Arizona. The University of Arizona Press, Tucson. 240 pp.

Morris, R.H., D.P. Abbott, and E.C. Haderlie (eds.). 1980. Intertidal Invertebrates of California. Stanford University Press, Stanford, CA. 658 pp.

Mowbray, T. 1999. American Wigeon (*Anas americana*). *In* The Birds of North America, No. 401 (A. Poole and F. Gill, eds.). The Birds of North America Inc., Philadelphia, PA 32 pp.

Moyle, P.B. 2002. Inland Fishes of California. University of California Press, Berkeley and Los Angeles, CA. 446 pp.

Nagy, K.A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. Ecol. Monogr. 57:111-128.

Nagy, K.A. 2001. Food requirements of wild animals: predictive equations for free-living mammals, reptiles, and birds. Nutrition Abstracts and Reviews, Series B: Livestock Feeds and Feeding. 71(10):21R-31R.

NatureServe. 2001. Internet address: http://www/natureserve.org

Nevada Division of Wildlife. 1998. Comments on LCR MSCP Preliminary Species Conservation Goals: Bird Species. Prepared by C. Tomlinson, nongame biologist, Nevada Division of Wildlife, Las Vegas, Nevada.

Nichols, J, S. Bradbury, and J. Swartout. 1999. Derivation of wildlife values for mercury. Journal of Toxicology and Environmental Health, Part B, 2:325-355.

Norheim, G. and A. Froslie. 1978. The degree of methylation and organ distribution of mercury in some birds of prey in Norway. Acta Pharmacol. and Toxicol. 43:196-204.

Ohmart, R.D. and R.W. Smith. 1973. North American Clapper Rail (Rallus longirostris). Literature Survey With Special Consideration Being Given to the Past and the Present Status of *yumanensis*. USBR, Contract No. 14-06-300-2409.

Ohmart, R.D. and R.E. Tomlinson. 1977. Foods of western Clapper Rails. Wilson Bulletin. 89(2):332-336.

Page, G.W., L.E. Stenzel, W.D. Shuford, and C.R. Bruce. 1991. Distribution and abundance of the snowy plover on its western North American breeding grounds. J. Field Ornithol. 62(2):245-255.

Page, G.W., J.S. Warriner, J.C. Warriner, and P.W.C. Paton. 1995. Snowy Plover (*Charadrius alexandrinus*). *In* The Birds of North America, No. 154 (A. Poole and F.Gill, eds.). The Academy of Natural Sciences, Philadelphia, PA, and The Ornithologists' Union, Washington, D.C.

Palmer, R.S. (ed.). 1988. Handbook of North American Birds. Vols. 4 & 5. Yale University Press, New Haven, CT.

Patten, M.A., G. McCaskie, P. Unitt. In Press. Birds of the Salton Sea: Status, Biogeography, and Ecology.

Piest, L. And J. Campoy. 1998. Report of Yuma Clapper Rail Surveys at Cienega de Santa Clara, Sonora. Unpublished Report.

Pray, R.H. 1954. Middle Pacific Coast region. Audubon Field Notes. 8:326-327.

Proctor, N.S. and P.J. Lynch. 1993. Manual of ornithology: avian structure and function. Yale University Press. 340 pp.

Reidman, M.L. and J.A. Estes. 1988. A review of the history, distribution and foraging ecology of sea otters, Chapter 2. *in* G.R. VanBlaricom and J.A. Estes (eds.), The Community Ecology of Sea Otters. Springer-Verlag, Berlin, Germany. pp. 4-21.

Reidman, M.L. and J.A. Estes. 1990. The sea otter (*Enhydra lutris*): behavior, ecology, and natural history. Biological Report 90(14). U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C. 102 pp.

Rosenberg, K.V., R.D. Ohmart, W.C. Hunter, and B.W. Anderson. 1991. Birds of the LCR Valley. University of Arizona Press, Tucson, AZ.

Roth, V. and W. Brown. 1980. Arthropoda: Arachnida (Mites, Spiders, and Scorpions), Chapter 23 *in* R.C. Brusca (ed.), Common Intertidal Invertebrates of the Gulf of California. The University of Arizona Press, Tuscon, Arizona. pp. 347-355.

Ruhlen, T. and S. Abbott. 2000. Distribution, protection, and reproductive success of Snowy plovers at Point Reyes National Seashore in 2000. Point Reyes Bird Observatory, Stinson Beach, California.

Schwarzbach, S., . Henderson, and J.S. Albertson. 1996. Assessing risk from methyl mercury in tidal marsh sediments to reproduction of California clapper rails (*Rallus longirostris obsoletus*) using threshold diet and egg/sediment ratio approaches. Poster for Society of Environmental Toxicology and Chemistry, November 1996, Washington, D.C.

Schwarzbach, S. and T. Adelsbach. 2002. Assessment of ecological and human health impacts of mercury in the Bay-Delta watershed. Subtask 3B: Field assessment of avian mercury exposure in the Bay-Delta ecosystem. Final Report to the CALFED Bay-Delta Mercury Project, 39 pp.

Schwarzbach, S.E., J.D. Albertson, and C.M. Thomas. (in press). Factors affecting reproductive success of the California clapper rail (*Rallus longirostris obsoletus*) in San Francisco Bay. 34 pp.

Setmire, J.G., J.C Wolfe, and R.K. Stroud. 1990. Reconnaissance investigation of water quality, bottom sediment, and biota associated with irrigation drainage in the Salton Sea area, California, 1986-1987. U.S. Geological Survey Water-Resources Investigative Report, 89-4102.

Shepardson, D.I. 1909. Notes on the least tern. Oologist. 26:152.

Sibley, C.G. 1952. The birds of the south San Francisco Bay region. Oakland Public Museum. 42 pp.

Slotton, D.G., T.H. Suchanek, and S.M. Ayers. 2000. Delta wetlands restoration and the mercury question: year 2 findings of the CALFED UC Davis Delta mercury study. Contributed paper for the IEP Newsletter, Interagency Ecological Program for the San Francisco Estuary. 13(4):34-44.

Snyder, N.F. and J.W. Wiley. 1976. Sexual size dimorphism in hawks and owls of North America. Orni. Monogr. 20.

Spalding, M.G., P.C. Frederick, H.C. McGill, S.N. Bouton, and L.R. McDowell. 2000a. Methylmercury accumulation in tissues and effects on growth and appetite in captive great egrets. Journal of Wildlife Diseases. 36(3):411-422.

Spalding, M.G., P.C. Frederick, H.C. McGill, S.N. Bouton, L.J. Richey, I.M. Schumacher, C.G.M. Blackmore, and J. Harrison. 2000b. Histologic, neurologic, and immunologic effects of methylmercury in captive great egrets. Journal of Wildlife Diseases. 36(3):423-435.

Stalmaster, M. V., R. L. Knight, B. L. Holder, and R. J. Anderson. 1985. Bald eagles. Pps. 269-290 in E. R. Brown, ed. Management of wildlife and fish habitats in forests of western Oregon and Washington: Part 1- Chapter narratives. USDA Forest Service, Pacific North West Station, Portland, Oregon.

Stalmaster, M. V. and J. L. Kaiser. 1998. Effects of recreational activity on wintering bald eagles. Wildlife Monographs 137:1-46.

Storey, A., W. Montevecchi, H. Andrews, and N. Sims. 1988. Constraints on nest site selection: A comparison of predator and flood avoidance in four species of marsh-nesting birds (Genera: *Catoptrophorus*, *Larus*, *Rallus*, and *Sterna*). Journal of Comparative Psychology 102: 14-20.

Sykes, G. 1937. The Colorado Delta. *American Geographic Society Special Publication*, 19.

Thelander, C., and M. Crabtree. 1994. Life on the edge: a guide to California's endangered natural resources. Biosystem Books, Santa Cruz, CA. 550pp.

Thompson, B.C., J.A. Jackson, J. Burger, L.A. Hill, E.M. Kirsch, and J.L. Atwood. 1997. Least Tern (*Sterna antillarum*). *In* The Birds of North America, No. 290 (A. Poole and F. Gill, eds.). The Academy of Naturla Sciences, Philadelphia, PA, and The American Ornithologists' Union, Washington, D.C. 32 pp.

Thompson, D.R. 1996. Mercury in birds and terrestrial mammals, Chapter 14 *in* W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood (eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Lewis Publishers, Clemson, SC.

Tucker, M.A. and A.N. Powell. 1999. Snowy plover diets in 1995 at a coastal southern California breeding site. Western Birds. 30:44-48.

U.S. Bureau of Land Management. 2001. Revised Biological Opinion on Transportation and Delivery of Central Arizona Project Water to the Gila River Basin in Arizona and New Mexico and its Potential to Introduce and Spread Nonnative Aquatic Species, (2-21-90-F-119a). April 17.

U.S. Department of Energy. 1993. Toxicological Benchmarks for Wildlife. ES/ER/TM-86. Environmental Restoration Division, ORNL Environmental Restoration Program, Oak Ridge, TN. 55 pp + appendices.

_____. 1994. Toxicological Benchmarks for Wildlife: 1994 Revision. ES/ER/TM-86/R1. Health Sciences Research Division and Environmental Sciences Division, Oak Ridge, TN. 84 pp + appendices.

_____. 1995. Toxicological Benchmarks for Wildlife: 1995 Revision. ES/ER/TM-86/R2. Risk Assessment Program, Lockheed Martin Energy Systems, Inc., Oak Ridge, TN. 28 pp + appendices.

_____. 1996. Toxicological Benchmarks for Wildlife: 1996 Revision. ES/ER/TM-86/R3. Risk Assessment Program, Health Sciences Research Division, Oak Ridge, TN. 29 pp + appendices.

U.S. Department of the Interior. 1998. Guidelines for Interpretation of the Biological Effects of Selected Constituents in Biota, Water, and Sediment. Report No. 3

U.S. Environmental Protection Agency. 1993. Wildlife Exposure Factors Handbook, Volume I. EPA/600/R-93/187a. Office of Research and Development. Washington, DC

_____. 1995a. Trophic Level and Exposure Analyses for Selected Piscivorous Birds and Mammals, Volume I: Analyses of Species in the Great Lakes Basin. Office of Water. Washington, DC

_____. 1995b. Trophic Level and Exposure Analyses for Selected Piscivorous Birds and Mammals, Volume III: Appendices. Office of Water. Washington, DC

_____. 1995c. Great Lakes Water Quality Initiative Technical Support Document for Wildlife Criteria. EPA-820-B-95-009. Office of Water. Washington, DC

_____. 1995d. Great Lakes Water Quality Initiative Criteria Documents for the Protection of Wildlife. EPA-820-B-95-008. Office of Water. Washington, DC

_____. 1996. Amphibian toxicity data for water quality criteria chemicals. EPA/600/R-96/124. National Health Environmental Effects Research Laboratory, Corvallis, Oregon.

_____. 1997a. Mercury Study Report to Congress Volume VI: An Ecological Assessment for Anthropogenic Mercury Emissions in the United States. EPA-452/R-97-008. Office of Research and Development. Washington, DC

_____. 1997b. Mercury Study Report to Congress Volume VII: Characterization of Human Health and Wildlife Risks from Mercury Exposure in the United States. EPA-452/R-97-009. Office of Research and Development. Washington, DC

U.S. Fish and Wildlife Service. 1976. The literature of the western clapper rails. Special Scientific Report - Wildlife No. 194. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C. 21 pp.

_____. 1983. Yuma Clapper Rail Recovery Plan. U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 51 pp.

_____. 1984. The salt marsh harvest mouse/California clapper rail recovery plan. U.S. Fish and Wildlife Service, Portland, Oregon. 122 pp.+ appendices.

_____. 1985a. Recovery plan for the California least tern (*Sterna antillarum browni*). U.S. Fish and Wildlife Service, Portland, Oregon. 112 pp.

_____. 1985b. Recovery plan for the Light-footed Clapper Rail. U.S. Fish and Wildlife Service, Portland, Oregon. 121 pp.

_____. 1985c. Recovery plan for the San Francisco garter snake (*Thamnophis sirtalis tetrataenia*). U.S. Fish and Wildlife Service, Portland, Oregon. 77 pp.

_____. 1986. Recovery plan for the Pacific bald eagle. U.S. Fish and Wildlife Service, Portland, Oregon. 163 pp.

_____. 1999. Draft recovery plan for the Giant garter snake (*Thamnophis gigas*). U.S. Fish and Wildlife Service, Portland, Oregon. ix+ 192 pp.

_____. 2001. Western snowy plover (*Charadrius alexandrinus nivosus*) Pacific coast population draft recovery plan. U.S. Fish and Wildlife Service, Portland, Oregon. xix+ 630 pp.

_____. 2002. Recovery plan for the California Red-legged frog (*Rana aurora draytonii*). U.S. Fish and Wildlife Service, Portland, Oregon. viii+ 173 pp.

_____. 2003. Final revised recovery plan for the souther sea otter (*Enhydra lutris nereis*). U.S. Fish and Wildlife Service, Portland, Oregon. xi+ 165 pp.

U.S. Fish and Wildlife Service and National Marine Fisheries Service. 2000. Biological opinion on the effects of the U.S. Environmental Protection Agency's final promulgation of the California Toxics Rule on listed species and critical habitats in California. U.S. Department of the Interior, Fish and Wildlife Service, Sacramento, California and U.S. Department of Commerce, National Marine Fisheries Service, Long Beach, California. 236 pp.+ appendices.

U.S. Forest Service. 2000. Draft Bald Eagle Mangement Plan, Lassen National Forest, Recovery Zone 26, Lake Almanor Area, Lake Almanor and the Upper Feather River. Chester, CA. 30 pp.

Varoujean, D.H. 1972. A study of the California clapper rail in Elkhorn Slough, 1972. Report to the California Dept. of Fish and Game. 9 pp.

Vermeer, K., F.A.J. Armstrong, and D.R.M. Hatch. 1973. Mercury in aquatic birds at Clay Lake, Western Ontario. J. Wildl. Manage. 37(1):58-61.

Walsh, P.M. 1990. The use of seabirds as monitors of heavy metals in the marine environment, Chapter 10 *in* R.W. Furness and P.S. Rainbow (eds.), Heavy Metals in the Marine Environment. CRC Press, Boca Raton, Florida.

Wang, J.C.S. 1986. Fishes of the Sacramento-San Joaquin estuary and adjacent waters, California: A guide to the early life histories. Interagency Ecological Study Program for the Sacramento-San Joaquin Estuary. Tech. Rept. 9. (FS/B10-4ATR 86-9). Internet address: http://elib.cs.berkeley.edu/kopec/tr9/.

Warriner, J.S., J.C. Warriner, G.W. Page, and L.E. Stenzel. 1986. Mating system and reproductive success of a small population of polygamous snowy plovers. Wilson Bull. 98(1):15-37.

Webber, H.M. and T.A. Haines. 2003. Mercury effects on predator avoidance behavior of a forage fish, golden shiner (*Notemigonus crysoleucas*). Environmental Toxicology and Chemistry. 22(7):1556-1561.

West, J.M. and J.B. Zedler. 2000. Marsh-creek connectivity: fish use of a tidal salt marsh in southern California. Estuaries. 23(5):699-710.

Wiener, J. G. and D. J. Spry, 1996. Toxicological significance of mercury in freshwater fish, Chapter 13 *in* W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood (eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. Special Publication of the Society of Environmental Toxicology and Chemistry, Lewis Publishers, Boca Raton Florida, USA. 494 pp.

Wiener, J.G. D.P. Krabbenhoft, G.H. Heinz, and A.M. Scheuhammer. 2002. Ecotoxicology of mercury, Chapter 16 *in* D.J. Hoffman, B.A. Rattner, G.A. Burton, Jr., and J. Cairns, Jr, (eds.), Handbook of Ecotoxicology, 2nd edition. CRC Press, Boca Raton, Florida, USA, pp. 409-463.

Wilbur, S. 1974. The status of the light-footed clapper rail. American Birds 28: 868-870.

Wilson, D.E., M.A. Bogan, R.L. Brownell, Jr., A.M. Burdin, and M.K. Maminov. 1991. Geographic variation in sea otters, *Enhydra lutris*. Journal of Mammalogy. 72(1):22-36.

Winger, P.V., C. Siekman, T.W. May, and W.W. Johnson. 1984. Residues of organochlorine insecticides, polychlorinated biphenyls, and heavy metals in biota from the Apalachicola River, Florida, 1978. J. Assoc. Off. Anal. Chem. 67:325-333.

Wobeser, G., N.O. Nielsen, and B. Schiefer. 1976a. Mercury and mink I. The use of mercury contaminated fish as food for ranch mink. Can. J. Comp. Med. 40:30-33.

Wobeser, G., N.O. Nielsen, and B. Schiefer. 1976b. Mercury and mink II. Experimental methyl mercury intoxication. Can. J. Comp. Med. 40:34-45.

Wolfe, M.F., S. Schwarzbach, and R. A. Sulaiman. 1998. Effects of mercury on wildlife: a comprehensive review. Environmental Toxicology and Chemistry. 17(2):146-160.

Wolfe, M.F. and D. Norman. 1998. Effects of waterborne mercury on terrestrial wildlife at Clear Lake: evaluation and testing of a predictive model. Environmental Toxicology and Chemistry. 17(2):214-227.

Wren, C.D., H.R. MacCrimmon, and B.R. Loescher. 1983. Examination of bioaccumulation and biomagnification of metals in a Precambrian shield lake. Water, Air, and Soil Pollution. 19:277-291.

Wren, C.D., D.B. Hunter, J.F. Leatherland, and P.M. Stokes. 1987. The effects of polychlorinated biphenyls and methylmercury, singly and in combination on mink. II: Reproduction and kit development. Arch. Environ. Contam. Toxicol. 16:449-454.

Zeiner, D. , W. Laudenslayer, Jr., K. Mayer, M. White. Editors. 1990. California's Wildlife. Volume 2. Birds. State of California, Department of Fish and Game. Sacramento, California. 731 pp.

Zembal, R. and J.M. Fancher. 1988. Foraging behavior and foods of the light-footed clapper rail. Condor. 90:959-962.

Zembal, R. 1989. The light-footed clapper rail (*Rallus longirostris levipes*). U.S. Fish and Wildlife Service Publication. 2pp.

Zembal, R., B. Massey, and J. Fancher. 1989. Movements and activity patterns of the light-footed clapper rail. Journal of Wildlife Management 53: 39-42.

Zembal, R., S. Hoffman, and J. Bradley. 1998. Light-footed clapper rail management and population assessment, 1997. California Dept. of Fish and Game, Bird and Mammal Conservation Program Rep. 98-01. 23pp.

Zembal, R., and S. Hoffman. 2001. Light-footed clapper rail management, study, and translocation, 2001. Report to Naval Base Ventura County, U.S. Fish and Wildlife Service, and California Dept. of Fish and Game. Report prepared for California State University, Long Beach Foundation and Eldorado Audubon Society, Long Beach, California.

XII.B. PERSONAL COMMUNICATIONS

Heinz, G.H. 2002, 2003. Wildlife Biologist. U.S. Department of the Interior, U.S. Geological Survey, Patuxent Wildlife Research Center, Laurel, Maryland.

Schwarzbach, S.E. 2003. Fish and Wildlife Administrator. U.S. Department of the Interior, U.S. Geological Survey, Western Ecological Research Center Headquarters, Sacramento, California.

APPENDIX Federally Listed Threatened (T) and Endangered (E) Species in California Potentially At Risk From Methylmercury in Aquatic Ecosystems

Birds:
(T) Bald Eagle
(E) California Least Tern
(E) California Clapper Rail
(E) Yuma Clapper Rail
(E) Light-Footed Clapper Rail
(T) Western Snowy Plover

Amphibians and Reptiles:
(T) California Red-Legged Frogs
(T) Giant Garter Snake
(E) San Francisco Garter Snake

Fish:
(T) Coho Salmon (and Critical Habitat)
- (T) Central CA (and Critical Habitat)
- (T) So. OR/Northern CA (and Critical Habitat)

(T&E) Chinook Salmon (and Critical Habitat)
- (T) Central Valley Spring ESU (and Critical Habitat)
- (T) CA Coast ESU (and Critical Habitat)
- (E) Winter Run (and Critical Habitat)

(T&E) Steelhead Trout (and Proposed Critical Habitat and Critical Habitat)
- (PT) Northern CA ESU
- (T) Central CA Coast ESU (and Critical Habitat)
- (T) Central Valley ESU (and Critical Habitat)
- (T) South Central CA Coast ESU (and Critical Habitat)
- (E) Southern CA ESU (and Critical Habitat)

(T) Little Kern Golden Trout (and Critical Habitat)
(T) Paiute Cutthroat Trout
(T) Lahonton Cutthroat Trout
(E) Bonytail Chub (and Critical Habitat)
(E) Unarmored Threespine Stickleback (and Proposed Critical Habitat)
(E) Shortnose Sucker (and Proposed Critical Habitat)
(E) Lost River Sucker (and Proposed Critical Habitat)
(T) Sacramento Splittail

Mammals:
(T) Southern Sea Otter