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Southern Resident Killer Whales

Updated June 2021 based on data available through December 2020.

Declining Trend

Annual monitoring of the Southern Resident Killer Whale population has shown fluctuations in population size over time, from a starting point of 68 identified individuals in 1976, to a peak of 98 individuals in 1995. The population then declined to 78 individuals in July 2000, followed by a period of moderate growth to 89 individuals in July 2006.

Since 2006, the population has generally declined and has not shown signs of recovery, with only 74 individuals as of December 2020. This trend, along with biological condition of the Southern Resident Killer Whale population, acoustic stressors, vessel impacts, the consistently low availability of Chinook salmon, and exposure to contaminants, indicate that this population is facing increasing threats to its survival and recovery.

On this page:

Photo courtesy of NOAA Fisheries.

About Southern Resident Killer Whales

Killer whales, or orcas, are top predators and cultural icons of the Salish Sea. During the spring, summer and fall months, killer whales can be seen regularly in these waters.

Southern Resident Killer Whales have been listed as endangered species in both the U.S. and Canada, and their population is closely tied to the overall health of the ecosystem.

Critical Habitat

Critical habitat is especially important to maintain as it provides the features, functions and attributes required to support the species' survival or recovery. Such habitat provides for sustained feeding and foraging, resting, socialization, reproduction, rearing, and migration.

Coastal watersheds that are not currently designated as critical habitat are also important for Southern Resident Killer Whales and their prey. Southern Resident Killer Whale critical habitat in Canadian waters was expanded in 2018, and in the United States, the National Oceanic and Atmospheric Administration is proposing to expand Southern Resident Killer Whale habitat beyond the Salish Sea.

Social Organization

Killer whale societies are organized into a series of social units according to maternal genealogy. How much each unit is related to another gets progressively weaker from the smallest social unit (matriline) through the largest (community).

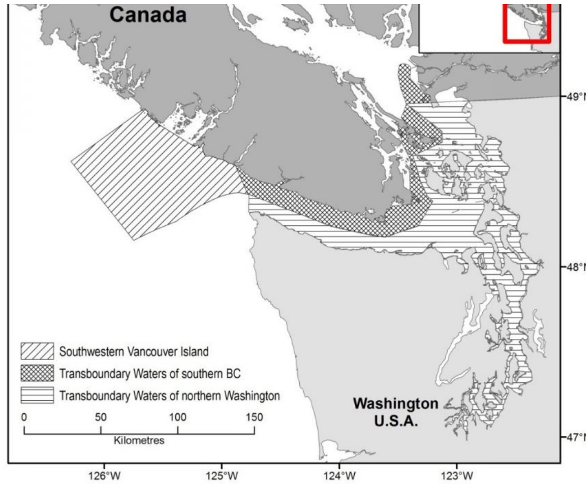
Matriline

Matriline is the smallest killer whale social unit. A matriarch (older female) and all of her descendants (including sons, daughter, and grandchildren) are referred to as a matriline. Sons and daughters stay with their mother throughout their lives, even after they have offspring of their own. In 2010, Southern Resident Killer Whales were organized along 19 matrilines, and more recent estimates report that up to 25 matrilines may now exist.

Pod

A pod is a group of related matrilines that travel, forage, socialize, and rest together. As pods grow in size over time, they may split into new pods. Southern Resident Killer Whales are comprised of three pods: J-Pod, K-Pod and L-Pod.

<https://epa.gov/sites/default/files/2020-11/salish-sea-srkw-critical-habitat.jpg>



Identified critical habitat for the Southern Resident Killer Whales. Canadian and transboundary waters in British Columbia are protected under the *Species at Risk Act*. Washington transboundary waters are protected under the *Endangered Species Act*. Source: Fisheries and Oceans Canada. Click on image for larger view.

An aerial view of the entire I16 matriline of Northern Resident Killer Whales taken in 2014. Photo credit: NOAA Fisheries, Vancouver Aquarium.

Clan

A clan is a group of pods that share similar calls or dialects. Pods with very similar dialects are more closely related than those with different features in their dialects. All

Southern Resident Killer Whales belong to J-Clan.

Community

Community is the top level of killer whale social structure. Resident killer whales that share a common range and that associate at least occasionally are considered to be members of the same community. Pods from one community have rarely or never been seen to travel with those from another community even though their ranges partly overlap. There are two communities found in the Salish Sea: Northern Resident Killer Whales and Southern Resident Killer Whales.

Ecotype

Although they are currently recognized as a single species, three distinct ecotypes of killer whale can be found in the Salish Sea. These ecotypes of killer whale do not interbreed, and differ in their behavior, calls, genetics, morphology, and prey preferences. Resident killer whales (split into Northern and Southern residents) primarily eat salmon, while Transient killer whales (also called Bigg's killer whales) eat marine mammals and seabirds. The third ecotype of killer whale, Offshore killer whales, eat sharks and other fishes and are rarely seen in the Salish Sea. Differences in appearance, such as dorsal fin shapes and markings on the backs of the whales, can be used to tell the ecotypes apart.

Sustainable Perspectives

Killer whales and coastal Indigenous people have lived together in the Salish Sea for at least 5000 years.

Although remains of many marine mammals can be found at historical Salish village sites, killer whales are rarely found at these sites. This has been attributed to their special significance in aboriginal culture. In British Columbia, non-First Nation anglers once considered killer whales to be nuisances and competition for salmon. About 1 in 4 killer whales that were captured in the 1960s and 1970s showed evidence of having been previously shot and wounded.

By the late 1960s, attitudes toward killer whales shifted as people around the world observed captive killer whales as intelligent mammals. By the 1970s, the North American environmental movement helped foster compassion for killer whales. In 1980, increased public interest supported the first commercial whale watching excursions in Johnstone Strait. Now whale watching generates hundreds of millions of dollars annually in the U.S. and Canada.

Counting Killer Whales

National Marine Fisheries Service research vessel observing a "spy hopping" Southern Resident Killer Whale off San Juan Island, Washington. Photo credit: NOAA Northwest Fisheries Science Center.

The counting of individual killer whales began in 1972 and 1973 with the collection of information on killer whales and their movements near Vancouver Island. Using photographs of killer whales showing identifying features, including some submitted by citizens. Researchers at Fisheries and Oceans Canada were able to develop the first estimates of killer whale population size for British Columbia. Those early researchers wrote in 1976 that their efforts would be enhanced by the further efforts planned in Washington state.

The photo-identification studies that started in the State of Washington in 1976 have now continued annually for 44 years. The Center for Whale Research records encounters with killer whales, including the Southern Resident Killer Whales. Researchers now know about each individual in the Southern Resident Killer Whale population. According to the Center for Whale Research, the Southern Resident Killer Whales are now the best-studied marine mammals in the world.

On January 24, 2020, the Center for Whale Research reported that L41, one of the most prolific breeding males from L-pod, had gone missing and is now feared dead. A confirmed loss of another whale would leave the killer whale population at 72 animals. Immediately after the published update, local media in British

Columbia and Washington both reported on the discouraging news.

The Seattle Times described the whale’s contribution to the Southern Resident Killer Whale population: "L41 fathered 21 orca babies with 11 different females."It is not clear yet if L41 is truly gone, as the Vancouver Sun's interview with a marine mammal researcher noted that, "males help locate prey and spread out and communicate information."

The Center for Whale Research summed up the hope of many in the Salish Sea in a follow-up media release: "We are hopeful that L41 is alive somewhere and returns to the subgroup, but he did live to a ripe old age and fathered more baby whales than any other whale in the community."

In the fall of 2020, the Center for Whale Research reported two killer whale births that were announced widely in American, Canadian, and even the international media. The health of the calves appear good and they have been named Phoenix J57, birthed by Tahlequah J35; and Crescent J58, birthed by Eclipse J41. These births mark the first since the last healthy recorded birth to the southern residents in May 2019, and bring the total SRKW population to 74 - including 24 in J Pod, 17 in K Pod and 33 in L Pod.

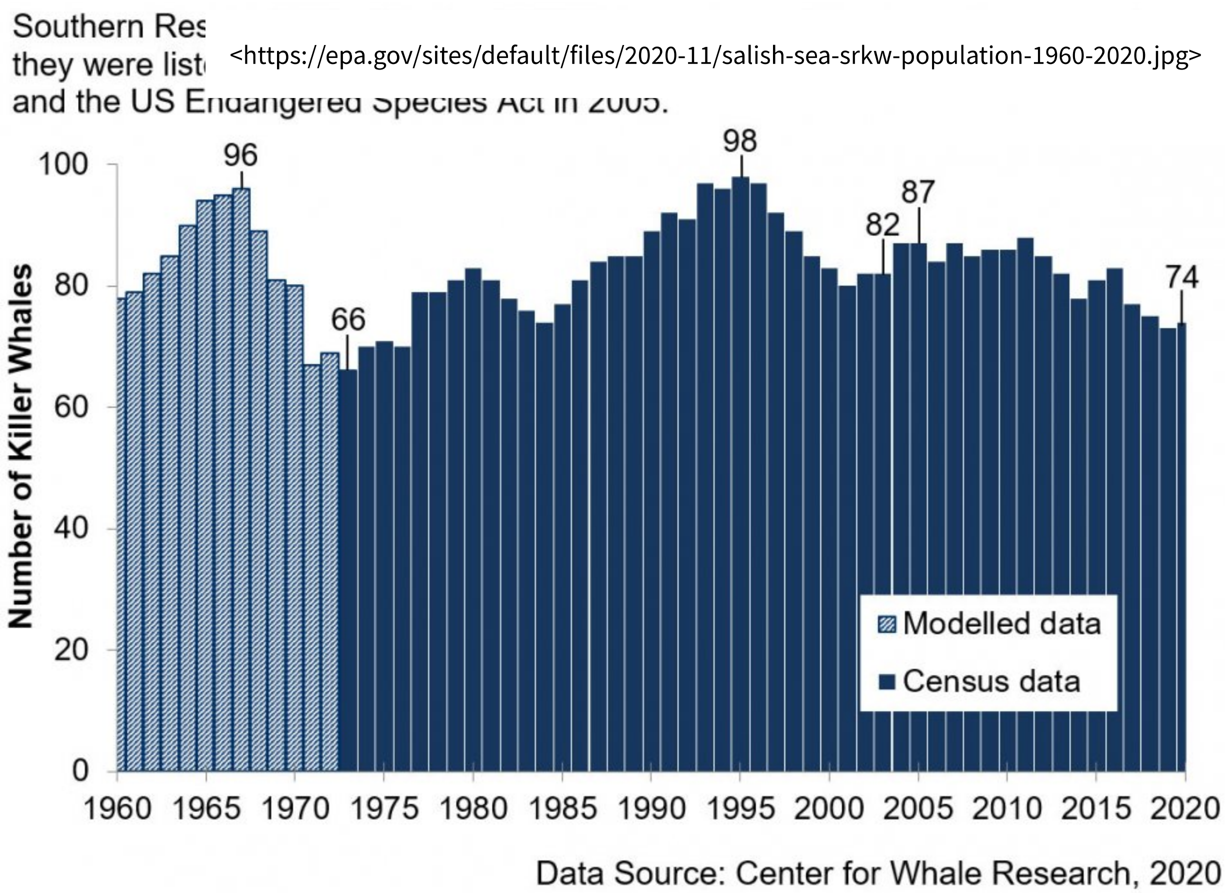
What's Happening?

From 1973 to 2019, the Southern Resident Killer Whale population showed periods of both growth and decline. When researchers conducted the first population census in 1973, 66 whales were sighted. It is unknown what historic population sizes may have been before researchers started tracking the Southern Resident Killer Whale population.

The population increased by 48% relative the first whale census in 1973, to a count of 98 individuals in 1995, and then dropped 16% to a count of 80 individuals in 2001, prompting the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) to designate the Southern Resident Killer Whales as Endangered. The U.S. began the process of listing them as endangered species under the Endangered Species Act (ESA), with the listing finalized in 2005. In Canada, Southern Resident Killer Whales were listed under the new Species at Risk Act legislation in 2003.

By December 2020, the Southern Resident Killer Whale population had declined to a 40-year low count of 74 individuals. This is more than a 25% decline from the observed peak population size in 1995. There were killer whale births at the start of 2019, with one birth occurring in January and another in May. These births raised the population to 76 individuals, which was encouraging. However over the remainder of 2019, three whales were declared missing and presumed deceased. Now the number of Southern Resident Killer Whales is approaching the observed lowest count, recorded in 1973.

<https://epa.gov/sites/default/files/2020-11/salish-sea-srkw-population-1960-2020.jpg>



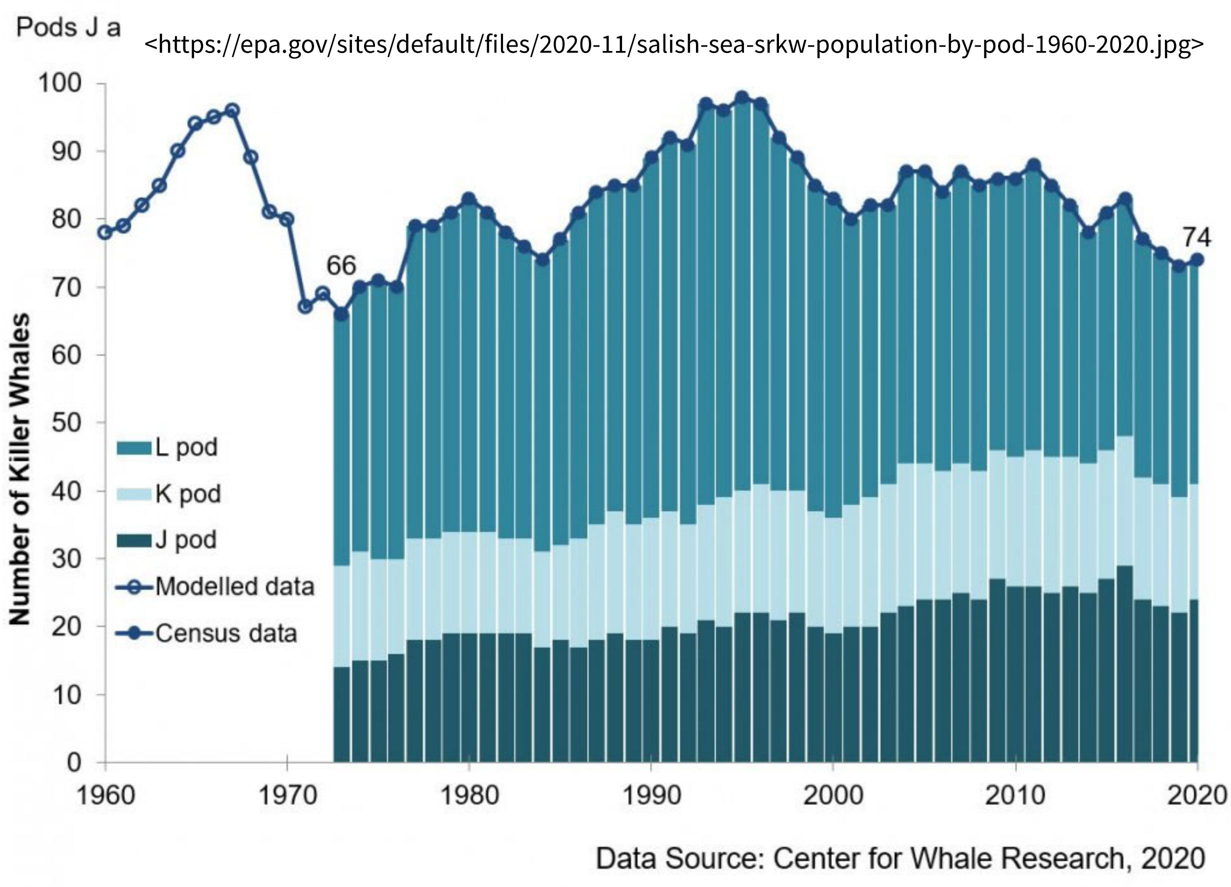
Graph showing the number of Southern Resident Killer Whales from 1960-2020. Click on image for larger view.

The three pods within the Southern Resident Killer Whale population have shown different dynamics in the number of whales over time. Since the observed peak population size of 98 Southern Residents in 1995, the size of K-pod has remained consistent with about 17 individuals. The size of J-pod increased after 1995 to a peak of

29 members in 2016, but most recently the size of J-pod has returned to 24 individuals, which is the same size as it was recorded in 1995.

Since 1995, L-pod has lost 25 individuals, contributing the most to the overall decline in the population count for Southern Resident Killer Whales. L-pod has the highest proportion of older females. Older female killer whales contribute to a pod's success by assisting with hunting and increase the chances of survival of juveniles, but older females that are no longer reproducing limit the ability to add new juveniles to the pod's population. L-pod also appears to have high juvenile mortality and a male sex-ratio bias among juveniles. These factors may represent further challenges to the recovery of the L-pod.

<https://epa.gov/sites/default/files/2020-11/salish-sea-srkw-population-by-pod-1960-2020.jpg>



Graph showing the number of Southern Resident Killer Whales by pod from 1960-2020. Click on image for larger view.

Why Is It Important?

The health of killer whale populations is important for many reasons. Killer whales are culturally, spiritually, and economically important to the Salish Sea. They are featured prominently in the stories and art of the Coast Salish people and other Indigenous peoples of the northwest coast of North America. And, for some Salish peoples, killer whales are family members that live under the water.

Killer whales are viewed as an indicator species for the Salish Sea. The decline in local killer whale populations may indicate stressors that eventually will affect the whole ecosystem.

Killer whales are also important for local tourism. In 2008, the total expenditures from whale watching in British Columbia was estimated at $118 million USD. A 2013 survey of Salish Sea whale watching companies ranked killer whales as the most important wildlife species for their clients and company marketing. As of 2018, whale watching in the Puget Sound region alone provides an economic contribution of over $216 million USD annually and supports over 1,800 jobs.

Why Is It Happening?

Recent declines in Southern Resident Killer Whale populations are linked to reduced prey availability, and other threats such as chemical pollution, or acoustic and physical disturbance from vessels and other noise sources. These factors have an even greater impact on the Southern Resident Killer Whale population when they are combined than could be predicted by studying any one factor by itself. Studies have shown that all threats need to be a part of population size models for Resident Killer Whales in order to match the observed changes in Resident Killer Whale abundance over time.

Southern Resident Killer Whales rely heavily on healthy populations of salmon – particularly Chinook – which are declining across the Salish Sea and Pacific coast. For all of June 2019, Southern Resident Killer Whales were not spotted in the Salish Sea for the first time on record. The Southern Resident Killer Whales did arrive in July 2019, though. Changes in the spring presence patterns for Southern Resident Killer Whales in the Salish Sea has been linked to Chinook salmon abundance patterns for the period of 1994-2016. An overall change in the Salish Sea community is happening however, as the observed presence of transient killer whales, which eat marine mammals rather than fish, increased from 2011-2017 in the Salish Sea.

Understanding the problem of prey abundance is complicated because most Southern Resident Killer Whale pods spend most of the time each year outside the Salish Sea, in environments where it can be challenging to carry out research. While conditions in the Salish Sea and its watersheds are important to enhance and protect, conditions in the Pacific and coastal watersheds from Central California through Vancouver Island are also important to enhance and protect. Southern Resident Killer Whales also forage near the outlets of the Sacramento River, the Klamath River, and the Columbia River watersheds, which historically supported large runs of salmon that the killer whales rely on as food.

Changing age demographics and gender ratios within the small Southern Resident Killer Whale population can compound these factors and have pronounced influence over social dynamics such as mating, group foraging, and social care of calves. Further, the greater genetic diversity loss in stable or declining populations and inbreeding is becoming an increasing concern for Southern Resident Killer Whale survival and reproductive success.

An estimated 48 Southern Resident Killer Whales were removed from the Salish Sea between 1962 and 1977, due to live capture activities for aquariums all over the world. Washington and British Columbia served as the primary source of captive killer whales because inland waters offered fewer escape routes, shallower waters made netting easier, and a large network of shore observers provided updates on movements of the whales.

Current Threats to Killer Whale Recovery

Prey Availability

Survival and birth rates in Southern Resident Killer Whales have shown a close correlation with coast-wide abundance of salmon. The abundance of their preferred prey, Chinook salmon, has declined from historical levels in the Salish Sea. Fraser River Chinook salmon have been shown to make up about 80% of killer whales' summer diet when they are in the Salish Sea, and Fraser River populations are currently of conservation concern due to substantial declines over the last decade. In the fall, Southern Resident Killer Whale pods in Puget Sound show more Chum and Coho salmon in their diet. Low Chinook salmon availability has been linked to failed pregnancies in Southern Resident Killer Whales.

<https://epa.gov/sites/default/files/2020-11/salish-sea-srkw-diet.jpg>

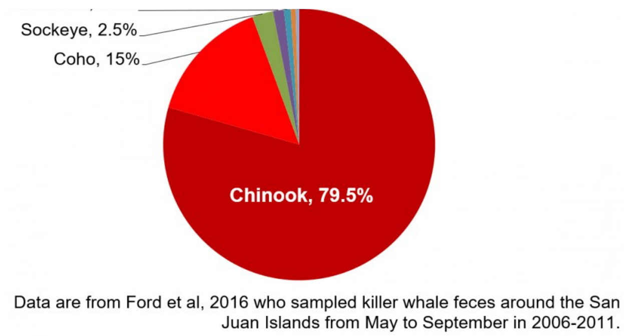


Chart showing Southern Resident Killer Whale diet during summer months when the whales are most present in the Salish Sea. Click on image for larger view.

Southern Resident Killer Whales also consume Chinook salmon from the Columbia, Sacramento, and Klamath rivers along with other coastal river systems outside of the Salish Sea ecosystem, and a small proportion of other fish species throughout their range. These other river systems contribute prey during winter months when coastal foraging is particularly important to local pods of killer whales, making up the largest portion of the Southern Resident Killer Whale's annual diet.

Pollution and Contaminants

High levels of persistent organic pollutants (e.g. PCBs and DDT, which were banned from use in Canada and the U.S. long ago) and newer pollutants like those found in flame retardants (PBDEs), may be preventing the population of Southern Resident Killer Whales from increasing at a rate required for recovery. Individuals have been found to carry some of the highest PCB concentrations reported in animals, with levels in blubber exceeding those known to affect the health of other marine mammals. Other contaminant levels, such as the levels of DDT and PBDEs, are also found in high levels, especially in juvenile killer whales.

When trapped in blubber, contaminants have little impact on the killer whale's health. However, when killer whales are food-deprived, they rely on their blubber to survive. When this fat is used, harmful pollutants accumulated in the blubber over time are released into the whales. These pollutants, in addition to malnutrition, may cause pregnancy failure in Southern Resident Killer Whales and may affect the killer whale's immune system function.

Vessel Traffic, Noise and Risk of Oil Spills

The cumulative impacts of vessel presence and noise may interfere with the ability of Southern Resident Killer Whales to communicate and find food. Southern Resident Killer Whales may avoid areas in their foraging range that have high levels of vessel disturbance and may spend additional energy and swim faster to avoid vessels. Studies indicate that whales expend more energy and find less food when vessels are present than when they are not present, making it harder to find enough food for growth and successful reproduction when salmon are scarce. Whales adapt and find prey when there are high noise levels in their background environment by increasing the loudness of their calls, which may use additional energy while they hunt for food.

What's Being Done About It?

Many agencies and groups are working to address threats to the Southern Resident Killer Whales by focusing on efforts that address prey availability, environmental contaminants, acoustic and physical disturbance from shipping and boat traffic, and oil spills. In recent years, governments in the United States and Canada have both contributed over one billion dollars to efforts aiming to protect and recover Southern Resident Killer Whales, in addition to ongoing spending specifically targeted at salmon recovery.

Examples

Below are examples of the type of work being done by government agencies to help protect killer whale populations:

The following links exit the site

Conducting Research, Science and Monitoring

Current information is essential to inform decision-making, adaptive management and implementation of actions to recover the Southern Resident Killer Whales. It will be important to use an adaptive management approach to track effectiveness of implemented actions, assess unintended consequences, monitor ongoing ecosystem change and adjust future policies and investments based on the findings. Continued monitoring of killer whales under existing sightings networks and observation programs in Canada and the United States contributes updated and current information on the population size of the killer whales as they move.

Coordinating Marine Mammal Response Networks

Several partners in Canada agreed that there was a need to unite marine mammal response networks across Canada, in efforts to support the work of Fisheries and Oceans Canada's response activities. The Canadian Marine Animal Response Alliance brings together several organizations across Canada to further rescue, research, and outreach activities.

Funding Investments from the United States

In May 2019, the Washington State Governor signed 5 orca recovery bills into law, which aim to decrease vessel noise and traffic, educate boaters about whale watching, more safely transport oil, support Chinook salmon populations, and decrease toxics pollution. The Governor's 2019-2021 operating budget includes $1.1 billion USD for enforcement of these laws, salmon habitat restoration, salmon hatcheries, and toxics cleanup and prevention.

Funding Investments from Canada

In 2018, the Government of Canada introduced its 5-year, $167.4 million (CAD) Whales Initiative . Funds from this initiative are used to improve killer whale prey availability, reduce vessel disturbance, increase environmental monitoring for whales and noise, strengthen compliance and enforcement with whale-related regulations, and build partnerships with other organizations. These funds are in addition to Canada's 2016 $1.5 billion (CAD) Oceans Protection Plan , to help protect Canadian waters and marine life, including killer whales. In October 2018, another $61.5 million (CAD) was allocated by Fisheries and Oceans Canada for additional measures to protect and support the recovery of the Southern Resident Killer Whale .

Collaborating to Increase Understanding

The U.S. National Oceanic and Atmospheric Administration (NOAA) Fisheries is working with Fisheries and Oceans Canada to evaluate a report by an independent science panel that evaluated effects of salmon fisheries on Southern Resident Killer Whales <https://www.fisheries.noaa.gov/west-coast/endangered-species-conservation/effects-salmon-fisheries-southern-resident-killer-whales>. They are also working toward understanding other potential factors such as where the whales are in the winter when they are outside of the Salish

Sea, demographics, mating patterns and inbreeding effects.

Collaborating to Inform Management Actions

Several initiatives have been focused recently on protecting Southern Resident Killer Whales including:

- The Washington State Governor established the Southern Resident Orca Task Force <https://www.governor.wa.gov/issues/issues/energy-environment/southern-resident-orca-recovery/task-force> in March 2018 to develop recommendations for killer whale recovery. This task force invited participants from many U.S. states, local governments, and Canadian government agencies.
- In 2018, the Government of Canada formed several technical working groups focused on the key threats to Southern Resident Killer Whales, with representatives comprised of policy, technical and scientific experts from the federal government, Indigenous groups, environmental groups, and industry. Recommendations from these working groups, along with consultations with Indigenous groups, stakeholders, environmental organizations and the public, led to the implementation of a number of additional management measures in 2019 for the Southern Resident Killer Whale. Management measures focused on prey availability and acoustic and physical disturbance including the creation of three interim sanctuary zones in Canadian Southern Resident Killer Whale critical habitat. These efforts continue, with implementation of enhanced measures to protect Southern Resident Killer Whales .
- The Vancouver Fraser Port Authority is leading the Enhancing Cetacean Habitat and Observation (ECHO) Program to better understand and manage the impact of shipping activities on at-risk whales throughout the southern coast of British Columbia. The ECHO program also has several U.S. and Canadian agency collaborators.

- In 2019, a conservation agreement for Southern Resident Killer Whales under Section 11 of the Government of Canada's Species at Risk Act was signed with the goal to reduce the acoustic and physical disturbance from large commercial vessels and tugs that operate in killer whale critical habitat. The five-year agreement was the first for a marine aquatic species and was signed by Fisheries and Oceans Canada, Transport Canada, the Vancouver Fraser Port Authority, the Chamber of Shipping of British Columbia, the Shipping Federation of Canada, Cruise Lines International Association, the Council of Marine Carriers, the International Ship Owners Alliance of Canada, and the Pacific Pilotage Authority.

Whale Watching Laws, Marine Mammal Regulations, Interim Measures and Guidelines

The Canadian Government, U.S. National Ocean and Atmospheric Administration, and Washington State have adopted vessel regulations to reduce vessel disturbances and manage traffic. Organizations such as Straitwatch (for Victoria and the Southern Gulf Islands) and Soundwatch (for the San Juan Islands) provide details about these rules and guidelines for vessels near whales. Be Whale Wise provides up to date information on guidelines and regulations for boating around marine mammals. The measures that are in place include:

- As of May 2019, a Washington State law to protect Southern Resident Orca Whales restricts boats and kayaks to slow to no-wake speeds (slower than a speed of 7 knots) within a half-nautical mile of the Southern Resident Killer Whales, though there are several exemptions for safety and other reasons.
- Also enacted in May 2019, Washington State law requires that vessels in Washington must stay at least 300 yards away from either side of killer whales and 400 yards behind or in the advancing paths of killer whales, and must disengage engines if the separation distance is less than this.
- In 2018, amendments to the Marine Mammal Regulations under Canada’s Fisheries Act make it mandatory to stay 200 metres away from all killer whales in Canadian Pacific waters year-round. These regulations along with those for other marine mammals and any exceptions that may apply can be explore online at Fisheries and Oceans Canada's website.

- From June 2019 to October 2019, through an Interim Order under the Canada Shipping Act, the Government of Canada required all boaters to stay a minimum distance of at least 400 metres away from all killer whales when in the Canadian critical habitat for the Southern Resident Killer Whales. Canada also put Interim Sanctuary Zones in place in 3 locations (Swiftsure Bank, Pender Island and Saturna Island). These measures were in effect from June 1, 2019 – October 31st, 2019. Consultations on the continuation of these measures were held at the end of 2019 and the measures were renewed in 2020.

Improving Land Leasing Activities

The Washington Department of Natural Resource's Aquatic Reserves Program <https://www.dnr.wa.gov/managed-lands/aquatic-reserves> is working to protect aquatic environments from aquatic land leasing activities, with emphasis on protecting habitat and species such as killer whales. As well, the State of Washington enacted legislation to phase out the aquaculture of Atlantic salmon in marine net-pens by 2022.

Collaborative Marine Response Plans

NOAA Fisheries has developed an Oil Spill Emergency Response Plan for killer whales in partnership with the Washington Department of Fish and Wildlife and the SeaDoc Society. In addition, the Canada–United States Joint Marine Pollution Contingency Plan , an agreement between the Canadian Coast Guard and the United States Coast Guard, provides a framework for Canada–U.S. cooperation in response to marine pollution incidents threatening coastal waters of both countries and for major incidents in one country where the assistance of the neighboring country is required. Other spill-response collaborative initiatives include the Salish Sea Shared Waters Forum held in 2018, 2019, and 2020 that were facilitated by the Pacific States/British Columbia Oil Spill Task Force . Other spill response activities in Canada and the United States are also occurring at the local government level.

Learn More

The following links exit the site

- Be Whale Wise (Regulations and guidance for keeping proper distance from whales.)

- Government of Canada's Species at Risk Public Registry – Southern Resident Killer Whales
- Environment and Climate Change Canada - Reducing the Threat of Contaminants to Southern Resident Killer Whales/Contaminants Technical Working Group
- U.S. National Oceanic and Atmospheric Administration (NOAA) - Killer Whales <https://www.fisheries.noaa.gov/west-coast/endangered-species-conservation/southern-resident-killer-whale-orcinus-orca>
- B.C. Cetacean Sightings Network (Wild Whales)
- Puget Sound Vital Signs - Orcas <https://vitalsigns.pugetsoundinfo.wa.gov/vitalsign/detail/19>
- Center for Whale Research
- University of British Columbia Marine Mammal Research Unit (MMRU)
    - Availability of Prey for Southern Resident Killer Whales - 2017 MMRU Technical Workshop Proceedings (PDF) (64 pp, 2 MB, About PDF <https://epa.gov/home/pdf-files>)
- NOAA's Saving the Southern Residents Story Map
- Governor Inslee's Orca Task Force <https://www.governor.wa.gov/issues/issues/energy-environment/southern-resident-orca-recovery/task-force>
- Fisheries and Oceans Canada's Marine Environmental Quality Initiative
- Marine Mammal Commission Southern Resident Killer Whale <https://www.mmc.gov/priority-topics/species-of-concern/southern-resident-killer-whale/>
- Fisheries and Oceans Canada 2020 Management Measures to Protect Southern Resident Killer Whales
- NOAA Oil Spill Response and Killer Whales <https://response.restoration.noaa.gov/oil-and-chemical-spills/oil-spills/resources/oil-spill-response-and-killer-whales.html>

Six Things You Can Do To Help

1. Get involved in efforts to **protect and restore salmon habitat** in your community. Chinook salmon are especially important to Resident killer whale populations in the Salish Sea.

2. Killer whales are sensitive to noise and disturbance from boats. Consult Be Whale Wise before getting out on the water and follow the laws and guidelines in Washington and British Columbia waters. **Give killer whales space** and enjoy whale watching from land. Check out thewhaletrail.org for the best spots to see whales from shore.
3. When boating, **prevent contaminants from entering local waters** by using sewage pump-out services and reducing grey water discharge. Reduce your driving whenever possible. Cars and trucks can release contaminants onto roadways that eventually end up into the Salish Sea when rainwater rinses the contaminants away. As well, vehicle exhaust contains airborne contaminants that can contribute to ocean acidification.
4. Choose to **eat sustainably-harvested salmon** and other seafood to help protect wild fish populations. Check for a certification symbol on food packaging or menus, such as Ocean Wise Sustainable Seafood . When fishing, be aware of relevant local and national regulations that may restrict what species or amounts you can take.
5. Keep plastics, medications and toxic chemicals out of our waterways. Do your part to **dispose of unused medicine and chemicals properly**. Never dump them into household toilets and sinks or outside where they can get into ditches or storm drains. Consult safe disposal of prescription drug programs in Canada or the Washington's Safe Medication Return Program <https://www.doh.wa.gov/forpublichealthandhealthcareproviders/healthcareprofessionsandfacilities/safemedicationreturnprogram> for pharmaceutical take-back programs. And when possible, use non-toxic cleaning products. Most public wastewater treatment systems are not designed to remove medicines or household chemicals. See if your community has a household hazardous waste collection facility that will take your old or unused chemicals.
6. **Report your sightings of killer whales**. In British Columbia waters, report your whale sightings to the BC Cetacean Sightings Network . Your reports are used to reduce ship strikes and contribute to Science. The Orca Network, located in Washington State, also maintains a database of sightings of Killer Whales sighted in the Salish Sea.

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For 20-plus years, EPA has failed to regulate 'forever chemicals'

By **Scott Faber** (/news-insights/our-experts/scott-faber) (EWG)

JANUARY 9, 2020

The Environmental Protection Agency was first alerted to the health hazards of toxic fluorinated chemicals, known as PFAS, in 1998. In the decades since, the agency has failed to set enforceable regulations on PFAS in drinking water, food, food packaging and a wide array of other everyday consumer goods.

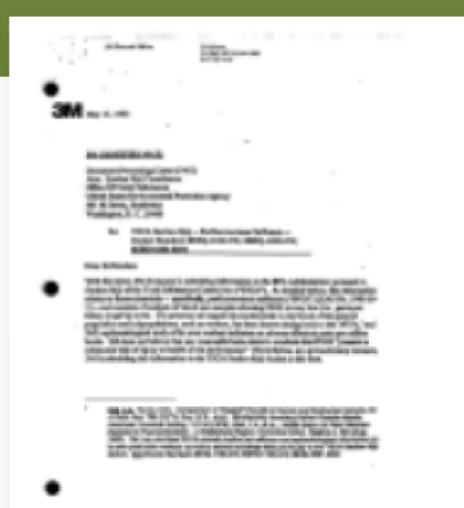
In 2009 and 2019, the EPA announced toothless PFAS "action plans" that fall far short of what is needed to protect Americans, even as reams of studies have linked some PFAS to cancer, reproductive and immune system harm, thyroid disease and other serious health impacts.

DOWNLOAD ALL DOCUMENTS (https://static.ewg.org/reports/2020/pfas-epa-timeline/EPA-PFAS-Timeline-10-21.pdf)

1998

3M alerts EPA that PFOS, the PFAS chemical in Scotchgard, builds up in blood.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/1998_3M-Alerts-EPA.pdf?_ga=2.24803514.1253861871.1649070681-2123137255.1639662520)



1998

3M sends rat studies to EPA, showing liver damage from PFAS exposure.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/1998_Publication-Strategy.pdf?_ga=2.133396622.1253861871.1649070681-2123137255.1639662520)

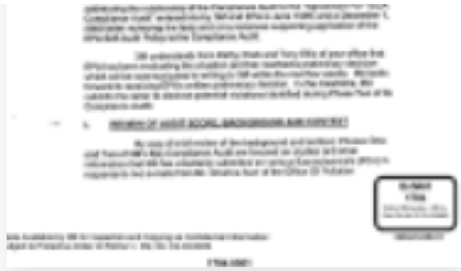
1999

EPA AUDIT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2001_EPA-3M-AuditResponse.pdf?_ga=2.93418525.1253861871.1649070681-2123137255.1639662520)

1999

EPA begins audit of 3M studies.

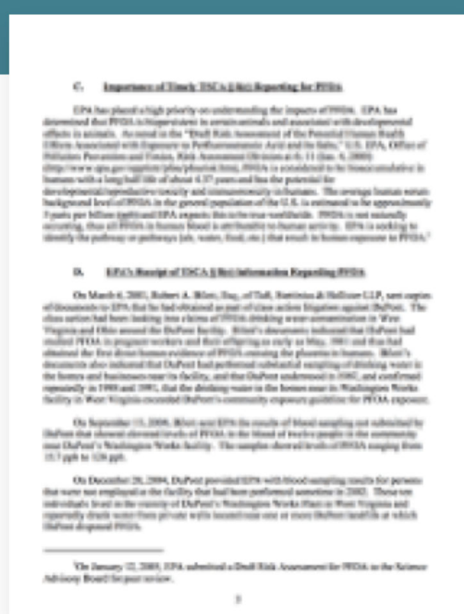
3M RESPONSE (https://static.ewg.org/reports/2020/pfas-epa-timeline/2001_3M-TSCA-SubstantialRiskAlert.pdf?_ga=2.87080856.1253861871.1649070681-2123137255.1639662520)



2001

Attorney Rob Bilott provides EPA with secret DuPont documents on PFOA, the PFAS chemical in Teflon.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2001_Bilott-Submits-to-EPA.pdf?_ga=2.124851338.1253861871.1649070681-2123137255.1639662520)



2001

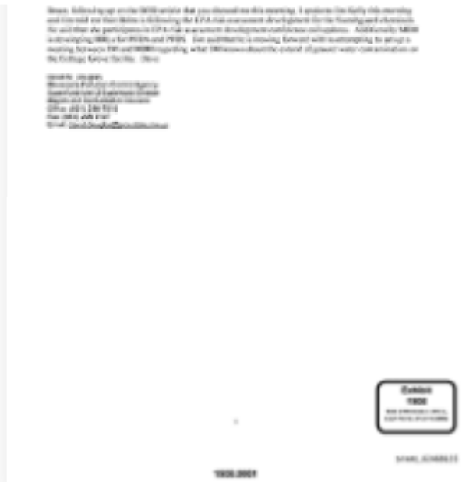
3M submits PFOS toxicity studies to EPA.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2001_3M-ToxStudies-EPA.pdf?_ga=2.57178026.1253861871.1649070681-2123137255.1639662520)

2002

EPA initiates a "priority review" of PFOA.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2003_EPA-Risk-Assesment.pdf?_ga=2.122327819.1253861871.1649070681-2123137255.1639662520)



2005

DuPont urges EPA to "restate safety of [PFOA] products and no health effects."

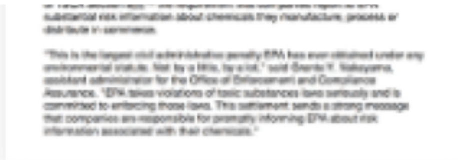
VISIT SITE (https://theintercept.com/2015/08/20/teflon-toxin-dupont-slipped-past-epa/)

2005

EPA fines DuPont $10.25 million for failing to report "substantial risk of injury to human health" from PFOA.

[DOWNLOAD DOCUMENT](https://static.ewg.org/reports/2020/pfas-epa-timeline/2005_EPA_Fines_DuPont_Over_PFOA.pdf?_ga=2.123845386.1253861871.1649070681-2123137255.1639662520) (https://static.ewg.org/reports/2020/pfas-epa-timeline/2005_EPA_Fines_DuPont_Over_PFOA.pdf?_ga=2.123845386.1253861871.1649070681-2123137255.1639662520)

Reference News Release: EPA Settles PFOA Case Against DuPont for Largest Environmental Administrative Penalty in Agency History



2006

EPA brokers a voluntary agreement with DuPont, 3M and other companies to phase out the use of PFOS and PFOA. Announcing the agreement, EPA says "to date, EPA is not aware of any studies specifically relating current levels of PFOA exposure to human health effects."

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2006_EPA-StewardshipProgram.pdf?_ga=2.163322428.1253861871.1649070681-2123137255.1639662520)

2006

Following **requests from DuPont** (https://static.ewg.org/reports/2020/pfas-epa-timeline/2006_DuPont-Leverages-EPA.pdf), EPA tells consumers that it **is safe to use** (https://static.ewg.org/reports/2020/pfas-epa-timeline/2006_EPA-PFOA_Safe.pdf) products made with PFAS.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2006_DuPont-Leverages-EPA.pdf?_ga=2.27811772.1253861871.1649070681-2123137255.1639662520)

2006

3M shares hundreds of secret documents with EPA, resulting in more than $1.5 million in penalties.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2006_3M-1.5Million-Fine.pdf?_ga=2.136549168.1253861871.1649070681-2123137255.1639662520)

2006

EPA Science Advisory Board draft report finds PFOA to be a "likely human carcinogen."

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2006_EPA-SAB-LikelyCarcinogen.pdf?_ga=2.122400907.1253861871.1649070681-2123137255.1639662520)

2009

EPA publishes a “provisional health advisory” for PFOA and PFOS.

[DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2009_EPA-HealthAdvisory.pdf?_ga=2.27414716.1253861871.1649070681-2123137255.1639662520)](https://static.ewg.org/reports/2020/pfas-epa-timeline/2009_EPA-HealthAdvisory.pdf?_ga=2.27414716.1253861871.1649070681-2123137255.1639662520)

SHARES

2009

EPA publishes first PFC action plan.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2009_EPA-Action-Plan.pdf?_ga=2.69294992.1253861871.1649070681-2123137255.1639662520)

2012

EPA requires one-time monitoring by public water systems for some PFAS chemicals.

Addition.pdf?_ga=2.162550972.1253861871.1649070681-2123137255.1639662520)

2015

EPA proposes a Significant New Use Rule (SNUR) for long-chain PFAS chemicals – as of February 2020, yet to be implemented.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2015-SNUR.pdf?_ga=2.91446298.1253861871.1649070681-2123137255.1639662520)

2016

EPA sets a non-enforceable "health advisory" level of 70 parts per trillion for PFOA and PFOS in drinking water – far above what independent researchers say is safe.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2016_LHA-70ppt.pdf?_ga=2.58334122.1253861871.1649070681-2123137255.1639662520)

EPA

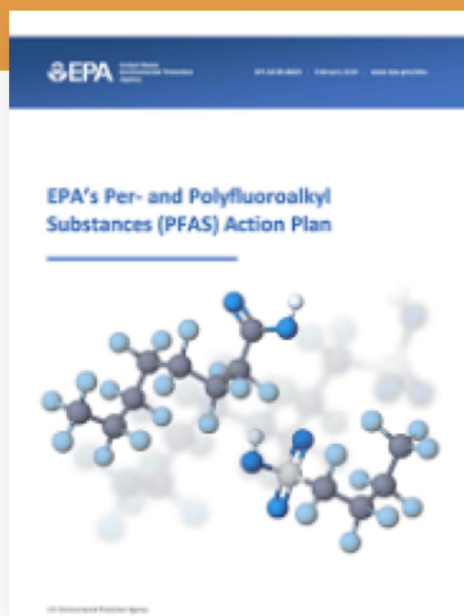
FACT SHEET

PFOA & PFOS Drinking Water Health Advisories

2019

EPA issues second PFAS Action Plan.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2019_PFAS-Action-Plan.pdf?_ga=2.125459338.1253861871.1649070681-2123137255.1639662520)



2019

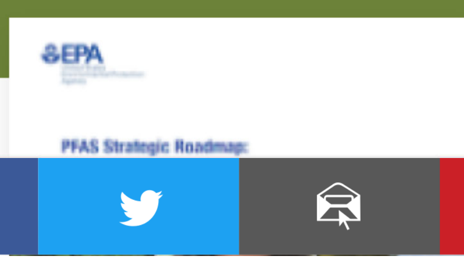
EPA misses self-assigned deadline to issue a plan to set an enforceable legal limit for PFOA and PFOS in drinking water by the end of 2019.

Letter.pdf?_ga=2.57695402.1253861871.1649070681-2123137255.1639662520)

2021

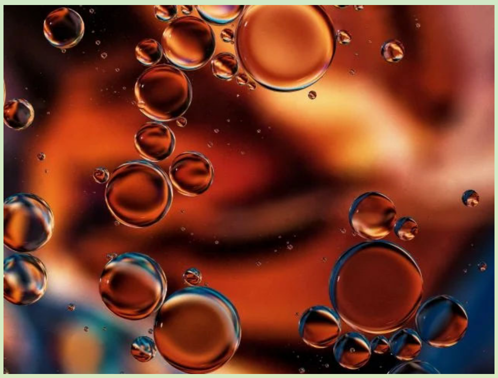
EPA releases its "PFAS Roadmap," detailing a timeline for the agency to set drinking water standards, wastewater treatment guidelines, health assessments and hazardous substance designations for several PFAS chemicals.

DOWNLOAD DOCUMENT (https://static.ewg.org/reports/2020/pfas-epa-timeline/2021_pfas-roadmap_final-508.pdf?_ga=2.169900192.1253861871.1649070681-2123137255.1639662520)



TOPICS

Learn about these issues



(/areas-focus/toxic-chemicals)

Toxic Chemicals (/areas-focus/toxic-chemicals)

Chemical companies aren't required to test chemicals for safety before they go on the market. We offer resources to help you make better, safer decisions.

(/areas-focus/toxic-chemicals/pfas-chemicals)

PFAS Chemicals (/areas-focus/toxic-chemicals/pfas-chemicals)

DuPont's Teflon changed our lives, but also polluted our bodies. Today, Teflon-like compounds called PFAS are found in the blood of almost all Americans. These "forever chemicals" pollute water, don't break down, and remain in the environment and people for decades.

U.S. Department of the Interior
Fish and Wildlife Service

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

Prepared By:

Daniel Russell
U.S. Fish and Wildlife Service
Environmental Contaminants Division
Sacramento Fish and Wildlife Office

Sacramento, California
October, 2003

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Literature citation should read as follows:

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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.**

<u>**Discussion**</u>

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 Califomia 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:

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

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

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

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

$$\mathbf{FDTL2 = DC / [(\%TL2) + (\%TL3 \times MTL3) + (\%TL4 \times MTL3 \times MTL4)]} \quad \textbf{(3)}$$

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

$$\mathbf{FDTL3 = FDTL2 \times MTL3} \quad \textbf{(4)}$$

$$\mathbf{FDTL4 = FDTL3 \times MTL4} \quad \textbf{(5)}$$

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.

$$\mathbf{FDTL2} = \text{TRC} / [(\%\text{TL2}) + (\%\text{TL3} \times \text{MTL3}) + (\%\text{TL4} \times \text{MTL3} \times \text{MTL4})]$$

$$\mathbf{FDTL2} = 0.3 \text{ mg/kg} / [(0.217) + (0.457 \times 5.7) + (0.326 \times 5.7 \times 4)]$$

$$\mathbf{FDTL2} = 0.3 / 10.247$$

$$\mathbf{FDTL2 = 0.029 \ mg/kg}$$

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

$\mathbf{FDTL2 = 0.029 \ mg/kg}$
$\mathbf{FDTL3} = 0.029 \times 5.7 = \mathbf{0.165 \ mg/kg}$
$\mathbf{FDTL4} = 0.165 \times 4.0 = \mathbf{0.660 \ mg/kg}$

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:

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

$$\mathbf{0.3 \ mg/kg = (.217 \times 0.029 \ mg/kg) + (.457 \times 0.165 \ mg/kg) + (.326 \times 0.66 \ mg/kg)}$$

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:

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

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:

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

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. <u>Southern Sea Otter</u> (*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. <u>California Least Tern</u> (*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) = $2.601 \times (\text{body weight in g})^{0.640}$
FMR = $2.601 \times 45^{0.640}$
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:

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

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

IV.D. <u>Light-footed Clapper Rail</u> (*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. California 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.*, - $0.09 \times 0.176 \times 100 = 1.58\%$).

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:

$$\text{FMR (kcal/day)} = 2.601 \times \text{body weight (g)}^{0.640}$$
$$\text{FMR} = 2.601 \times 5250^{0.640}$$
$$\mathbf{FMR = 625\ kcal/day}$$

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.

$$ME_{fish} = 1.2\ \text{kcal/g} \times 0.79 = \mathbf{0.948\ kcal/g\ fish}$$

$$ME_{birds} = 1.9\ \text{kcal/g} \times 0.78 = \mathbf{1.482\ kcal/g\ birds}$$

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:

$$\text{FMR} = [Y(g) \times 1.482(\text{kcal/g birds})] + [4.88Y(g) \times 0.948\ \text{kcal/g fish}]$$

$$625\ \text{kcal/day} = 1.482Y + 4.626Y$$

$$625\ \text{kcal/day} = 6.108Y$$

$$Y = 102\ \text{g birds consumed/day}$$

$$4.88Y = 498\ \text{g fish consumed/day}$$

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:

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

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 × FDTL2) + (%TL3 × FDTL3) + (%TL4 × FDTL4) + (%OB × FDOB) + (%PB × 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. <u>California Clapper Rail</u>

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 California 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. <u>Fish</u>

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 malfomation 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.

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XII.B. PERSONAL COMMUNICATIONS

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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

DRAFT

Biological Opinion
on
EPA's Proposed Program of Continuing Approval or Promulgation of New Cyanide Criteria in State and Tribal Water Quality Standards

U.S. Fish and Wildlife Service
Arlington, Virginia
January 15, 2010

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1.0 LIST OF ACRONYMS

ACR	Acute to Chronic Ratio
BE	Biological Evaluation
BMP	Best Management Practice
CCC	Criteria Continuous Concentration (chronic criterion)
CFR	Code of Federal Regulations
CMC	Criteria Maximum Concentration (acute criterion)
CN	Cyanide
CPP	Continuing Planning Process
CWA	Clean Water Act
EC	Effects Concentration
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FAV	Final Acute Value
FR	Federal Register
HCN	Hydrogen Cyanide
ICE	Interspecies Correlation Estimate
ISO	International Organization for Standardization
LC	Lethal Concentration
LCL	Lower Confidence Limit
LOEC	Lowest Observable Effects Concentration
MATC	Maximum Acceptable Toxicant Concentration
MLE	Maximum Likelihood Estimate
NOEC	No Observable Effects Concentration
NPDES	National Pollution Discharge Elimination System
NPS	Non-Point Source
OECD	Organisation for Economic Co-operation and Development
SETAC	Society for Environmental Toxicology and Chemistry
TMDL	Total Maximum Daily Load
WQM	Water Quality Management
WQS	Water Quality Standard

Formal Draft Biological Opinion.

2.0 INTRODUCTION

This document transmits the U.S. Fish and Wildlife Service's (Service) biological opinion based upon our review of EPA's proposed program of continuing approval or promulgation of acute and chronic cyanide criteria in State and Tribal water quality standards and their effects on endangered and threatened species and designated critical habitats in accordance with section 7 of the Endangered Species Act of 1973, as amended (16 U.S.C. 1531 *et seq*.)(ESA). Your March 23, 2007, request for formal consultation was received on March 26, 2007.

Your request for formal consultation also included a request for our concurrence that the proposed acute and chronic cyanide criteria are not likely to adversely affect any endangered and threatened species or their critical habitats listed in section 9.2.1 of your March 23, 2007, *Biological Evaluation of Aquatic Life Criteria—Cyanide* (CN BE). According to information presented in the CN BE, available data suggest that cyanide is toxic to aquatic plants only at concentrations well above the proposed criterion concentrations. For that reason, we concur with your finding. We determined the proposed action may affect but is not likely to adversely affect numerous threatened and endangered animal species. The species for which we make this determination and our rationale are provided in Appendix B.

This biological opinion is based on information provided in: (1) your March 23, 2007, CN BE; (2) your July 31, 2006, *Draft Framework for Conducting Biological Evaluations of Aquatic Life Criteria: Methods Manual* (BE Methodology); (3) your October 29, 2004, *Draft Methodology for Conducting Biological Evaluations of Aquatic Life Criteria: Methods Manual*; (4) the February 22, 2001, *Memorandum of Agreement Between the Environmental Protection Agency, Fish and Wildlife Service and National Marine Fisheries Service Regarding Enhanced Coordination Under the Clean Water Act and Endangered Species Act; Notice* (66 FR 11202); and (5) additional information contained in Service files. A complete administrative record of this consultation is on file at the Service's Headquarters Office in Arlington, Virginia.

Scope of this Biological Opinion

This biological opinion evaluates the effects of EPA's program of continuing approval of cyanide criteria adopted in State water quality standards under the Clean Water Act (CWA) on listed species and their critical habitats. More precisely, this biological opinion evaluates at a program level the potential effects to listed species and critical habitat from exposure to cyanide at EPA's recommended CWA section 304(a) criteria. The assumptions made in this biological opinion about the potential for exposure at the criteria values may not apply consistently to all State and Tribal water quality standards or even all waters within a State or Tribal boundary. State and Tribal water quality standards are comprised of 4 elements: (1) designated uses; (2) criteria sufficient to protect those uses; (3) an antidegradation policy; and (4) general policies. The other components of a water quality standard, besides the criteria, may invalidate the assumptions made here about exposure.

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Because the assumptions made in this biological opinion about the potential for listed species' exposure at the criteria values may not apply consistently to all State and Tribal water quality standards or even all waters within a State or Tribal boundary, this biological opinion does not include incidental take exemptions. Given the uncertainty about such exposure, we are unable to analytically determine at the program scale the likelihood (as opposed to the potential) of such take occurring. Therefore, it will be necessary for EPA to conduct subsequent, step-down ESA section 7 consultations with the Service on individual State and Tribal water quality standards to determine if incidental take exemptions associated with State and Tribal implementation of cyanide criteria in their water quality standards are warranted.

We anticipate much of the analysis presented in this biological opinion will carry over, so that the tiered consultation on State and Tribal water quality standards need only focus on potential effects of elements that were not fully considered here. Because we address criteria in this biological opinion, tiered consultation would likely focus on the various General Policies being implemented by each State and Tribe and the need to incorporate considerations for listed species and critical habitats into those policies, where necessary.

The Service expects that the EPA will notify affected States and Tribes of the need, in their next triennial review, to review their water quality standards for consistency with the findings presented in this biological opinion. EPA should work with States and Tribes to coordinate and consult directly with the Service's local Field Offices in conducting this review. In this review, EPA will, in consultation with the Service, evaluate waters of the United States within the range of applicable listed species and critical habitats considered in this biological opinion to determine whether:

1. there exists a designated use to which aquatic life criteria apply;
2. State-adopted criteria are consistent with this biological opinion; and,
3. general policies adopted by States or Tribes and approved by EPA would alter the effects analysis in this biological opinion.

This tiered consultation will involve a review of the effects of the proposed cyanide criteria on listed species and critical habitats at a more refined scale that incorporates the four elements of the State's or Tribe's water quality standards as approved. As noted above, it is at this scale that we will determine if the effects analysis includes findings that support an incidental take exemption(s).

3.0 CONSULTATION HISTORY

On February 22, 2001, the Service and the National Marine Fisheries Service (NMFS; collectively, the Services), and EPA noticed in the Federal Register the 2001, Memorandum Of Agreement (MOA) referenced above which, among other things, described our plan for conducting ESA section 7 consultations on EPA's recommended aquatic life criteria.

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In January 2004, the Services and EPA decided to proceed with a data call for the first batch of pollutants that would be reviewed in consultation, while continuing to work on the *Draft Methodology for Conducting Biological Evaluations of Aquatic Life Criteria--Methods Manual.*

On May 14, 2004, the Services and EPA issued data calls to regional staff and NMFS science center staff requesting information and data on cyanide, ammonia, chromium III and chromium VI. The data call requested the regions and science centers to send relevant studies to our Headquarters Offices by June 30, 2004.

On November 12, 2004, the Services received by email from EPA a November 5, 2004, revised *Draft Methodology for Conducting Biological Evaluations of Aquatic Life Criteria--Methods Manual* (dated October 29, 2004, on the document). This version represented a methodology developed collaboratively, and which had been peer reviewed by subject experts outside of the Federal government.

In December 2004, the Service and EPA exchanged comments on recommended revisions to the November draft methodology. EPA also informed the Services that they had received a draft BE for cyanide from their contractor and were reviewing the document to ensure the contractor had followed the BE methodology accurately.

On January 19, 2005, EPA transmitted by email the January 19, 2005, *Biological Evaluation of Aquatic Life Criteria—Cyanide, Part 1: Toxicity Analysis and Preliminary Effects Assessment*.

On May 3, 2005, the Services transmitted by email comments on EPA's January 19, 2005, draft biological evaluation for cyanide criteria.

On January 31, 2006, the Service received a January 26, 2006, letter from EPA requesting the Service to review a January 26, 2006, draft CN BE for its "completeness" in fulfilling the information requirements for ESA section 7 consultation.

On April 18, 2006, the Service transmitted a letter to EPA responding to EPA's January 26, 2006, consultation request.

In a June 29, 2006, letter, EPA requested the Service's concurrence that the proposed approvals of cyanide criteria were not likely to adversely affect 455 threatened and endangered species and their critical habitats.

On August 1, 2006, EPA electronically transmitted a July 31, 2006, *Draft Framework for Conducting Biological Evaluations of Aquatic Life Criteria: Methods Manual*, which they used to support their effects determinations.

On November 28, 2006, the Service responded by letter to EPA's consultation request with a recommendation that EPA initiate formal consultation.

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On March 23, 2007, EPA requested formal consultation and provided the CN BE, which concluded their action was not likely to adversely affect listed species or designated critical habitats.

On May 29, 2007, the Service transmitted a letter acknowledging the initiation of formal consultation.

On August 6, 2007, the Service transmitted a letter to EPA requesting an extension of formal consultation to November 6, 2007.

On August 17, 2007, the Service received an August 15, 2007, letter from EPA offering to extend the consultation period to September 30, 2007.

On May 5-9, 2008, the Services met with EPA to conduct a "Kaizen" "lean event." The purpose of the meetings was to analyze the cyanide consultation process from the development of a biological assessment through the anticipated completion of formal consultation in an effort to find efficiencies in the process. The Services and EPA also discussed coordination and communication with respect to the national consultation on cyanide and local consultation on EPA promulgation of Oregon water quality standards.

On June 12, 2008, the Services and EPA met to follow up on the Kaizen lean event. Subsequent follow up meetings were cancelled until the Services completed draft biological opinions.

BIOLOGICAL OPINION

This biological opinion does not rely on the regulatory definition of "destruction or adverse modification" of critical habitat at 50 C.F.R. 402.02. Instead, we have relied upon the statutory provisions of the ESA to complete the following analysis with respect to critical habitat.

4.0 DESCRIPTION OF THE PROPOSED ACTION

Background

Section 304(a)(1) of the CWA directs EPA to publish criteria for water quality that accurately reflect the latest scientific knowledge on a number of factors, including "…the kind and extent of all identifiable effects on health and welfare including, but not limited to, plankton, fish, shellfish, wildlife, plant life, shorelines, beaches…which may be expected from the presence of pollutants in any water body, including groundwater;" "on the concentration and dispersal of pollutants, or their byproducts," and on "the effects of pollutants on biological community diversity, productivity, and stability." EPA's CWA section 304(a) cyanide aquatic life criteria recommendations are published in *Ambient Water Quality Criteria for Cyanide – 1984* (EPA 1985). EPA describes the published criteria as a criterion maximum concentration (CMC) and criterion continuous concentration (CCC) for freshwater and saltwater:

Freshwater CMC (as free cyanide) = 22 µg/L
Freshwater CCC (as free cyanide) = 5.2 µg/L
Saltwater CMC (as free cyanide) = 1.0 µg/L
Saltwater CCC (as free cyanide) = 1.0 µg/L

For cyanide, the CMC ("acute" criterion) represents an estimated concentration in fresh or salt water to which aquatic organisms and their uses should not be affected unacceptably if the one-hour average concentration does not exceed this value more than once every three years on average, except possibly where locally important species are more sensitive (EPA 1985). The CCC ("chronic" criterion) represents an estimated concentration in either fresh or salt water to which aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration does not exceed the CCC more than once every three years on average, except possibly where locally important species are more sensitive (EPA 1985).

Section 303(c)(2)(B) of the CWA requires States, Tribes, and U.S. territories (hereafter referred to collectively as States as defined by EPA in 40 CFR 131.3(j)) to adopt into their water quality standards numeric criteria for toxic pollutants listed under section 307(a) of the CWA for which section 304(a) criteria have been published if the presence of these pollutants is likely to affect a water body's use. States can adopt criteria under section 303(c) that differ from EPA's 304(a) criteria values whenever adequately justified, but States generally choose to adopt, verbatim, EPA's 304(a) criteria and rely on the criteria document for their scientific justification. Once adopted into State or Tribal water quality standards, criteria help form the legal basis for implementing CWA programs to control pollution and achieve the goals and requirements of the CWA.

Proposed Program Action

EPA would approve State or Tribal-adopted water quality standards for cyanide criteria, or promulgate Federal water quality standards for cyanide criteria for U.S. waters that are identical to or more stringent than EPA's recommended CWA section 304(a) aquatic life criteria for cyanide (EPA 1985). EPA's cyanide criteria document (EPA 1985) serves as the States', Tribes' and EPA's scientific justification that the recommended criteria values are sufficient to achieve designated uses that protect aquatic life under the CWA.

All States and Tribes have already either adopted into their state water quality standards cyanide criteria identical to EPA's recommended 304(a) cyanide aquatic life criteria (EPA 1985) and EPA has approved them, or EPA promulgated cyanide criteria for the States and Tribes identical to their 304(a) criteria. For some water bodies, States and Tribes have adopted and EPA has approved site-specific criteria less stringent than the CWA 304(a) criteria, but EPA has not included continuing approval of less stringent criteria as part of the proposed action considered in this consultation.

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This biological opinion does not evaluate or provide ESA section 7 compliance for past EPA actions involving cyanide criteria for water quality standards or the past, present, and future effects of those actions on listed species and critical habitats. Past actions and their effects are relevant to describing the Status and Environmental Baseline of listed species and critical habitats. In this biological opinion the Service evaluates the proposed action in the context of EPA's continuing oversight role under the CWA and its discretion and authority to approve or promulgate State and Tribal water quality standards and affect implementation of CWA programs to achieve the goals and requirements of the CWA and the requirements of section 7(a)(2) of the ESA.

Interrelated and Interdependent Activities

The effects of EPA's approval or promulgation of cyanide criteria in State and Tribal water quality standards must be understood in the larger context of the CWA. This larger context is framed by Congress' stated objective, goals, and policies of the CWA and the programs and activities authorized by the CWA and implemented by EPA, States, Tribes and local governments.

The objective of the CWA is to restore and maintain the chemical, physical, and biological integrity of the Nation's waters (CWA section 101(a)). In order to achieve this objective, the CWA articulates goals to ultimately eliminate the discharge of pollutants into navigable waters, with an interim goal of water quality that provides for the protection and propagation of fish, shellfish, and wildlife. Congress also articulated in section 101 of the CWA its policy to prohibit the discharge of pollutants in toxic amounts, and its policy that programs be developed and implemented so that the goals of the CWA could be met through the control of point and nonpoint sources of pollution.

Water quality standards are the States' and Tribes' goals for individual water bodies and provide the regulatory basis for control decisions under the CWA (40 CFR 130.0(b)). States, Tribes and EPA plan and implement CWA programs to manage point and non-point sources of pollution to attain the goals established in State and Tribal water quality standards. Control measures are implemented by issuing permits, building publicly-owned treatment facilities, instituting best management practices for nonpoint sources of pollution, and other means.

In the Water Quality Standards Handbook (EPA 1994), EPA illustrates how the various elements of the CWA and its regulations work together to manage pollution. Figure 1, reproduced from the handbook, describes the eight stages of the water quality-based approach to pollution control. Each stage represents a major CWA program with specific regulatory requirements and guidance. This figure is intended to illustrate how the different programs fit into the overall water quality control scheme.

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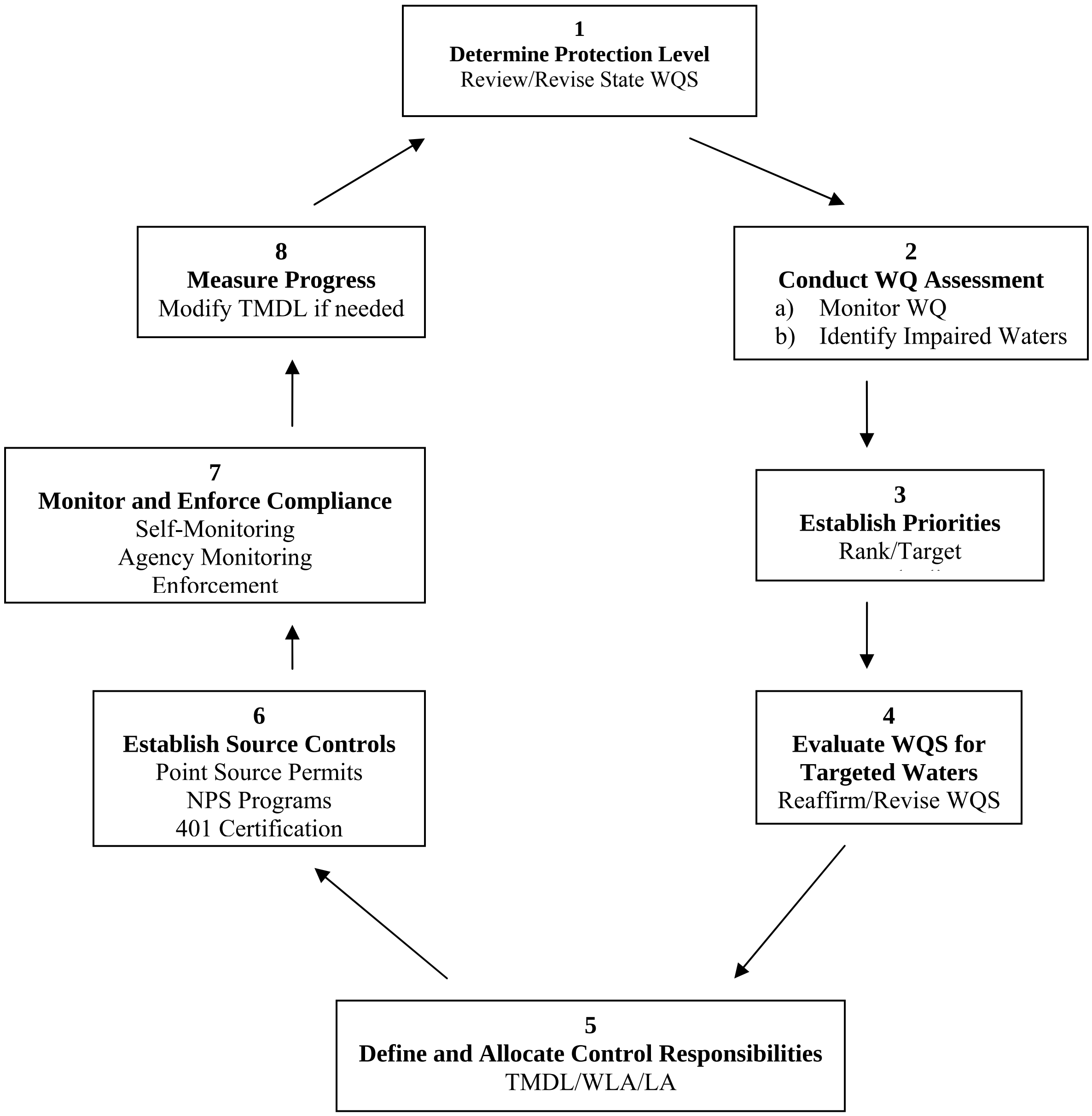
Figure 1. Eight stages of EPA's approach to water quality-based pollution control.



1. **Determine Protection Level**. States and Tribes adopt, and EPA approves, water quality standards to protect public health or welfare, enhance the quality of water, and serve the purposes of the CWA. A water quality standard defines the water quality goals of a water body, or portion thereof, by designating a use or uses, by setting criteria necessary to protect the uses, and by preventing degradation of water quality through antidegradation provisions. States and Tribes may, at their discretion, include general policies which allow for exceedances in discharges

under specific circumstances. Development and review/revision of standards is directed by the Water Quality Standards Regulation (40 CFR part 131), described in greater detail below.

2. **Conduct Water Quality Assessment**. Once State and Tribal water quality standards establish the level of protection to be afforded to a water body, States and Tribes are required to conduct water quality monitoring to identify waters not meeting the standards. Section 305(b) of the CWA requires States and Tribes to prepare a water quality inventory every 2 years to document the status of water bodies that have been assessed. Under section 304(l), States and Tribes identify all surface waters adversely affected by toxic, conventional, and nonconventional pollutants from point and non-point sources. Under section 314(a), States and Tribes identify publicly-owned lakes for which uses are known to be impaired by point and non-point sources. Section 319 requires States and Tribes to perform nonpoint source (NPS) assessments of navigable waters, including the identification of impaired and threatened waters and the activities causing impairment. NPS assessment reports and management programs are subject to EPA approval and oversight. The collective assessment efforts contribute to the States' and Tribes' section 303(d) listing of waters for which effluent limitations and other pollution control requirements are not stringent enough to implement a water quality standard.

3. **Establish Priorities**. Once waters needing additional controls have been identified, States and Tribes are required to submit for EPA review their priority rankings of waters in need of total maximum daily load (TMDL) development.

4. **Evaluate WQS for Targeted Waters**. At this point in the water quality management process, States and Tribes have targeted priority water quality-limited water bodies. EPA recommends that States and Tribes re-evaluate the appropriateness of the water quality standards for the targeted waters if: 1) States or Tribes have not conducted in-depth analyses of appropriate uses and criteria; 2) changes in the uses of the water body may require changes in the standard; 3) more recent water quality monitoring show the standard is being met; and, 4) site-specific criteria may be appropriate because of specific local environmental conditions or the presence of species more or less sensitive than those included in the national criteria data set.

5. **Define and Allocate Control Responsibilities**. For water quality limited waters, States and Tribes must establish a TMDL that quantifies pollutant sources, and a margin of safety, and allocates allowable loads to the contributing point and non-point source discharges so that the water quality standards are attained. EPA recommends States and Tribes develop TMDLs on a watershed basis.

6. **Establish Source Controls**. Once a TMDL has been established and the appropriate source loads developed, implementation should proceed. The first step is to update the "water quality management plan," described below. Next, point

and nonpoint source controls should be implemented to meet waste load allocations and load allocations, respectively. The NPDES permitting process is used to limit effluent from point sources. Construction decisions regarding publicly-owned treatment works must also be based on the more stringent of technology-based or water quality-based limitations. In the case of nonpoint sources, State, Tribal and local laws may authorize the implementation of nonpoint source controls, such as best management practices (BMPs) or other management measures.

7. **Monitor and Enforce Compliance**. Monitoring is essential to water quality-based decision making. Point source dischargers are required to provide reports on compliance with NPDES permit limits. A monitoring requirement can be put into the permit as a special condition as long as the information is collected for the purposes of writing a permit limit. Effective monitoring programs are also required for evaluating nonpoint source control measures and EPA provides guidance in implementing and evaluating nonpoint source control measures. EPA and States and Tribes are authorized to bring civil or criminal action against facilities that violate their NPDES permits. State nonpoint source programs are enforced under State law and to the extent provided by State law.

8. **Measure Progress**. If the water body achieves the applicable State or Tribal water quality standards it may be removed from the section 303(d) list of waters needing TMDLs. If water quality standards are not met, the TMDL and allocation of load and waste loads must be modified.

The water quality-based approach to pollution control is implemented by EPA, and the States and Tribes consistent with specific statutory and regulatory requirements. The regulatory requirements are articulated in: the *Water Quality Planning and Management* regulation (40 CFR 130) (50 FR 1779, January 11, 1985, as amended 54 FR 14359 April 11, 1989; 57 FR 33049, July 24, 1992; 59 FR 13817, March 23, 1994; 65 FR 17170, March 31, 2000; and, 66 FR 53048, October 18, 2001); and the *Water Quality Standards* regulation (40 CFR 131)(48 FR 51405, November 8, 1983, as amended 56 FR 64893, December 12, 1991; 59 FR 64344, December 14, 1994; 60 FR 15386, March 23, 1995; 65 FR 24653, April 27, 2000). Separately, the *Water Quality Guidance for the Great Lakes System* regulation (40 CFR 132)(60 FR 15387, March 23, 1995, as amended 65 FR 47874, August 4, 2000; 65 FR 59737, October 6, 2000; 65 FR 66511, November 6, 2000; 65 FR 67650, November 13, 2000) constitutes the guidance for States in the Great Lakes system; and other regulations promulgated by EPA.

State Water Quality Standards

The CWA directs States and authorized Tribes to adopt water quality standards for all their waters and submit their standards to EPA for review and approval or disapproval (of all or part)(CWA section 303(a)(1)-(2), (c)). The CWA further requires that a State shall at least once every three years hold public hearings for the purpose of reviewing applicable water quality standards and, as appropriate, modifying and adopting standards (i.e., conduct triennial reviews). Consultation with EPA is one of the first steps States and Tribes take when beginning the triennial review process (EPA 1994). The triennial review process is a forum through which EPA would review and approve or disapprove proposed revisions to State and Tribal water quality standards.

By statute, State and Tribal water quality standards consist of the designated uses of waters and the water quality criteria needed to achieve the designated uses. Section 303(d) and 118(c) of the CWA requires States and Tribes to establish an antidegradation policy. The *Water Quality Standards Regulation* (40 CFR part 131) defines the regulatory requirements of the water quality standards program and adds a provision for States and Tribes to adopt general policies that affect the application and implementation of State and Tribal water quality standards. For example, States and Tribes may adopt policies concerning mixing zones, water quality standards variances, and critical flows for water quality-based permit limits. General policies provide a mechanism for permitting a discharge where numeric water quality criteria may be exceeded, or low flows below which criteria do not apply, or where a standard is not being attained, a variance from the standard would enable permit issuance as long as progress towards attainment is being made. The designated uses, criteria, antidegradation policy, and general policies, together, define the level of protection afforded to a water body or portion thereof under the CWA.

In a January 27, 2005, memorandum (EPA 2005) EPA concluded that ESA section 7 consultation does not apply to EPA's approvals of State and Tribal antidegradation policies because EPA's approval action does not meet the "Applicability" standard defined in the regulations implementing section 7 of the ESA (50 CFR 402.03). Section 402.03 of the ESA section 7 consultation regulations (50 CFR part 402) states that section 7 and the requirements of 50 CFR part 402 apply to all actions in which there is discretionary Federal involvement or control. EPA concluded that they are compelled to approve State and Tribal antidegradation policies if State or Tribal submissions meet all applicable requirements of the *Water Quality Standards Regulation* (40 CFR part 131) and lack discretion to implement measures that would benefit listed species. Consequently, in its analyses the Service can not rely on antidegradation policies in State and Tribal water quality standards to provide protection over and beyond approved water quality criteria. EPA will treat new or revised antidegradation policies as being inapplicable to consultation and thus existing antidegradation policies can be changed by States or Tribes independent of any review of existing water quality criteria.

<u>Water Quality Monitoring</u>

States and Tribes are required to establish water quality monitoring, including collection and analysis of physical, chemical, and biological data to determine abatement and control priorities; developing and reviewing water quality standards, total maximum daily loads, wasteload allocations and load allocations; assessing compliance with NPDES permits by dischargers; reporting information to the public through the section 305(b) report and reviewing site-specific monitoring efforts (40 CFR 130.4).

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Continuing Planning Process

Section 303(e) of the CWA requires States and Tribes to have in place a "continuing planning process" (CPP) approved by EPA and requires EPA to periodically review a State's or Tribe's planning process for conformity to the requirements of the CWA. Section 303(e)(3) states that EPA shall approve any CPP that will result in plans for all navigable waters that include, but are not limited to, eight elements. EPA's regulations implementing this section (40 CFR 130) add a ninth element:

1. effluent limitations and schedules of compliance at least as stringent as those required by section 301(b)(1), section 301(b)(2), section 306, and section 307, and at least as stringent as any requirements contained in any applicable water quality standard in effect under section 303;
2. the incorporation of all elements of any applicable areawide waste management plans (CWA section 208) and applicable basin plans (CWA section 209);
3. total maximum daily load for pollutants in accordance with section 303(d);
4. procedures for revision;
5. adequate authority for intergovernmental cooperation;
6. adequate implementation, including schedules of compliance, for revised or new water quality standards under CWA section 303(c);
7. controls over the disposition of all residual waste from any water treatment processing;
8. an inventory and priority ranking of need for waste treatment works to meet applicable requirements of CWA sections 301 and 302; and,
9. a process for determining the priority of permit issuance.

By statute and regulation, an approved CPP is a prerequisite for EPA's approval of a permitting program under Title IV of the CWA.

Water Quality Management Plans

The CWA and its implementing regulations (40 CFR 130) require States and Tribes to establish Water Quality Management (WQM) Plans to prioritize and direct implementation of CWA programs. The following are required elements of State or Tribal WQM Plans:

1. total maximum daily loads;
2. effluent limitations;
3. municipal and industrial waste treatment;
4. nonpoint source management and control;
5. identification of management agencies capable of carrying out responsibilities;
6. implementation measures necessary to carry out the plan;
7. programs for the control of dredge or fill material;
8. identification of relationship to basin plans developed under CWA section 209;
9. identification of programs for control of ground water pollution.

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WQM Plans are produced and regularly updated on the basis of water quality assessments. Consistency with WQM Plans is a requirement for issuance of permits under CWA section 402 and grants made under the municipal construction grants program. States and Tribes must certify by letter for EPA approval that WQM Plan updates are consistent with all other parts of the plan (40 CFR 130).

In summary, the effects of EPA's approval or promulgation of cyanide criteria in State or Tribal water quality standards must be understood in the larger context of the CWA. This larger context is framed by Congress' stated objective, goals, and policies of the CWA and the programs and activities authorized by the CWA and implemented by EPA, States, Tribes and local governments to achieve these objectives, goals, and policies.

State water quality standards establish the level of protection afforded to water bodies or water body segments. Water quality standards include the designated uses assigned to water bodies, criteria to achieve the designated uses, an antidegradation policy to protect higher quality waters, and general policies which provide for exceedances of criteria values in specified circumstances. The approval or promulgation of cyanide criteria is either interrelated to and/or interdependent with the other elements of State or Tribal water quality standards. The proposed action analyzed in this biological opinion must be compared with the water quality standards as they are approved for individual States and Tribes. Separate, tiered consultations would be necessary to address discrepancies and quantify and exempt incidental take, as appropriate.

The programs of the water quality-based approach to pollution control are targeted to assessing compliance with standards and instituting changes to achieve compliance through modifications to allowable discharges or to the standards themselves. The adaptive management of water quality, articulated in the water quality-based approach to pollution control, is driven by continuing planning processes and water quality management planning and implementation. Because the planning and implementation of CWA programs by EPA, States, Tribes and local governments is targeted towards achieving State or Tribal water quality standards, these activities are necessarily part of a larger action, and thus interrelated to the approval of criteria in State or Tribal water quality standards.

5.0 ACTION AREA

The action area for this consultation consists of all waters subject to the CWA, including "territorial seas," which extend seaward a distance of three miles from the coast, and habitats effected by administration of the CWA. The action area also includes all other parts of watersheds that serve as sources to waters subject to the CWA where activities contribute to point and non-point sources of pollution and are subject to compliance with the CWA. This action area includes such waters and habitats within and surrounding Indian country, the 50 States, and all U.S. territories.

6.0 STATUS OF THE SPECIES AND CRITICAL HABITAT

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Information on the status of affected listed species and critical habitat is presented in two parts. The species and critical habitats considered and the states encompassing their distribution are listed in Table 1, and individual species and critical habitat accounts are presented in Appendix A. Because the action area for this consultation encompasses the entire range of affected listed species and critical habitats, no Environmental Baseline section is needed or presented.

In the Status of the Species and Critical Habitat section of a biological opinion the Service presents biological or ecological information relevant to formulating the opinion, and characterizes the current condition of the species or critical habitat, the factors responsible for that condition, and the survival and recovery needs of the species and the intended conservation function of critical habitat. This information is derived from listing documents and recovery plans or subsequent biological opinions as new information becomes available. The Status of the Species and Critical Habitat evaluation establishes key biological context for evaluating the effects of a proposed Federal action and any cumulative effects for purposes of making an ESA section 7(a)(2) determination(s).

Aquatic life criteria address the CWA goals and policy of attaining water quality that provides for the protection and propagation of fish, shellfish, and wildlife, and prohibiting the discharge of toxic pollutants in toxic amounts (CWA Sec. 101(a)(2) & (3)). The CWA policy of prohibiting the discharge of toxic pollutants in toxic amounts provides a foundation for characterizing EPA's discretion in recommending aquatic life cyanide criteria under CWA section 304(a) and approval of cyanide criteria in state water quality standards. Although most approved water quality standards include a statement prohibiting the discharge of toxic pollutants in toxic amounts, the criteria by which this standard presumably is enforced, the CCC and the CMC, are defined in such a way that adverse effects are limited but not prohibited. \

To fully assess the impacts of the proposed action, it is important to consider the status of the species and the current state of their aquatic environments as this provides a context for the jeopardy analysis. Although the baseline condition includes the past and present impacts of activities in the action area, it does not include the future impacts of the action under review in this consultation. Because the action area for this consultation (i.e. all waters of the U.S., including territories) encompasses the entire ranges for the affected listed species and critical habitats, it is difficult to fully assess 1) the past and present impacts of all Federal, State, or private actions and other human activities in the action area; 2) the anticipated impacts of all proposed Federal projects in the action area that have already undergone formal or early section 7 consultation; and 3) the impact of State or private actions occurring simultaneously with this consultation for each species included in this opinion. As such, we will broadly describe the status of the aquatic environments within the action area as a means for establishing a baseline condition from which to analyze the effect of the action.

Section 304(a)(1) of the CWA requires EPA to develop criteria for water quality for the protection of aquatic life as well as for human health. EPA develops these criteria as numeric limits on the amounts of chemicals that can be present in river, lake, or stream water to protect aquatic organisms from death, slower growth, reduced reproduction, and the accumulation of harmful levels of toxic chemicals in their tissues that may also adversely affect consumers of such organisms. Aquatic life criteria have been developed for almost 50 pollutants to date and serve as a basis for the development of state water quality standards. Waters that do not meet these standards are considered to be "impaired" under section 303(d) of the CWA. The most recent National Water Quality Inventory Report to Congress (EPA 2009) presents a summary finding of the 2004 state water quality reports covering 16% of the nation's 3.5 million miles of rivers and streams, 39% of the nation's 41.7 million acres of lakes, ponds, and reservoirs, and 29% of the nation's 87,791 square miles of bays and estuaries. For waters included in the assessment, 44% of the rivers and streams, 64% of the lakes ponds and reservoirs, and 29% of the bays and estuaries were reported as impaired or not clean enough to support their designated uses.

The sources of degradation vary by water type but included pathogens, habitat alterations, mercury, and organic enrichment/oxygen depletion from both known and unknown or unspecified sources. An assessment by the U.S. Geological Survey (USGS) on the presence and concentration of pesticide in the nation's streams and ground water found that concentrations of pesticides were frequently greater than water-quality benchmarks for aquatic life and fish eating wildlife. Specifically, USGS found that 57% of 83 agricultural streams, 83% of 30 urban streams, and 42% of 65 mixed-land-use streams had concentrations of at least one pesticide that exceeded one or more aquatic-life benchmarks at least one time during the year. The most frequently detected pesticide compounds included atrazine, deethylatrazine, and metolachlor (USGS 2006).

Over 83,000 chemicals are currently listed in the Toxic Substances Control Act Inventory and more than 87,000 chemicals including pesticides, commodity chemicals, naturally occurring non-steroidal estrogens, food additives, cosmetics, nutritional supplements, and representative mixtures have been identified as potential endocrine disruptors by the Endocrine Disruptor Screening and Testing Advisory Committee (1998) convened by EPA. For most of these chemicals, scientific data is insufficient or unavailable for evaluating their potential individual and combined impacts on aquatic environments.

Table 1. Listed species and critical habitats considered in this biological opinion along with their listing status and distribution on a state-by-state basis. E=endangered; T=threatened; CH=critical habitat; XN=(non-essential experimental population).

Common Name	Scientific Name	Status	Distribution
	FISH		
	Acipenseridae		
Gulf sturgeon	*Acipenser oxyrinchus desotoi*	T, CH	AL, FL, LA, MS
Kootenai River white sturgeon	*A. transmontanus*	E, CH	ID, MT
Pallid sturgeon	*Scaphirhynchus albus*	E	AR, IA, IL, KS, KY, LA, MO, MS, MT, ND, NE, SD, TN

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Common Name	Scientific Name	Status	Distribution
Alabama sturgeon	*S. suttkusi*	E, CH	AL, MS
	Amblyopsidae		
Ozark cavefish	*Amblyopsis rosae*	T	AR, MO, OK
Alabama cavefish	*Speoplatyrhinus poulsoni*	E, CH	AL
	Atherinidae		
Waccamaw silverside	*Menidia extensa*	T, CH	NC
	Catostomidae		
Modoc sucker	*Catostomus microps*	E, CH	CA, OR
Santa Ana sucker	*Catostomus santaanae*	T, CH	CA
Warner sucker	*Catostomus warnerensis*	T, CH	OR
Shortnose sucker	*Chasmistes brevirostris*	E	CA, OR
Cui-ui	*Chasmistes cujus*	E	NV
June sucker	*Chasmistes liorus*	E, CH	UT
Lost River sucker	*Deltistes luxatus*	E	CA, OR
Razorback sucker	*Xyrauchen texanus*	E, CH	AZ, CA, CO, NM, NV, UT
	Cotidae		
Pygmy sculpin	*Cottus paulus*	T	AL
	Cyprinidae		
Blue shiner	*Cyprinella caerulea*	T	AL, GA, TN
Beautiful shiner	*Cyprinella Formosa*	T, CH	AZ, NM
Devils River minnow	*Dionda diaboli*	T, CH	TX
Spotfin chub	*Erimonax monachus*	T, CH, XN	AL, NC, TN, VA
Slender chub	*Erimystax cahni*	T, CH, XN	TN, VA
Mojave tui chub	*Gila bicolor mohavensis*	E	CA
Owens tui chub	*Gila bicolor snyderi*	E, CH	CA
Borax Lake chub	*Gila boraxobius*	E, CH	OR
Humpback chub	*Gila cypha*	E, CH	AZ, CO, UT
Sonora chub	*Gila ditaenia*	T, CH	AZ
Bonytail chub	*Gila elegans*	E, CH	AZ, CA, CO, NV, UT
Gila chub	*Gila intermedia*	E, CH	AZ, NM
Yaqui chub	*Gila purpurea*	E, CH	AZ
Pahranagat roundtail chub	*Gila robusta jordani*	E	NV
Virgin River Chub	*Gila seminuda (=robusta)*	E, CH	AZ, NV, UT
Rio Grande silvery minnow	*Hybognathus amarus*	E, CH	NM, TX
Big Spring spinedace	*Lepidomeda mollispinis pratensis*	T, CH	NV
Little Colorado spinedace	*Lepidomeda vittata*	T, CH	AZ
Spikedace	*Meda fulgida*	T, CH	AZ, NM
Moapa dace	*Moapa coriacea*	E	NV
Palezone shiner	*Notropis albizonatus*	E	AL, KY
Cahaba shiner	*Notropis cahabae*	E	AL
Arkansas River shiner	*Notropis girardi*	T, CH	AR, KS, NM, OK,

Common Name	Scientific Name	Status	Distribution
			TX
Cape Fear shiner	*Notropis mekistocholas*	E, CH	NC
Pecos bluntnose shiner	*Notropis simus pecosensis*	T, CH	NM
Topeka shiner	*Notropis topeka (=tristis)*	E, CH	IA, KS, MN, MO, NE, SD
Oregon chub	*Oregonichthys crameri*	E	OR
Blackside dace	*Phoxinus cumberlandensis*	T	KY, TN
Woundfin	*Plagopterus argentissimus*	E; CH, XN	AZ, NM, NV, UT
Colorado pikeminnow (=squawfish)	*Ptychocheilus lucius*	E; CH, XN	AZ, CA, CO, NM, UT
Ash Meadows speckled dace	*Rhinichthys osculus nevadensis*	E, CH	NV
Kendall Warm Springs dace	*Rhinichthys osculus thermalis*	E	WY
Loach minnow	*Tiaroga cobitis*	T, CH	AZ, NM
	Gasterosteidae		
Unarmored threespine stickleback	*Gasterosteus aculeatus williamsoni*	E	CA
	Gobiidae		
Tidewater goby	*Eucyclogobius newberryi*	T, CH	CA
	Goodeidae		
White River springfish	*Crenichthys baileyi baileyi*	E, CH	NV
Hiko White River springfish	*Crenichthys baileyi grandis*	E, CH	NV
Railroad Valley springfish	*Crenichthys nevadae*	T, CH	NV
	Osmeridae		
Delta smelt	*Hypomesus transpacificus*	T, CH	CA
	Percidae		
Slackwater darter	*Etheostoma boschungi*	T, CH	AL, TN
Vermilion darter	*Etheostoma chermocki*	E	AL
Relict darter	*E. chienense*	E	KY
Etowah darter	*E. etowahae*	E	GA
Fountain darter	*E. fonticola*	E, CH	TX
Niangua darter[1]	*E. nianguae*	T, CH	MO
Watercress darter	*E. nuchale*	E	AL
Okaloosa darter	*E. okaloosae*	E	FL
Duskytail darter	*E. percnurum*	E, XN	KY, TN, VA
Bayou darter	*E. rubrum*	T	MS
Cherokee darter	*E. scotti*	T	GA
Maryland darter	*E. sellare*	E, CH	MD
Bluemask darter	*E. sp.*	E	TN
Boulder darter	*E. wapiti*	E, XN	AL, TN
Amber darter	*Percina antesella*	E, CH	GA, TN
Goldline darter	*P. aurolineata*	T	AL, GA

Common Name	Scientific Name	Status	Distribution
Conasauga logperch	*P. jenkinsi*	E, CH	GA, TN
Leopard darter	*P. pantherina*	T, CH	AR, OK
Roanoke logperch	*P. rex*	E	VA, NC
Snail darter	*P. tanasi*	T, CH	AL, GA, TN
	Poeciliidae		
Big Bend gambusia	*Gambusia gaigei*	E	TX
San Marcos gambusia	*Gambusia georgei*	E, CH	TX
Clear Creek gambusia	*Gambusia heterochir*	E	TX
Pecos gambusia	*Gambusia nobilis*	E	NM, TX
Gila topminnow	*Poeciliopsis occidentalis*	E	AZ, NM
	Salmonidae		
Bull trout	*Salvelinus confluentus*	T, CH	CA, ID, MT, NV, OR, WA
Little Kern Golden trout	*Oncorhynchus aquabonita whitei*	T, CH	CA
Apache trout	*O. apache*	T	AZ
Lahontan Cutthroat trout	*O. clarkii henshawi*	T	CA, NV, OR, UT
Paiute Cutthroat trout	*O. clarkii seleniris*	T	CA
Greenback Cutthroat Mountain trout	*O. clarkii stomias*	T	CO
Gila trout	*O. gilae*	T	AZ, NM
Atlantic salmon	*Salmo salar*	E	ME
	AMPHIPODS		
Illinois cave amphipod	*Gammarus acherondytes*	E	IL
Noel's amphipod	*G. desperatus*	E	NM
	MUSSELS		
Cumberland elktoe	*Alasmidonta atropurpurea*	E, CH	KY, TN
Dwarf wedgemussel	*A. heterodon*	E	CT, MA, MD, NC, NH, NJ, NY, PA, VA, VT
Appalachian elktoe	*A. raveneliana*	E, CH	NC, TN
Fat three-ridge	*Amblema neislerii*	E, CH	FL, GA
Ouachita rock pocketbook	*Arkansia wheeleri*	E	AR, OK
Birdwing pearlymussel	*Conradilla caelata*	E, XN	AL, TN, VA
Fanshell	*Cyprogenia stegaria*	E, XN	AL, IL, IN, KY, OH, TN, VA, WV
Dromedary pearlymussel	*Dromus dromas*	E, XN	AL, KY, TN, VA
Chipola slabshell	*Elliptio chipolaensis*	T, CH	AL, FL
Tar River spinymussel	*E. steinstansana*	E	NC
Purple bankclimber	*Elliptoideus sloatianus*	T, CH	FL, GA
Cumberlandian combshell	*Epioblasma brevidens*	E, XN, CH	AL, KY, TN, VA
Oyster mussel	*E. capsaeformis*	E, XN, CH	AL, GA, KY, NC, TN, VA

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Common Name	Scientific Name	Status	Distribution
Curtis pearlymussel	*(E. florentina curtisii)*	E	AR, MO
Yellow blossom	*E. florentina florentina*	E, XN	AL, TN
Tan riffleshell	*E. florentina walkeri*	E	KY, TN, VA
Upland combshell	*E. metastriata*	E, CH	AL, GA, TN
Catspaw	*E. obliquata obliquata*	E, XN	AL, IL, IN, KY, OH, TN
White catspaw	*E. o. perobliqua*	E	IN, OH
Southern acornshell	*E. othcaloogensis*	E, CH	AL, GA, TN
Southern combshell	*E. penita*	E	AL, MS
Green blossom	*E. torulosa gubernaculums*	E	TN, VA
Northern riffleshell	*E. t. rangiana*	E	IN, KY, MI, OH, PA, WV
Tubercled blossom	*E.a torulosa torulosa*	E, XN	AL, IL, IN, KY, TN, WV
Turgid blossom	*Epioblasma turgidula*	E, XN	AL, TN
Shiny pigtoe	*Fusconaia cor*	E, XN	AL, TN, VA
Finerayed pigtoe	*Fusconaia cuneolus*	E, XN	AL, TN, VA
Cracking pearlymussel	*Hemistena lata*	E, XN	AL, IN, KY, PA, TN, VA
Pink mucket	*Lampsilis abrupta*	E	AL, AR, IL, IN, KY, LA, MO, OH, PA, TN, WV
Fine-lined pocketbook	*Lampsilis altilis*	T, CH	AL, GA, TN
Higgins eye	*Lampsilis higginsii*	E	IA, IL, MN, MO, WI
Orangenacre mucket	*Lampsilis perovalis*	T, CH	AL, MS
Arkansas fatmucket	*Lampsilis powelli*	T	AR
Speckled pocketbook	*Lampsilis streckeri*	E	AR
Shinyrayed pocketbook	*Lampsilis subangulata*	E, CH	AL, FL, GA
Alabama lampmussel	*Lampsilis virescens*	E, XN	AL, TN
Carolina heelsplitter	*Lasmigona decorata*	E, CH	NC, SC
Scaleshell mussel	*Leptodea leptodon*	E	AR, MO, OK
Louisiana pearlshell	*Margaritifera hembeli*	T	LA
Alabama moccasinshell	*Medionidus acutissimus*	T, CH	AL, GA, MS
Coosa moccasinshell [1]	*Medionidus parvulus*	E, CH	GA, TN
Gulf moccasinshell	*Medionidus penicillatus*	E, CH	FL, GA
Ochlockonee moccasinshell	*Medionidus simpsonianus*	E, CH	FL, GA
Ring pink	*Obovaria retusa*	E, XN	AL, IN, KY, PA, TN
Littlewing pearlymussel	*Pegias fibula*	E	KY, NC, TN, VA
White wartyback pearlymussel	*Plethobasus cicatricosu*	E, XN	AL, IN, KY, TN
Orangefoot pimpleback	*Plethobasus cooperianus*	E, XN	AL, IL, IN, KY, PA, TN
Clubshell	*Pleurobema clava*	E, XN	AL, IN, KY, MI, OH, PA, WV
James spinymussel	*Pleurobema collina*	E	NC, VA, WV
Black clubshell	*Pleurobema curtum*	E	MS

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Common Name	Scientific Name	Status	Distribution
Southern clubshell	*Pleurobema decisum*	E, CH	AL, GA, MS
Dark pigtoe	*Pleurobema furvum*	E, CH	AL
Southern pigtoe	*Pleurobema georgianum*	E, CH	AL, GA, TN
Cumberland pigtoe	*Pleurobema gibberum*	E	TN
Flat pigtoe	*Pleurobema marshalli*	E	AL, MS
Ovate clubshell	*Pleurobema perovatum*	E, CH	AL, MS
Rough pigtoe	*Pleurobema plenum*	E, XN	AL, IN, KY, PA, TN, VA
Oval pigtoe	*Pleurobema pyriforme*	E, CH	FL, GA
Heavy pigtoe	*Pleurobema taitianum*	E	AL
Fat pocketbook	*Potamilus capax*	E	AR, IL, IN, KY, LA, MO, MS
Alabama heelsplitter	*Potamilus inflatus*	T	AL, LA
Triangular kidneyshell	*Ptychobranchus greeni*	E, CH	AL, GA, TN
Rough rabbitsfoot	*Quadrula cylindrica strigillata*	E, CH	TN, VA
Winged mapleleaf	*Quadrula fragosa*	E, XN	AR, MN, OK, WI
Cumberland monkeyface	*Quadrula intermedia*	E, XN	AL, TN, VA
Appalachian monkeyface	*Quadrula sparsa*	E, XN	TN, VA
Stirrupshell	*Quadrula stapes*	E	AL, MS
Pale lilliput	*Toxolasma cylindrellus*	E	AL, TN
Purple bean	*Villosa perpurpurea*	E, CH	TN, VA
Cumberland bean	*Villosa trabalis*	E, XN	AL, KY, TN, VA
	AMPHIBIANS		
	Ambystomatidae		
Reticulated flatwoods salamander	*Ambystoma bishop*	E, CH	AL, FL, GA
Frosted flatwoods Salamander	*A. cingulatum*	T, CH	FL, GA, SC
California tiger salamander	*A. californiese* Central California DPS	T, CH	CA
California tiger salamander	*A. californiese* Santa Barbara County DPS	E, CH	CA
California tiger salamander	*A. californiense* Sonoma County DPS	E	CA
Santa Cruz long-toed salamander	*A. macrodactylum croceum*	E	CA
Sonora tiger salamander	*A. tigrinum stebbinsi*	E	AZ
	Plethodontidae		
San Marcos salamander	*Eurycea nana*	T, CH	TX
Barton Springs salamander	*E. sosorum*	E	TX
Texas blind salamander	*Typhlomolge rathbuni*	E	TX
	Bufonidae		
Wyoming toad	*Bufo baxteri*	E	WY

Common Name	Scientific Name	Status	Distribution
Arroyo toad	*B. californicus*	E, CH	CA
Houston toad	*B. houstonensis*	E, CH	TX
	Eleutherodactylidae		
Guajon	*Eleutherodactylus cooki*	T, CH	PR
	Ranidae		
California red-legged frog	*Rana aurora draytonii*	T, CH	CA
Chiricahua leopard Frog	*R. chiricahuensis*	T	AZ, NM
Mountain yellow-legged frog	*R. muscosa*	E, CH	CA, NV

7.0 EFFECTS OF THE ACTION

Our analysis of the effects of the proposed action on listed species and critical habitats includes a discussion of the sources of cyanide, an explanation of our assumptions about cyanide exposure, an overview of cyanide toxicity, an analysis of effects to listed fish, followed by analyses of effects to listed amphibians and invertebrates including freshwater mussels. The taxon-specific analyses (i.e. fish, amphibians, invertebrates, mussels) considered information on cyanide toxicity, factors influencing toxicity, approaches for estimating effects on individuals, and discussions of population-level responses. Our analyses were dependent on the available information, which varied among taxa. For fish, the available chronic toxicity data enabled us to develop a method for estimating the magnitude of effect on fish species. For other taxa we exercised best professional judgement to estimate effects based on existing data. These sections provide the basis for our discussion of effects to individual listed species and their designated critical habitat.

General Sources of Cyanide

Cyanide is ubiquitous in the environment, and enters waterways as a point source and nonpoint source of pollution. Cyanide is produced synthetically to support industrial uses, it is found naturally in such foods as oil of bitter almonds, cassava, cherry pits, and various microorganisms produce cyanide (Leduc 1984, Eisler 2000, Dzombak et al. 2006). Cyanides are used widely in ore-extraction, steel and heavy metal industries (e.g., electroplating), the manufacture of synthetic fabrics and plastics, some chlorination treatment plants, as a pesticide and as an intermediate ingredient in herbicides, in road salts, and until recently some fire retardants (see Table 2). Anthropogenic sources contribute the vast majority of cyanide in the environment. Certain activities directly release cyanide into the environment, or cyanide may be produced as the byproduct of activities. Industrial activities that produce cyanide as a byproduct include municipal waste and sludge incineration and coking and gasification of coal, to name a few. Metal industries and organic chemical industries are major contributors of cyanide into the freshwater aquatic environment, whereas, atmospheric cyanide, a by-product of forests fires, may be the primary source of oceanic cyanide (Leduc 1984, EPA 2005, Dzombak et al. 2006).

Compared to storm water runoff, urban roadway snow exposed to traffic and winter maintenance practices has a much greater capacity to accumulate and retain heavy metals and other pollutants. In a study of urban highway sites, concentrations of cyanide and metals were orders of magnitude higher than at the control sites and exceeded storm water runoff concentrations by one to two orders of magnitude. Cyanide levels, although demonstrating some variability, remained relatively constant at all sites, averaging 154 µg/L, or cyanide concentrations increased according to the increased application of deicing salts that contained cyanide compounds as anti-caking agents (Glenn and Sansalone 2002).

A study on the effect of cyanide on the anaerobic treatment of synthetic wastewater noted that cyanide is produced on an industrial scale of 2–3 million tons per year and, therefore is in many different industrial waste waters. The concentrations encountered in industrial waste generally are in the range 0.01–10,000 mg/L, most of it in complexed species of cyanide, which are less toxic than free cyanide. Cyanide contamination also occurs in the processing of agricultural crops containing high concentrations of this compound, such as cassava (Gijzen et al. 2000). Systematic surveys of large wastewater effluents in Southern California support a low estimate of free cyanide in wastewaters. In different years reported from 1992 – 2002, mean cyanide concentrations in effluents ranged from <2 to 30 µg/L (Steinberger and Stein 2003).

A more widespread risk of cyanide poisoning of aquatic life is likely from biomass burning (e.g., burning waste biomass for energy conversion, crop burning, prescribed forest fires and wildfires). Barber et al. (2003) examined releases of cyanides from biomass burning and their effect on surface runoff water. In laboratory test burns, available cyanide concentrations in leachate from residual ash were much higher than in leachate from partially burned and unburned fuel and were similar to or higher than a 96-h median lethal concentration (LC50) for rainbow trout (45 µg/L). Free cyanide concentrations in stormwater runoff collected after a wildfire in North Carolina averaged 49 µg/L, an order of magnitude higher than in samples from an adjacent unburned area (Barber et al. 2003).

According to the Toxics Release Inventory, cyanide compound releases to land and water totaled about 1.5 million lbs. between 1987 and 1993, of which about 65 percent was to water. The largest releases (combined land and water releases) occurred in California and Pennsylvania. Releases in California were land-based, while the releases in Pennsylvania occurred primarily in water (EPA 2005). A review of STORET data in 1981, reported the mean cyanide concentration in surface waters of the United States was less than 3.5 µg/L, with concentrations in some industrial areas exceeding 200 µg/L (Dzombak et al. 2006). Coincidentally, production of HCN, one of most toxic forms of cyanide in water, has more than doubled in the United States since the early 1980s (from 330,000 tons per year in 1983 to 750,000 tons per year in 2001).

Table 2. Forms of cyanides and their uses.

Form	Uses	Reference
Cyanide salts (potassium	Steel manufacturing & heat-treating facilities Metal cleaning, electroplating	IPCS; Leduc 1984; EPA 2005

cyanide, sodium cyanide)	Ore-extraction (gold-mining, coke extraction)	IPCS; Leduc 1984; EPA 2005
	Dyeing, printing of photographs	IPCS; EPA 2005
	Production of resin monomers (acrylates)	IPCS
	Fire retardants[1]	Little and Calfee 2002
	Anti-caking agent for road salts	Dzombak et al. 2006
	Pharmaceuticals (antibiotics, steroids, chemotherapy)	Dzombak et al. 2006
Hydrogen cyanide, organocyanides, metallo-cyanide compounds	Fumigant/pesticide	IPCS, Dzombak et al. 2006
	Herbicides (dichlobenil, bromoxynil, bantrol)	EPA 2005, Dzombak et al. 2006
	Road salts	EPA 2005
	Production of other cyanides (e.g., sodium cyanide for gold mining)	EPA 2005, Dzombak et al. 2006
	Pyrolysis of paper, wool, polyurethane	IPCS
	Chelating agents for water and wastewater treatment	EPA 2005, Dzombak et al. 2006
	Production of clear plastics	Dzombak et al. 2006
	Methionine[2] for animal food supplement	Dzombak et al. 2006

[1] As of 2007 sodium ferrocyanide was no longer an accepted ingredient in fire retardants (Long Term Retardents) used in the U.S. (U.S. Forest Service. 2007. Specification 5100-304c Long Term Retardant, Wildland Firefighting)
[2] Hydrogen cyanide is used in the manufacturing of methionine

The risk to aquatic environments from cyanide releases depends on several factors including: the form and concentration of cyanide released, water pH, the presence of metallic trace elements like iron, degree of solar radiation, air and water temperatures, and the presence of natural cyanide sinks (Dzombak et al. 2006). Aqueous cyanide readily evolves from hydrogen cyanide, metallocyanide complexes, and organocyanides, and from metal-cyanide solids. Solid forms of cyanide may exist in the soil of sites for years, and once exposed to water may result in dissolved cyanide reaching ground water and eventually surface waters (see Dzombak et al.'s [2006] discussion about the industrial legacy of cyanide box wastes at thousands of former manufactured gas plants in the United States). Free cyanide readily biodegrades to carbon dioxide and the ammonia ion but its fate depends upon water temperatures, dissolved oxygen levels, mixing, and nutrients (Young et al. 2006).

While available free cyanide is the primary toxic agent in water and is the form expressed by the aquatic life criteria reported by EPA (1985; see Table 1), total cyanide is most commonly measured in discharges (EPA 1985, Dzombak et al. 2006). Measurements are frequently conducted via colorimetric, titrimetric, or electrochemical finish techniques (Dzombak et al. 2006). Measurements of total cyanide are limited to detection in a reagent water matrix of about 1 to 5 µg/L and do not measure: cyanates, thocyanates, most organic-cyanide compounds, and most cobalt and platinum cyanide complexes (Dzombak et al. 2006). Problems with sample storage, regulatory criteria, and the methods for testing and their sensitivity are a concern (Eisler 2000, Dzombak et al. 2006). Eisler (2000) notes that due to the volatilization of cyanide, periodic monitoring is not informative (for example, monitoring once per quarter [see EPA 2006]). Consequently, Eisler (2000) and others recommend that continuous monitoring systems are necessary, with particular emphasis on industrial dischargers, to understand the fate and transport, critical exposures, and relative contributions of human and natural sources of cyanide in the aquatic environment.

It should be noted that EPA's request for ESA section 7 consultation on the aquatic life criteria for cyanide is the first consultation request on its recommended aquatic life criteria. Cyanide will co-occur in waters of the United States in mixture with numerous other pollutants. These other pollutants may or may not have criteria established under section 304(a) of the CWA.

Basis for Assuming Exposure to Cyanide at Criteria Concentrations

We based our effects analysis in this Biological Opinion on the premise that the proposed action could be implemented fully. Listed aquatic species and their critical habitats could be exposed to cyanide concentrations in the water column at concentrations consistent with the CMC and CCC. These criteria, as approved by EPA in State and Tribal water quality standards, become the "protection level" to which the water quality-based approach to pollution control is applied. In freshwater systems, this protection level is met if the one-hour average concentration in waters does not exceed 22 ug/L cyanide more than once every three years on average; AND, the four-day average concentration does not exceed 5.2 ug/L cyanide more than once every three years on average. For saltwater systems, the one-hour average concentration should not exceed 1.0 ug/L more than once every three years on the average.

Our rationale for assuming exposure to cyanide at the CMC and CCC includes the following:

1. Section 7 consultation is future-oriented, focusing on the potential effects of the *proposed* action.
2. The CMC and CCC represent the basis for administering water quality programs under the water quality-based approach to pollution control, including monitoring to determine whether waters are attaining designated uses, 303(d) listing of impaired waters, and the development and implementation of TMDLs.
3. We can make no reasonable assumption that any additional protections afforded by antidegradation policies will persist in the future, given EPA's conclusion that section 7 consultation does not apply to EPA approval of new or revised antidegradation policies.
4. There are no other elements of the action proposed that would limit the timing, duration, frequency, or magnitude of exposure of listed species and critical habitats to cyanide pollution other than those defined by the CMC and CCC. General Policies could have the effect of relaxing limits on exposure.

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5. Approval of cyanide concentrations at the CMC and CCC can result in exposures that vary through time and space or occur uniformly throughout waterbodies. Exposure to cyanide pollution at the CMC and CCC could occur to portions of populations, whole populations, or the full range of a species.

Physiology of Cyanide Toxicity

The adverse effects of cyanide on fish and other aquatic species have been well documented (Leduc 1984, Eisler 2000, Gensemer et al. 2006, Lanno and Menzie 2006) and are discussed in subsequent sections of this document. Our knowledge of the basic mechanisms by which cyanide exerts its toxic effects, however, has been gained largely as a result of human health-related studies with rodents and other mammals. Recent reviews of the mammalian literature (ATSDR 2006, Borowitz et al. 2006) describe the physiological mechanisms for cyanide toxicity and are summarized below.

Cyanide is known to affect several organ systems including the central nervous system, heart, liver and kidney, although, the principal sites of action are the brain and heart. Cyanide is a potent respiratory toxin which inhibits mitochondrial respiration and can induce rapid lethal responses following acute exposures. Cyanide preferentially binds to the ferric iron atom of the metalloenzyme, cytochrome c oxidase, which is the terminal oxidase in the mitochondrial membrane respiratory chain (electron transport system). The binding of cyanide to the ferric iron atom inhibits the reaction by which electrons are transferred from reduced cytochrome to oxygen thereby blocking oxidative metabolism and ATP production. Blockage of oxidative metabolism can disrupt calcium homeostasis by stimulating calcium release from intracellular stores and enhance influx of extracellular calcium. Elevated cellular calcium activates numerous biochemical pathways including those that lead to nerve cell death via necrosis and apoptosis (programmed cell death). Cytotoxicity is common following acute lethal cyanide exposures. Impaired cellular oxygen utilization results in hypoxia, a shift from aerobic to anaerobic metabolism, and a build up of lactic acid. Because neural tissues have a high oxygen demand and cannot function under low oxygen, the brain is particularly sensitive. The effects of cellular hypoxia and lactate acidosis depress the central nervous system and, at lethal cyanide concentrations, lead to respiratory arrest and death.

Cyanide also binds to other metalloenzymes such as catalase, peroxidase, methemoglobin, hydroxocobalamin, phosphatase, tyrosinase, ascorbic acid oxidase, xanthine oxidase, and succinic dehydrogenase (ATSDR 2006). Modulation of these enzymes contributes to other manifestations of cyanide toxicity. For example, oxidative stress plays an important role in cyanide-induced neurotoxicity. Inhibition of antioxidant enzymes (e.g., catalase) promotes oxidative stress because these enzymes provide protection from the adverse effects (such as lipid peroxidation) that are caused by reactive oxygen species (ROS). In addition, elevated cellular calcium levels (described above) have been associated with increased concentrations of ROS (Borowitz et al. 2006). The effects of rising ROS and antioxident enzyme inhibition can exacerbate nerve cell damage. Cyanide can also cause the release of excitatory neurotransmitters (e.g., dopamine) in the brain and can initiate the release of catecholamines from the adrenals and adrenergic nerve terminals (Smith 1996).

Modulation of catecholamine biosynthesis and neurotransmitter release may help explain some of the acute and chronic neurological effects of cyanide (Borowitz et al. 2006).

The major route for cyanide elimination from the body is via enzyme-mediated thiocyanate formation in the kidney and excretion in urine. Rhodanase is the enzyme that catalyzes the transformation of thiosulfate to thiocyanate and its activity is limited by the availability of sulfur. Although thiocyanate (SCN^-) is the principle form of cyanide that is eliminated, it can also accumulate in tissues and is known to have antithyroidal properties. SCN^- inhibits iodine uptake by thyroid tissues and disrupts thyroid hormone homeostasis which can result in the development of goiter.

In the aquatic environment, the primary route of exposure for fish, amphibians, and aquatic invertebrates is via water. Cyanide enters the body through semipermiable membranes such as gills, egg capsules and other sites where gas exchange and osmoregulatory processes occur (Eisler 2000). Cyanide does not tend to bioaccumulate in aquatic biota so dietary uptake (at criteria concentrations) is not considered to be an important exposure pathway (Lanno and Menzie 2006).

7.1 Effects to Fish

Acute Toxicity to Fish

Knowledge of the acute lethal effects of cyanide on fish has been gained through observations following accidental spills, intentional field applications for lake/stream management, and controlled laboratory studies. Massive kills of fish and other aquatic organisms have been observed following accidental spills of cyanide from storage reservoirs, overturned rail tank cars, and other sources (Leduc 1984). Some of the most catastrophic releases have been from gold mine heap leaching operations, where cyanide is used in the gold extraction process (Wong-Chong et al. 2006). Releases from waste heap leaching pads and tailing storage ponds have discharged large quantities of cyanide into surface waters. In 1997, 245,000 gallons of cyanide solution leaked from a heap leach pad at the Gold Quarry Mine, NV into two nearby creeks, and 7 million gallons of treated leach solution (0.2 ppm cyanide) was released from storage ponds at the USMX Mine, UT into the East Fork of Beaver Dam Wash (Wong-Chong et al. 2006). One of the largest spills occurred in Baia Mare, Romania where 26 million gallons of cyanide-bearing tailings were released due to a failure in the tailings dam, killing an estimated 1,240 tons of fish in the Hungarian portion of the Tisza River alone (Wong-Chong et al. 2006). Because of its high toxicity and relatively short half-life, cyanide has been used by fishery managers for lake restoration (Leduc 1984) and for collecting fish from ponds, lakes and steams (Lewis and Tarrant 1960). Following treatment, poisoned fish exhibit several symptoms including increased ventilation, surfacing, gulping for air, frantic swimming in circles, convulsions, tremors and finally death (Leduc 1984). At high levels of exposure the onset of acute toxicity occurs rapidly, however, live fish that are rescued and transferred to clean water may survive (Leduc 1984).

Laboratory tests under controlled conditions have revealed that not all life stages of fish are equally sensitive to acute cyanide exposure, that cyanide toxicity can be modulated by abiotic factors, and that there is a wide range in sensitivity among aquatic organisms. Smith et al. (1978) conducted 96-hr acute toxicity tests with fathead minnow, bluegill, brook trout, and yellow perch and found that juveniles and fry were more sensitive to the lethal effects of cyanide than eggs. Bluegill, yellow perch, and brook trout juveniles were more sensitive than newly-hatched fry, where as, swim-up fry were the most sensitive fathead minnow life stage.

Smith et al. (1978) also reported that dissolved oxygen (DO) concentration and water temperature affect the susceptibility of these species to cyanide toxicity. Cyanide was more toxic when fish were held in water with lower DO concentrations. Considering that cyanide is a respiratory toxin that inhibits oxidative metabolism, it is not surprising that the effects are exacerbated under conditions where oxygen availability is limited. The authors also tested the effect of temperature on cyanide toxicity. LC_{50} values for juvenile brook trout, bluegill and yellow perch were lower at low temperatures than at higher temperatures, indicating a heightened sensitivity at lower temperatures. The combination of low DO and low temperature tended to produce conditions that rendered juveniles most susceptible to cyanide toxicity.

Kovacs (1979) found that the sensitivity of juvenile rainbow trout to cyanide was similarly influenced by temperature. He conducted 96-hr acute toxicity tests at three different temperatures (6, 12 and 18˚ C) and reported LC_{50} values of 28, 42, and 68 ug HCN/L, respectively. Thus, trout held at 6˚ C were 2.4 times more sensitive to cyanide than trout held at 18˚ C. These studies demonstrate that life stage as well as abiotic factors (DO and temperature) can influence acute cyanide toxicity. There is also considerable variability among aquatic taxa in terms of their intrinsic sensitivity to cyanide.

Standardized acute toxicity tests with cyanide have been conducted with numerous aquatic species. EPA compiled toxicity data for 83 species of aquatic animals and plants (61 freshwater species and 22 saltwater species) as part of their cyanide BE (EPA 2007). Based on this compilation, there appears to be a large range in sensitivity between the most sensitive (rock crab LC_{50} 4.89 ug CN/L) and the least sensitive species tested (river snail LC_{50} 760,000 ug CN/L). Freshwater species represented 9 phyla, 15 classes, 29 orders, 36 families, and 52 genera. Fishes were among the most sensitive freshwater taxa although there was substantial variability in sensitivity. Among the 24 freshwater fish species included in the list, there was a 33-fold difference in sensitivity between the most sensitive (rainbow trout, *Oncorhynchus mykiss*, LC_{50} 59 ug CN/L) and the least sensitive (bata, *Labeo bata*, LC_{50} 1970 ug CN/L). The 8 most sensitive fish species belong to 3 different families: Salmonidae (3 species, 3 genera); Percidae (2 species, 1 genera); and Centrarchidae (3 species, 3 genera). Because of the relatively low number of species that have been tested within these families, it is difficult to get a sense of the amount of intra-family variability in species sensitivity on the low end of the species sensitivity distribution. By contrast, the family Cyprinidae was well represented with 10 different species representing 8 genera. Among those 10 species, there is an 18-fold difference in sensitivity between the most sensitive (roach LC_{50} 108 ug CN/L) and the least sensitive (bata, *Labeo bata*, LC_{50} 1970 ug CN/L) species. Because of pronounced intra-family variation it is unlikely that the 8 species within the 3 most sensitive families represent the most sensitive species within those families.

Dwyer et al. (2005) conducted acute toxicity tests with 5 different water pollutants (carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin), 3 common test species (rainbow trout, fathead minnow, and sheepshead minnow) and 17 federally threatened, endangered or candidate fish species. They found that for some pollutants, threatened and endangered species in the following families were as or more sensitive than rainbow trout: Salmonidae (Apache trout, Greenback Cutthroat trout, and Lahontan Cutthroat trout), Percidae (Fountain darter and Greenthroat darter), Cyprinidae (Cape Fear shiner, Spotfin chub, and Colorado pikeminnow), and Acipenseridae (Atlantic sturgeon and Shortnose sturgeon). Based on these results, the authors recommended that for listed fish species which require greater protection (i.e., species that may be more sensitive than rainbow trout), a factor of 0.63 can be applied to the geometric mean of the rainbow trout LC_{50}, and that if even greater protection is desired a factor of 0.46 can be used (0.63 – 1 standard deviation). For cyanide, applying the adjustment factors of 0.63 and 0.46 to the rainbow trout LC_{50} (59 ug CN/L) would result in LC_{50} estimates of 27 to 37 ug CN/L for sensitive listed fish species.

Chronic Toxicity to Fish

Chronic cyanide toxicity tests have been conducted with relatively few fish species. However, available data indicate that cyanide not only reduces survival but also affects reproduction, growth, swimming performance, condition, and development (Table 3). Reproduction appears to be one of the most sensitive endpoints. Full and partial life cycle tests with fathead minnows and brook trout have shown that fish exposed to sublethal concentrations of cyanide spawned fewer eggs than non-exposed fish (Koenst et al. 1977, Lind et al. 1977). Fecundity was reduced by 57.8% and 46.9% (compared to controls) in female fathead minnows exposed to cyanide at 19.6 ug HCN/L (the LOEC) and 12.9 ug HCN/L (the NOEC), respectively. Similarly, the mean number of eggs spawned by brook trout was reduced by 53.3% at 11.2 ug HCN/L and by 17.7% at 5.7 ug HCN/L.

Kimball et al. (1978) exposed bluegill to cyanide (5.2 – 80 ug HCN/L) for 289 days and reported that no eggs were spawned in any of the cyanide treatments except for one spawning that occurred at the highest concentration. Although the single spawning is difficult to explain, the fact that spawning was completely inhibited in 42 of 43 cyanide-exposed females suggests that bluegill may be particularly sensitive. It is surprising, considering the overwhelming effects on reproduction, that a confirmatory study with bluegills has not been conducted over the past 30 years.

Cheng and Ruby (1981) studied the effects of pulsed exposures of cyanide on flagfish reproduction. Unlike the studies describe above, where fish were exposed over an extended period of time to a constant concentration, flagfish were exposed to sublethal concentrations of cyanide for 5-day pulses. Flagfish exposed to cyanide (65 ug/L) for 5 days following fertilization (i.e., as eggs) and then reared to maturity in clean water, spawned 25.6% fewer eggs than flagfish that had not been exposed. In another experiment by the same authors, flagfish that received a second 5-day pulse of cyanide as juveniles had an even greater reduction (39.3%) in the number of eggs spawned. These studies demonstrate that cyanide can affect an apical reproductive endpoint in fish.

The mechanism by which cyanide induces these reproductive effects is not fully understood. However, key physiological, biochemical, histological (morphological), and endocrine functions known to be involved in sexual maturation are affected by cyanide (Table 3).

Table 3. Chronic effects of cyanide on various fish species.

Species	Life stage	Exposure duration	Temp (C)	pH	HCN (ug/L)	CN (ug/L)	Effect	Reference
Atlantic Salmon *Salmo salar*	Adult females during late vitellogensis	12 days	7	6.8	5	4.8	Inhibition of vitellogenin uptake by ovaries: observed increased plasma vitellogenin, decline in gonad vitellogenin, and no change in liver vitellogenin.	Ruby et al. 1987
Atlantic Salmon *Salmo salar*	Egg/fry	103-112 day incubation plus 58 days post hatch	3.5 – 8.3	7.6	10 – 100 (nominal)		Teratagenic effects observed in fry: malformation and/or absence of the eyes, defects in mouth and vertebral column and yolk-sac dropsy (note: CN concentrations not measured in exposure tanks)	Leduc 1977
Bluegill *Lepomis macrochirus*	Adult and early life stages	289 days[1]	24.9	8.1	5.2 - 80	5.4 – 82.7	No spawning occurred among fish exposed to CN except for one female in the 82.7 ug/l treatment. There were 5 and 8 spawnings in the two controls, respectively.	Kimball et al. 1978
Bluegill *Lepomis macrochirus*	Egg/larvae	57 days	25	8.02	4.8 – 82.1	4.9 – 84.4	NOEC for early life stage survival 9.4 ug CN/L. LOEC was 19.9 ug CN/L (88% reduction in survival compared to controls).	Kimball et al. 1978
Brook Trout *Salvelinus fontinalis*	19 month-old adults	144 days	12.5	7.9	5.7 – 75.3	5.6 – 74.4	NOEC for fecundity (number of eggs spawned) 5.6 ug CN/L	Koenst et al. 1977
Brook Trout *Salvelinus fontinalis*	Sac fry/juvenile	90 days (from hatch to 90 post hatch)	9	7.9	5.6 – 77.2	5.5 – 76	NOEC for growth (weight of juveniles at 90 days post hatch) 21.4 ug CN/L.	Koenst et al. 1977
Brook Trout *Salvelinus fontinalis*	Sac fry/juvenile	90 days (from hatch to 90 post hatch)	9	7.9	5.5 - 77	5.4 – 75.6	Survival reduced by 15%, 25% and 70%, at 55, 66 and 77 ug CN/L, respectively.	Koenst et al. 1977
Fathead Minnow *Pimephales promelas*	Larvae through adult	256 days	24.95	8.075	5.8 – 100.7	6.0 – 103.9	NOEC for fecundity (number of eggs spawned) 13.3 ug CN/L. LOEC 20.2 ug CN/L (58% reduction in spawning compared to controls).	Lind et al. 1977
Flagfish *Jordanella floridae*	Egg through adult	5 days during embryo/ larvae stage	25	8.05	65 – 87	66.8 – 89.5	Onset of spawning delayed, estrus cycle[2] shortened, fecundity reduced in CN treatments 26% to 35% compared to controls	Cheng and Ruby 1981

Species	Life stage	Exposure duration	Temp (C)	pH	HCN (ug/L)	CN (ug/L)	Effect	Reference
Flagfish *Jordanella floridae*	Egg through adult	5 days & 5 days[3]	25	8.05	65 – 87	66.8 – 89.5	Onset of spawning delayed, estrus cycle[2] shortened, fecundity reduced in CN treatments 39 to 47% compared to controls	Cheng and Ruby 1981
Flagfish *Jordanella floridae*	Egg/larvae	From fertilization through hatching (~5 – 9 days)	25	8.05	65 – 150	66.8 – 154.3	Hatching Success: 89% (control), 86%, - 3% in CN treatments. Hatching delayed and pituitary gland size reduced in all CN treatments Eye Malformations (microphthalmia - reduced eye size, and monophthalmia - disintigration of the eye), 30%, (66.8 ug/L), 40% (77 and 89.5 ug/L)	Cheng and Ruby 1981
Rainbow Trout *Oncorhynchus mykiss*	Juvenile males	18 days	12.5	7.9	10, 30	9.8, 29.5	Spermatogenesis: reduced number of dividing spermatogonia, 13% reduction at 10 ug/L, 50% reduction at 30 ug/L.	Ruby et al. 1979
Rainbow Trout *Oncorhynchus mykiss*	150 – 300 g vitellogenic females	12 days	12.5	7.2	10	9.7	Reduction in plasma vitellogenin and GSI compared to controls	Ruby et al. 1986
Rainbow Trout *Oncorhynchus mykiss*	2–3 year-old	12 days	12.5[4] 10.7[5]	7.83[4] 7.78[5]	10	9.8	Increase in brain dopamine compared to control fish, decrease in mean oocyte diameter (19%) of vitellogenic females, and higher numbers of spermatogonial cysts in males. Two identical experiments, one in July the other in August.	Szabo et al. 1991
Rainbow Trout *Oncorhynchus mykiss*	200 - 350g vitellogenic female rainbow trout	7 days	12	7.2	10, 20, 30	9.7, 19.3, 29	Decrease in serum calcium at 9.7 and 19.3 ug/L	Da Costa, H. and Ruby, S.M. 1984
Rainbow Trout *Oncorhynchus mykiss*	2.5 year old vitellogenic female rainbow trout	12 days	12	7.6	10	9.7	20% reduction in female GSI, 33% reduction in plasma vitellogenin, 55% reduction in plasma 17B-estradiol (E2), 48% reduction in oocyte diameter, and a 70% reduction in plasma T3 compared to controls. Plasma T4 levels were lower but difference was not statistically significant.	Ruby, S.M. et al. 1993a.
Rainbow Trout *Oncorhynchus mykiss*	juveniles (35 - 43g)	20 days	10	7.8	10, 20	9.8, 19.5	At 9.8: 65% reduction in the frequency of stage 5 (most mature) oocytes, 2 fold increase in the frequency of atretic follicles. At 19.5: 62% reduction in the frequency of stage 5 oocytes, 1.9 fold increase in the frequence of atretic follicles.	Lesniak, J.A. and Ruby, S.M. 1982.

Species	Life stage	Exposure duration	Temp (C)	pH	HCN (ug/L)	CN (ug/L)	Effect	Reference
Rainbow Trout *Oncorhynchus mykiss*	2 year old sexually maturing males	12 days	11.5	7.9	10	9.8	Reduced # of spermatocytes, increased # of spermatogonial cysts, decreased # of basophils in pituitary, hypothesise effect of CN on hypthalmic-pituarity-gonadal axis	Ruby, S.M. et al. 1993b.
Rainbow Trout *Oncorhynchus mykiss*	Juveniles (3 g)	18 days	12.5	7.9	10	9.8	LOEC for growth measured as wet weight change over 18 days. NOEC < 9.8 ug CN/L	Dixon, D.G. and G. Leduc. 1981.
Rainbow Trout *Oncorhynchus mykiss*	Juveniles (12 g)	18 days	12.5	7.9	10	9.8	NOEC for growth measured as wet weight change over 18 days.	Dixon, D.G. and G. Leduc. 1981.
Rainbow Trout *Oncorhynchus mykiss*	Juveniles (3 g & 12 g)	18 days	12.5	7.9	10 – 30	9.8 – 29.5	Increased resting metabolic rate Liver damage:degenerative hepatic necrosis	Dixon, D.G. and G. Leduc. 1981.
Rainbow Trout *Oncorhynchus mykiss*	Juveniles (20g)	20 days	6 12 18	7.9	5 20 30	4.9 19.7 29.8	NOEC for mean specific growth rate (MSGR) based on dry weight. Author estimated thresholds at 6C, 12C and 18C to be < 4.9, 9.8 and 29.8 ug CN/L, respectively.	Kovacs, T.G. 1979.
Rainbow Trout *Oncorhynchus mykiss*	Juveniles (20g)	20 days	6,12, 18	7.9	5 – 45	4.9 – 44.7	Reduced swimming performance	Kovacs, T.G. 1979.
Sheepshead Minow *Cyprinodon variegatus*	Embryo/larvae	28 days	22.4		29 - 462		Survival of 28 day-old larvae reduced from 22.5% (29 ug/L) to 54.5% (462 ug/L) compared to controls. Author states that MATC[6] lies between 29 and 45 ug /L	Schimmel et al. 1981

[1]Exposure of first and second year spawners to HCN followed by 90-day exposure of eggs/larvae (second generation).
[2]Estrous cycle as defined by Cheng and Ruby 1981: "In this study, estrous cycle is defined as the duration of egg-laying starting from the first appearance of more than ten eggs through the occurrence of less than ten eggs on the mats."
[3] 5-day exposure during embryo/ larvae stage followed by a second 5 day exposure during juvenile stage.
[4]Experiment conducted in July.
[5]Experiment conducted in August.
[6]MATC – Maximum Acceptable Toxicant Concentration (the MATC is typically the geometric mean of the NOEC and LOEC).

Lesniak and Ruby (1982) reported abnormal oocyte development in sexually maturing female rainbow trout exposed to cyanide (10 and 20 ug HCN/L) for 20 days. Ovaries from cyanide-exposed fish contained fewer mature oocytes, exhibited altered patterns of secondary yolk deposition (in developing oocytes), had nearly twice the frequency of atresia (oocyte resorption), and had an overall reduction in the number of viable eggs.

Ruby et al. (1986) reported that vitellogenic female rainbow trout exposed for 12 days to 10 ug HCN/L had lower levels of plasma vitellogenin and a lower gonadosomatic index (GSI) compared to controls. In two similar studies, oocyte diameter (an indicator of gonadal growth and development) was reduced in sexually maturing female rainbow trout exposed for 12 days to 10 ug HCN/L (Ruby et al. 1993a, Szabo et al. 1991). Reduced oocyte diameter was accompanied by reductions in plasma vitellogenin,17β-estradiol (E2), and GSI (Ruby et al. 1993a), as well as increased whole brain dopamine levels (Szabo et al. 1991).

Dopamine has an inhibitory effect on gonadotropin-releasing hormone (GnRH) neurons in some fish species and it is GnRH that stimulates the release of gonadotropins (GtH I and GtH II) from the pituitary (Saligaut et al. 1999; Patino, R. 1997). GtH I and GtH II are believed to function similar to follicle-stimulating hormone and luteinizing hormone, respectively, in tetrapods (Patino, R. 1997). In female fish, GtH I acts on target cells in the gonad, stimulating E2 synthesis. E2 induces vitellogenin synthesis in the liver. Vitellogenin is the egg yolk precursor in fish that is produced by the liver, transported via blood, taken up by the ovaries, and incorporated into developing oocytes. GtH II also acts on the gonad by inducing the synthesis of maturation-inducing steroid (MIS). MIS induces oocyte maturational competence and ovulation (Park et al. 2007; Patino, R. 1997).

The control exerted by dopamine over gonadal maturation has been recognized by fish culturists, who have been successful in treating captive-reared fish with anti-dopaminergic drugs (which block dopamine receptors), such as pimozide and domperidone, to induce ovulation (Szabo et al. 2002, Park et al. 2007, Jensen 1993, Patino 1997). Thus, oocyte development, maturation and ovulation are under the control of gonadotropins and E2 which in turn, are modulated in part by GnRH and dopamine. This interaction between the neuroendocrine system and reproductive organs is referred to as the hypothalamus-pituitary-gonadal (HPG) axis (IPCS 2002).

Cyanide has also been shown to affect male reproductive processes. Exposure of male rainbow trout to cyanide concentrations of 10 and 30 ug HCN/L for 18 days disrupted spermatogenesis as evidenced by a reduction in the number of dividing spermatogonia and a blockage of mitotic progress (Ruby et al. 1979). Exposure of rainbow trout for 12 days to 10 ug HCN/L resulted in higher numbers of spermatogonial cysts in testes of male trout as well as higher levels of whole brain dopamine (Szabo et al. 1991). Ruby et al. (1993b) reported similar results where the number of spermatocytes decreased and the number of spermatocyte precursors (spermatogonial cysts) increased in two-year-old sexually maturing rainbow trout after a 12-day exposure to 10 ug HCN/L. There are indications that the transformation of spermatogonial cysts to spermatocytes is hormonally regulated through GtH along the HPG axis and that, within the pituitary, GtH is released from type I granular basophils (Ruby et al. 1993b). Histological examination of pituitary glands from cyanide-exposed fish showed a reduction in the number of type I granular basophils. Ruby et al. (1993b) suggested that elevated levels of brain dopamine may be responsible for the selective loss of type I granular basophils and subsequent alteration of spermatocyte formation.

Ruby et al. (1979, 1993b) and Szabo et al. (1991) hypothesized that cyanide acts through the HPG axis to affect reproduction in fish. Their studies (described above) demonstrated (1) that cyanide caused an increase in brain dopamine levels, consistent with neuronal effects observed on mammals, (2) that levels of reproductive hormones (E2) and egg-yolk precursors (vitellogenin) were altered following exposure to cyanide, (3) the selective loss of putative GtH releasing pituitary cells (type I granular basophils) and (4) retarded gonad development in cyanide-exposed male and female rainbow trout. Taken together, these results appear to be consistent with HPG axis involvement. Ruby et al. (1979, 1993b) and Szabo et al. (1991) also reported that these effects occurred following relatively short (12 to 18 days), sublethal exposures to cyanide. Whether these effects would result in the same type of reduced fecundity and spawning, as was observed in cyanide-exposed female fathead minnows (Lind et al. 1977), bluegill (Kimball et al. 1978), and brook trout (Koenst et al. 1977), was not addressed in the rainbow trout studies because they were terminated before the fish reached full sexual maturity. However, it does seem likely that these effects would occur.

Results from Cheng and Ruby (1981) indicate that continuous exposure of fish to cyanide through the spawning period may not be necessary to affect fecundity. Short-term, pulsed exposures of cyanide to flagfish were sufficient to induce subsequent effects on the number of eggs spawned, and exposed fish did not appear to recover once the exposure had ceased. Even exposure of eggs, one of the most tolerant life stages in terms of acute toxicity (Smith et al. 1979), resulted in latent effects on fecundity once embryos hatched and survived to maturity. This finding is not unexpected given that it is during early developmental stages that the HPG endocrine axis is set up and feedback sensitivity of the hypothalamus and pituitary gonadotropes to gonadal steroids is established (IPCS 2002). Although Cheng and Ruby (1981) did not measure specific indicators of endocrine axis function, they did find that the pituitary gland of cyanide-exposed flagfish embryos was significantly smaller than the pituitaries from control fish. It appears that cyanide, like many EDCs (endocrine disrupting compounds, IPCS 2002), may affect the "set up" of the HPG axis and that these early developmental effects may have long-term consequences on reproduction.

Chronic exposure of eggs and larvae to cyanide can result in reduced embryo/larvae survival and altered development. Leduc (1978) exposed newly fertilized Atlantic salmon eggs to cyanide at concentrations of 10 to 100 ug HCN/L, and observed teratogenesis, as well as, delayed hatching and reduced hatching success at higher concentrations. There was a dose-dependent increase in the frequency of abnormal fry, ranging from 5.8% to 18.5%. Abnormalities included malformed and/or absence of eyes, defects in the mouth and vertebral column and yolk-sac dropsy (*Hydrocoele embryonalis*, also known as blue sac disease). Similar eye abnormalities were reported by Cheng and Ruby (1981) in flagfish larvae exposed, as eggs, to cyanide at concentrations of 65, 75, 87, and 150 ug

HCN/L. Egg hatching success was also reduced and time to hatch was delayed in all cyanide treatments. In a 28-day embryo/juvenile toxicity test, sheepshead minnow survival was significantly reduced in all treatments >29 ug HCN/L (Schimmel 1981). Schimmel (1981) noted there was considerable embryonic mortality and that there was no larval mortality during the last two weeks of exposure, indicating a greater sensitivity during early development. Kimball et al. (1978) exposed bluegill eggs and larvae to cyanide at concentrations of 4.8 to 82.1 ug/HCN/L, and reported that most deaths occurred within the first 30 days after hatching. Survival was reduced in all cyanide treatments and the effects were statistically significant at cyanide concentrations >9.1 ug HCN/L.

As previously mentioned, cyanide effects oxidative metabolism, energy production, and thyroid function; all are important for normal growth and performance. Therefore, it is not unexpected that sublethal exposure of fish to cyanide has been shown to impact growth, condition and swimming performance. There is also evidence that the effect of cyanide on these physiological endpoints can be modulated by other factors such as diet/ration and temperature. When cichlids (*Cichlasoma bimaculatum*) were fed unlimited rations and exposed to cyanide for 24 days, those fish exposed to lower concentrations of cyanide (< 0.06 ug HCN/L) were larger than controls, where as, at higher concentrations weight gain was depressed (Leduc 1984). The increased weight gain in the low-dose treatments was attributed to higher food consumption, which was allowed to occur because ration was not restricted. Low-dose stimulation is a common effect across a broad range of chemical and non-chemical stressors (Calabrese 2008).

Dixon and Leduc (1981) held juvenile rainbow trout on restricted rations and exposed them to cyanide at concentrations of 10, 20, and 30 ug HCN/L for 18 days and observed significantly reduced weight gain in all treatments compared to controls. The effect was characterized by an initial decrease in specific growth during the first 9 days followed by a significant increase from day 9 through day 18. The growth surge during the latter half of the exposure period was not sufficient to offset early reductions. Cyanide-affected juvenile rainbow trout were in poorer condition, as indicated by lower fat content, and had higher respiration rates for several days post-exposure. In addition, juvenile rainbow trout in all cyanide treatments exhibited degenerative necrosis of hepatocytes (i.e., liver tissue damage) that increased in severity with the level cyanide exposure.

Kovacs (1979) held juvenile rainbow trout on restricted rations and exposed them to cyanide for 20 days. The results were similar to those reported by Dixon and Leduc (1981). Cyanide reduced the mean specific growth rate (MSGR) and affected-fish gained less fat during the exposure period. Kovacs (1979) also examined the effects of temperature on rainbow trout growth and sensitivity to cyanide. He found that the growth rate of rainbow trout was inversely related to holding temperature (6, 12 and 18˚C), as would be expected, and that trout held at colder temperatures were more sensitive to cyanide. The NOECs for MSGR were 5, 20, and 30 ug HCN/L for trout held at 6, 12, and 18˚C, respectively. Based on the exposure response curves, Kovacs (1979) estimated thresholds for effects on rainbow trout growth to be <5 ug HCN/L at 6˚C, 10 ug HCN/L at 12˚C, and 30 ug HCN/L at 30˚C.

Kovacs (1979) also evaluated the effects of cyanide on fish swimming performance. His results indicated that swimming performance was affected by cyanide and that the effect was also temperature-sensitive. Fish from the growth study described above were placed in swimming chambers and tested for swimming stamina. Among non-exposed trout, swimming stamina, measured as distance travelled (in meters), decreased with decreasing temperature (i.e., fish held at 6˚C travelled a shorter distance than fish held at 18˚C). Cyanide-exposed fish had reduced swimming stamina compared to non-exposed fish and the effect was more severe at colder temperatures. Based on the exposure-response regression equations reported by Kovacs (1979), the predicted reduction in swimming stamina (compared to controls) for fish exposed to cyanide at the chronic water quality criterion (5.2 ug CN/L) would be 52% at 6˚C, 20% at 12˚C, and 3% at 18˚C.

Cyanide has been shown to affect the swimming performance of other salmonid species, as well. Leduc (1984) calculated the cyanide concentration causing a 50% reduction in swimming ability based on original data collected for brook trout (Neil 1957) and coho salmon (*Oncorhynchus kisutch*) (Broderius 1970) to be 5 ug HCN/L and 7 ug HCN/L, respectively. Thus, chronic exposure of fish to cyanide at sublethal concentrations can affect growth, body condition, and swimming performance. Factors such as temperature and diet/ration can modulate cyanide toxicity, and, for some species-endpoint combinations, these effects may occur at or below the chronic cyanide criterion.

Effects of Pollutant Mixtures

Relatively few studies have been performed to measure the effects of free cyanide in combination with other contaminants. Concurrent exposure to cyanide and ammonia produced greater than additive effects to acute lethality in rainbow trout, salmon, and chub (Smith et al. 1979, Alabaster et al. 1983, and Douderoff 1976), and to chronic sublethal effects to growth in rainbow trout (Smith et al 1979). In rainbow trout and salmon, effects to acute lethality were 1.2 and 1.63 times greater, respectively, than would be expected by additivity. Concurrent exposure to cyanide and zinc also resulted in synergistic effects to acute lethality in fathead minnows, where toxicity was 1.4 times that predicted by additivity (Smith et al 1979). Although we are unable to quantify the effect of these synergistic mechanisms for this analysis, they should be considered when assessing effects of cyanide to aquatic organisms in waterways with elevated concentrations of ammonia and zinc.

Acute Effects Estimation for Listed Fish Evaluation Species

Of the 103 listed fish species that were evaluated for acute cyanide effects, only the 4 most sensitive (i.e. fountain darter, Apache trout, Lahontan cutthroat trout, and bull trout) were considered likely to be adversely affected (Appendix B). For these 4 species, the magnitude of effect resulting from acute exposure to cyanide at the CMC (22.4 ug CN/L) was estimated using regression analysis.

<u>Estimated acute effects for Fountain Darter</u>: Of the acute exposure-response regression equations listed in Appendix G there are six equations for juvenile mortality in a species of

fish, Yellow Perch, from the family Percidae (Smith et al. 1978). Because toxicity is inversely related to water temperature (Eisler 2000), the equation for the lowest tested temperature, 15 C, would be the most appropriate equation for estimating acute effects in the Fountain Darter. That equation of:

Probit (% juv. Mortality) = -17.790 + 11.650 (log (ug HCN /L))

yields an estimated Yellow Perch acute LC_{50} of 90.4 ug HCN /L, or 88.9 ug CN /L (at the test pH of 7.82). The estimated acute LC_{50} for Fountain Darter from the ICE lower 95% confidence value is 21.5 ug CN/L (Appendix B). This yields an acute $SSEC_x$ estimate of 92.6 ug CN/L, or 94.2 ug HCN /L (see chronic effects section and Appendix C for explanation of $SSEC_x$s). Entering that $SSEC_x$ value on an HCN basis into our probit-log regression equation yields an estimated effect level for Fountain Darter of 58.2% juvenile mortality at the CMC criterion.

<u>Estimated acute effects for Apache Trout</u>: Of the acute exposure-response regression equations listed in Appendix G there is only one for an *Oncorhynchus* species of salmonid (Broderius and Smith 1979). That equation is for tests with juvenile Rainbow Trout. That equation of:

Probit (% juv. Mortality) = 33.63 + 23.04 (log (mg CN /L))

yields an estimated Rainbow Trout acute LC_{50} of 57.2 ug CN /L. The estimated acute LC_{50} for Apache Trout from the ICE lower 95% confidence value is 16.5 ug CN/L (Appendix B). This yields an acute $SSEC_x$ estimate of 77.7 ug CN/L (see chronic effects section Appendix C for explanation of $SSEC_x$s). Entering that $SSEC_x$ value into our probit-log regression equation yields an estimated effect level for Apache Trout of >99.9% juvenile mortality at the CMC criterion.

<u>Estimated acute effects for Lahontan Cutthroat Trout</u>: Of the acute exposure-response regression equations listed in Appendix G there is only one for an *Oncorhynchus* species of salmonid (Broderius and Smith 1979). That equation is for tests with juvenile Rainbow Trout. That equation of:

Probit (% juv. Mortality) = 33.63 + 23.04 (log (mg CN /L))

yields an estimated Rainbow Trout acute LC_{50} of 57.2 ug CN /L. The estimated acute LC_{50} for Lahontan Cutthroat Trout from the ICE lower 95% confidence value is 22.8 ug CN/L (Appendix B). This yields an acute $SSEC_x$ estimate of 56.2 ug CN/L (see chronic effects section and Appendix C for explanation of $SSEC_x$s). Entering that $SSEC_x$ value into our probit-log regression equation yields an estimated effect level for Lahontan Cutthroat Trout of 43% juvenile mortality at the CMC criterion.

<u>Estimated acute effects for Bull Trout</u>: Of the acute exposure-response regression equations listed in Appendix G there are nine equations for juvenile mortality in a species of fish, Brook Trout, from the genus *Salvelinus* (Smith et al. 1978). Because toxicity is

inversely related to water temperature (Eisler 2000), the equation for the lowest tested temperature, 4 C, would be the most appropriate equation for estimating acute effects in the Bull Trout. That equation of:

Probit (% juv. Mortality) = -28.849 + 19.626 (log (ug HCN /L))

yields an estimated Brook Trout acute LC_{50} of 53.1 ug HCN /L, or 51.2 ug CN /L (at the test pH of 7.19). The estimated acute LC_{50} for Bull Trout from the ICE lower 95% confidence value is 15.7 ug CN/L (Appendix B). This yields an acute $SSEC_x$ estimate of 73.0 ug CN/L, or 75.6 ug HCN /L (see chronic effects section and Appendix C for explanation of $SSEC_x$s). Entering that $SSEC_x$ value on an HCN basis into our probit-log regression equation yields an estimated effect level for Bull Trout of 99.9% juvenile mortality at the CMC criterion.

Citing DeForest et al. (*in prep*.), Gensemer et al. (2007) noted that exposure-response curves for cyanide acute toxicity in fish are "quite steep". Gensemer et al.'s (2007) observation is supported by the low LC_{50}/LC_{10} ratios derived for this biological opinion from the exposure-response regression equations in Smith et al. (1978) and Broderius and Smith (1979). A combination of the steep response curves and the low estimated LC_{50} values for the listed evaluation species relative to the surrogate toxicity test species results in substantive estimates of adverse effects at the acute (CMC) criterion of 22.4 ug CN /L even though the acute effects assessment EC_as (13.77-20.00) were not far below the CMC (22.4).

Chronic Effects Estimation for Listed Fish Evaluation Species

Ideally, concentration (dose)-response data suitable for predictive modeling would be available for sensitive chronic endpoints for each of the listed species evaluated in this analysis (hereafter referred to as "listed evaluation species"). Such data do not exist for cyanide for any of our listed evaluation species. As recently reviewed by Gensemer et al. (2007), the current inventory of concentration-response data from chronic toxicity testing with cyanide consists of four datasets: one each for reproductive endpoints among the fathead minnow (*Pimephales promelas*; Lind et al. 1977) and the brook trout (Koenst et al. 1977); one for juvenile survivorship among bluegill (Kimball et al. 1978); and one for the sheepshead minnow (Schimmel et al. 1981). Upon closer inspection, Gensemer et al. (2007) found the dataset for the sheepshead minnow to be insufficient for meaningful predictive modeling and we agree with that conclusion. Thus, we are left with three datasets as the best available scientific basis for estimating toxic effects (or the lack thereof) at the chronic criterion value of 5.2 ug CN/L. In addition to our three useable concentration-response datasets, we also possess estimates of LC_{50} values for our listed evaluation species as per the procedures described in Appendix B.

Based on the above information, we took the following approach to evaluating the effects of the proposed action on the listed fish evaluation species:

(1) The three concentration-response data sets were transformed into the most precise, predictive concentration-response models that the data can support; these models were used to predict the response of chronic toxicity test species to proposed chronic CN exposure levels.

(2) The predicted response of a listed fish evaluation species to chronic CN exposures was considered to be the same as the response observed for a chronic toxicity test species at an adjusted chronic CN exposure level based on the ratio of their respective LC_{50} values (see example below).

Two assumptions were relied upon to predict the effect of proposed chronic CN exposure levels on the listed fish evaluation species:

(1) The relative differences in sensitivity to chronic CN exposures between the listed evaluation species and the chronic toxicity test species (fathead minnow, brook trout, and bluegill) are approximated by the ratio of their respective LC_{50} values; and
(2) The slopes of the concentration-response curves are also approximately comparable between the listed evaluation species and the chronic toxicity test species.

These assumptions create a clearly defined basis for a default hypothesis that allows for an analysis of the effects of the proposed action on listed fish evaluation species to proceed within the constraints of minimal data until such time as more data become available. As more data become available appropriate modification (or validation) of our default approach will be facilitated.

An example of applying the above methodology: suppose that one of our chronic toxicity test species is predicted to exhibit a 20% adverse effect from being exposed to a concentration of 5.2 ug CN/L. If a listed evaluation species happens to have an estimated LC_{50} value equal to that of the chronic toxicity test species, then a 20% adverse effect would also be predicted for the listed evaluation species. If the ratio of LC_{50} values was 1.5 (rather than 1.0) in the direction of greater sensitivity for the listed evaluation species than the chronic toxicity test species, then the predicted response at our concentration of interest of 5.2 ug/L for our listed evaluation species would be the same as the response observed for the chronic toxicity test species at a CN concentration 1.5 times 5.2 ug/L, which equals 7.8 ug/L. We refer to such predicted response values as surrogate currency equivalents (or $SSEC_x$ or SS_x values) for our listed evaluation species. In this example, the predicted adverse effect for our chronic toxicity test species at the $SSEC_x$ of 7.8 ug/L would be our surrogate currency predicted effect for the listed evaluation species at 5.2 ug CN/L (for one of three prediction models) for the purposes of this Biological Opinion. A more detailed derivation and explanation of the $SSEC_x$/ SS_x concept is provided in Appendix C.

Because groups of taxonomically related listed evaluation species were assigned identical LC_{50} values from the same ICE or SSD model, there are only 17 $SSEC_x$ values that need to be evaluated for any given (chronic toxicity test species) prediction model, but they are different for each prediction model (3 models x 17 values = 51 total $SSEC_x$ values of interest).

For the prediction model based on fathead minnow chronic toxicity data, the $SSEC_x$ values range from 6.7 to 45.8 ug CN/L (Table 4). As indicated by the entire range of $SSEC_x$ values being greater than 5.2 ug CN/L, all listed evaluation species have LC_{50} values that are more sensitive to cyanide than the fathead minnow LC_{50} value.

For the prediction model based on brook trout chronic toxicity data, the $SSEC_x$ values range from 4.2 to 28.4 ug CN/L (Table 4).

For the prediction model based on bluegill chronic toxicity data, the $SSEC_x$ values range from 6.1 to 41.7 ug CN/L (Table 4).

The $SSEC_x$ ranges indicated above define for each prediction model the range of cyanide concentrations over which model fit will be of most relevance to the effects of the proposed action on the listed evaluation species considered in this Biological Opinion. Detailed $SSEC_x$ -related results and the origins of the LC_{50} values used to calculate the $SSEC_x$ values are presented in Table 4 and Appendix D.

Table 4. Surrogate currency equivalents (SSECx) for each LC50 surrogate taxon/chronic toxicity test species combination. SSECx values were calculated using equation 5 in Appendix C. Surrogate taxa were used to estimate LC50 values for listed evaluation species except when measured values for the listed species were available (i.e. *Salmo salar*).

			Effects on Fecundity		Effects on Early Life Stage Survival
			Fathead Minnow SS LC_{50}=138.4 (ug CN/L)	Brook Trout SS LC_{50}=85.7 (ug CN/L)	Bluegill SS LC_{50}=126.1 (ug CN/L)
Surrogate taxa used to estimate listed species (LS) LC_{50}	$LSEC_x$ (ug CN/L)	LS LC_{50} (ug CN/L)	$SSEC_X$ (ug CN/L)	$SSEC_X$ (ug CN/L)	$SSEC_X$ (ug CN/L)
Actinopterygii (class)	5.2	66.5[1]	10.8	6.7	9.9
Cypriniformes (order)	5.2	84.55[1]	8.5	5.3	7.8
Family Catostomidae					
Xyrauchen texanus (species)	5.2	83.8[2]	8.6	5.3	7.8
Cyprinidae (family)	5.2	101.7[2]	7.1	4.4	6.4
Cyprinella monacha (species)	5.2	36.81[2]	19.6	12.1	17.8
Gila elegans (species)	5.2	50.9[2]	14.1	8.8	12.9
Notropis mekistocholas (species)	5.2	48.5[2]	14.8	9.2	13.5
Ptychocheilus lucius (species)	5.2	43.5[2]	16.6	10.3	15.1
Perciformes (order)	5.2	90.8[1]	7.9	4.9	7.2
Percidae (family)	5.2	42.3[2]	17.0	10.5	15.5
Etheostoma (genus)	5.2	40.0[2]	18.0	11.1	16.4
Etheostoma fonticola (species)	5.2	21.5[2]	33.4	20.7	30.5
Salmoniformes, Salmonidae					

Oncorhynchus (genus)	5.2	47.0[2]	15.3	9.5	13.9
O. apache (species)	5.2	16.5[2]	43.6	27.0	39.7
O. clarki henshawi (species)	5.2	22.8[2]	31.5	19.5	28.7
Salmo salar (species)	5.2	90[3]	8	5	7.3
Salvelinus (genus)	5.2	15.7[2]	45.8	28.4	41.7

[1] LC_{50} based on 5th percentile estimate from species sensitivity distribution (SSD), Table 2 – Cyanide BE.
[2] LC_{50} estimate based on lower bound of the 95% CI from ICE model (Appendix D).
[3] LC_{50} based on measured value from the Cyanide BE (Table 1).

Prediction Models

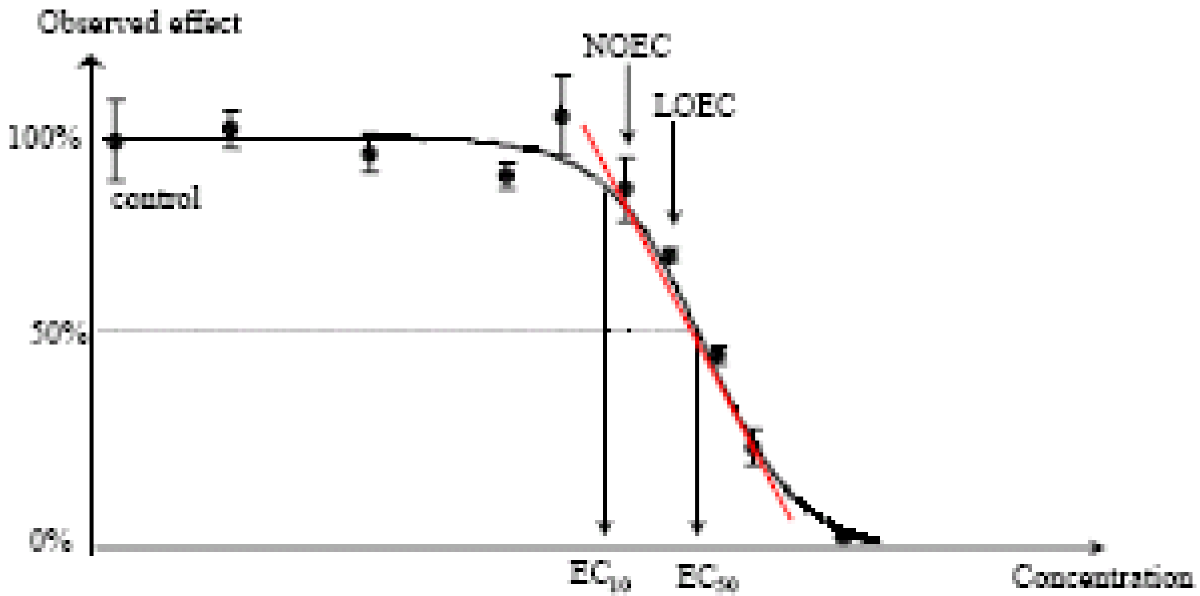
Statistical regression techniques were used to model or "fit" the relationship between cyanide concentrations and toxic effects to listed evaluation species based on data for the chronic toxicity test species. For nuances of statistical regression specific to toxicological applications, we relied substantively on two recent technical guidance documents: (1) Enivronment Canada (2005: "Guidance Document on Statistical Methods for Environmental Toxicity Tests"); and (2) OECD (2006: "Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application"). We also reviewed other relevant guidance such as that provided in the documentation for EPA's Toxicity Relationship Analysis Program (TRAP) (EPA 2002) and in discipline-specific statistical textbooks such as Gad and Weil (1988) and Sparks (2000).

As noted by Environment Canada (2005), an important principle of regression techniques is to keep the model simple, if that can reasonably be done. In completing this analysis, we have further incentive to follow that principle because we have a strong interest in evaluating the uncertainty (confidence) associated with point estimates and therefore an interest in avoiding what Environment Canada (2005) noted as the "...obstacle of calculating confidence intervals around nonlinear regression estimates..." Throughout this analysis we have been mindful of that because our models are not based on biological or chemical mechanisms of action, but are purely statistical constructs that have no mechanistic meaning. A statistical concentration-response model only serves to smooth the observed concentration-response to estimate effect concentrations by interpolating between treatment concentrations, and to provide a tool for assessing confidence intervals. Therefore, the choice of model is to some extent arbitrary (OECD 2006). That being noted, we constructed models that conformed to the non-arbitrary characteristics of the data we are working with and with statistical standard practices (such as data transformations). The degree of model fit achieved is an artifact of those specific decisions not the result of *post hoc* "model shopping" (EPA 2002).

Generic Concentration-reponse Relationship

Figure 1 illustrates a generic concentration-response relationship that typically takes on a sigmoidal form due to threshold effects on the low concentration end of the x-axis and to asymptotic effects at the high concentration end of the x-axis.

Figure 2. Generalized concentration-response relationship adapted from OECD (2006:Figure 3.2). Note that the illustrated curve is a plot fitted to a real dataset, thus the identification of NOEC and LOEC concentrations. For the purposes of this figure, consider the y-axis as a positive attribute that becomes diminished by toxicity, such as percent survivorship.



Note that the superimposed straight line in Figure 2 represents the region of concentrations that induce an intermediate toxic response that are well approximated by a linear fit. This "linear region" is strongest within one probit (also known as "normal equivalent deviate") either side of the median response concentration (EC_{50}), or roughly for concentrations that induce 16 to 84% response (Environment Canada 2005). The narrow ranges of $SSEC_x$ values that we need to evaluate can be expected to overwhelmingly fall within those boundaries as a result of the methods EPA used to set the chronic criterion at 5.2 ug CN/L; see the next section titled "Derivation of the Criterion Continuous Concentration (CCC)". Our approach is conceptually similar to the TRAP program's Piecewise Linear regression option (EPA 2002). Even with regard to the nonlinear regression options in TRAP, EPA (2002) provides a recommendation for segmented analysis when there is a focal region (or subset) of test concentrations of particular concern:

Within the limitations of this program, one useful approach can be to exclude (censor) high effects data from the analysis if (a) only low levels of effect are of interest and (b) there are sufficient low-to-moderate effects data to support a good analysis.

<u>Prediction Model based on the Fathead Minnow Dataset</u>

Lind et al. (1977) examined fathead minnow fecundity (number of eggs per spawn) and egg hatchability in relation to a series of cyanide treatments (concentrations). The experimental structure, as well as the fecundity results, is summarized in Table 5. There were five control replicates, and two replicates each for ten exposure concentrations. The response data are reasonably monotonic, especially within the intermediate response range covered by the lowest six treatments. Those treatments ranged (on a free cyanide basis) from 6 to 45.6 ug/L CN; a span that closely corresponds to the $SSEC_x$ range we want to evaluate (Table 4).

Table 5. Egg production of adult fathead minnows exposed for 256 days (from larvae through adult) to various concentrations of cyanide (from Lind et al. 1977; Table II).

Treatment HCN (ug/L)	Mean HCN (ug/L)	Free cyanide as CN (ug/L)	Mean eggs per female	Mean eggs per female per treatment	Reduction in the number of eggs per female - percent of control
Control			2530	3476	
Control			4483		
Control			3990		
Control			2718		
Control			3660		
5.7	5.8	6.0	1886	2512	27.7%
5.9			3138		
13.0	12.9	13.3[N]	1701	1845	46.9%
12.7			1989		
19.6	19.6	20.2[L]	1694	1468	57.8%
19.6			1241		
27.1	27.3	28.2	1093	1367	60.7%
27.5			1640		
36.0	35.8	36.9	678	1010	71.0%
35.6			1341		
43.7	44.2	45.6	2054	1124	67.7%
44.7			194		
62.5	63.5	65.6	74	72	97.9%
64.5			70		
73.1	72.8	75.1	573	319	90.8%
72.4			64		
81.5	80.7	83.3	266	243	93.0%
79.8			219		
96.1	100.8	103.9	0	0	100.0%
105.4			0		

[N] NOEC
[L] LOEC

To "build" our prediction model we transformed both the concentration data and the fecundity data for *a priori* reasons. We log-transformed the concentration data for two reasons: (1) statistically, toxicological tolerance distributions have long been confirmed as log-normal (OECD 2006); and (2) biologically, organisms experience toxicants on a log scale. Toxicological custom is to use log base-10 for the log transformations of test concentrations (Environment Canada 2005). Count data, such as "number of eggs per spawn" typically conform to a Poisson distribution rather than a normal distribution. To normalize such data for regression analysis a square-root transformation is recommended (EPA 2002). We used the square-root transformed response data for statistical analysis and then back-transformed the data for reporting results. This transformation does not change the model, but affects what the best parameter estimates and confidence limits are (EPA 2002). Thus, our model of choice is a log-square root linear regression over our focal segment (subset) of test concentrations.

In agreement with Gensemer et al.'s (2007) treatment of the same dataset, we collapsed the fecundity and egg hatchability endpoints into a single endpoint: "eggs hatched per spawn" which is the product of (eggs per spawn) x (egg hatchability) at each treatment concentration. We went a step further than Gensemer et al. (2007) and additionally applied a data-smoothing procedure to meet the assumption of monotonicity of response inherent in a linear regression. We did that by calculating three-point moving averages for both the fecundity and hatchability endpoints. This is a standard statistical technique for separating the "signal" from the "noise" in epidemiological and earth sciences (e.g., Rothman et al. 2008, Borradaile 2003).

TABLE 6. Fathead minnow input data for effects modeling.

Treatment (free ug CN/L)	Pooled mean eggs/female	Pooled Proportion Hatch[a]	Unsmoothed Pooled mean hatch/female[b]	3-pt moving average of proportion hatch	Smoothed Pooled mean hatch/female[b]	SQRT transform
Control Mean	3476	0.842	2927	0.763[c]	2652	51.5
6.00	2512	0.606	1522	0.754	1894	43.52
13.30	1845	0.813	1500	0.682	1258	35.47
20.20	1468	0.626	919	0.612	898	29.97
28.20	1367	0.396	541	0.527	720	26.83
36.90	1010	0.559	565	0.354	358	18.92
45.60	1124	0.108	121	0.271	305	17.46
65.60	72	0.147	11	0.149	11	3.31
75.10	319	0.192	61	0.181	58	7.62
83.30	243	0.204	50	0.132	32	5.66
103.90	0	0	0	0.068[c]	0	0

[a]Means weighted by replicate sample sizes; excludes hatchability result for Control B as per recommendation by Lind et al. (1977:264-265).

[b]Rounded to the nearest whole number.

[c]Based on double-weighted observed value; assuming any doses to the left of 0% response will be constant and any points to the right of 100% response will be constant.

[d]Final effects model based upon the shaded subset of data.

Although we didn't use the control data in our focal segment linear regression, we estimated where the smoothed data would cross the y-axis by double-weighting the control value, which then (along with its nearest neighboring data point) provided the basis of a three-point moving average for the "endpoint" of the concentration series. This double-weighting is justified conceptually because a treatment to the left of the controls on the concentration axis would be expected to respond the same as the controls (Environment Canada 2005). This enabled us to avoid comparing point estimates of eggs hatched per spawn from models fitted to smoothed data with "unsmoothed" control reference points. Note that our "smoothed" estimate of a control reference point was obtained using the actual data nearest to the y-axis and is not extrapolated from our estimated regression equation. Also note that we did not control-adjust the results prior to model fitting, a practice that leads to serious upward bias in EC_x point estimates (Environment Canada 2005, OECD 2006). A summary of response data smoothing and transformation is presented in Table 6.

The resulting log-square root focal segment linear regression model shows a very close fit to the data with an adjusted r-square of 0.964. The regression equation is:

Square-root (hatched eggs per spawn) = -30.19(LOG CN) + 68.36

The regression plot (Figure 3) and summary regression statistics (Table 7) are presented below. The regression was conducted using the multiple linear regression module of the *Statistica* software package (StatSoft 2006). Because we are dealing with small samples (six points in this case), we report the adjusted r-squared value which adjusts for the limited degrees of freedom in the model (StatSoft 2006).

Figure 3. Log- square root focal segment regression plot for fathead minnow fecundity x hatchability (= eggs hatched per spawn).

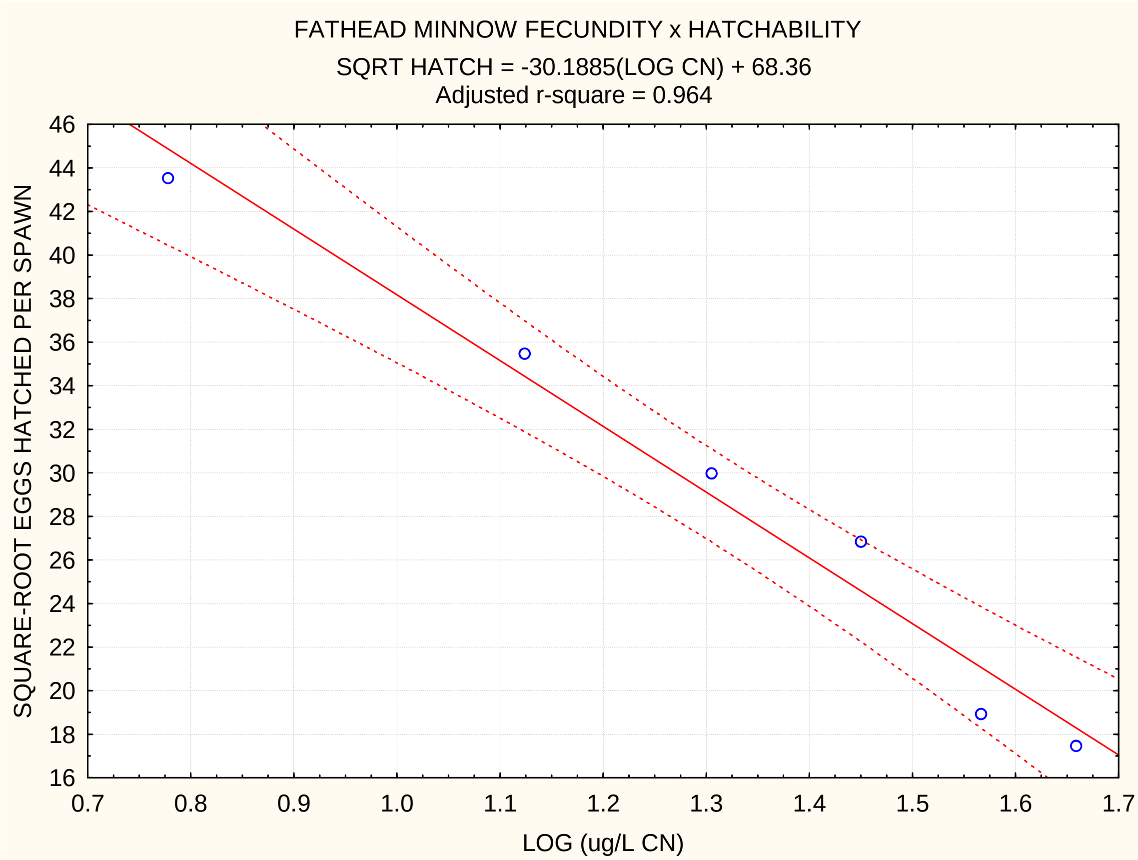


TABLE 7. Summary regression statistics.

Effects Surrogate	N	F value	p-level	Intercept	Std Err	p-level	Slope	Std Err	p-level
Fathead Minnow	6	134.6	<0.00032	68.36	3.505	0.000041	-30.19	2.602	0.00032
Brook Trout	5	12.34	<.039	24.85	2.595	0.0024	-6.594	1.877	0.039
Bluegill	5	11.75	<0.042	0.3514	0.9277	0.73	-2.533	0.7919	0.042

Prediction Model based on the Brook Trout Dataset

Koenst et al. (1977) examined brook trout fecundity (number of eggs per spawn) and egg viability in relation to a series of cyanide treatments (concentrations). The experimental structure, as well as the fecundity results, is summarized below (Table 8). There were two control replicates, and seven cyanide treatments. The lowest five treatments produced intermediate effects responses and covered a range of concentrations from 5.6 to 53.2 ug/L

CN; a span that closely corresponds to the $SSEC_x$ range we want to evaluate (Table 4). There was substantive variability in the results for the two control replicates. This lead to Koenst et al. (1977) excluding control replicate B, but noting that additional testing might indicate that the control results should be averaged. As noted in the footnote to Table 8, subsequent studies with brook trout (Holcombe et al. 2000) have confirmed that control replicate B should be averaged with control replicate A. For that reason, we used the control mean as our reference point for evaluating model predictions.

Table 8. Egg production of adult brook trout exposed to HCN for 144 days prior to the start of spawning (from Koenst et al., 1977).

HCN (ug/L)	Free cyanide as CN (ug/L)	Mean eggs spawned per female	Reduction in the number of eggs per female - percent of control*
Control A		502	
Control B		744	
Control Mean		623	
5.7	5.6	513	17.7%
11.2	11.1	291	53.3%
32.3	31.9	246	60.5%
43.6	43.1	442	29.1%
53.9	53.2	262	57.9%
64.9	64.1	124	80.1%
75.3	74.4	0	100.0%

* Reductions in the number of eggs spawned relative to controls were calculated using the Control mean (623 eggs per female). Koenst et al. (1977) performed the same calculation using only Control A (502 eggs per female) and reported that the MATC (Maximum Acceptable Toxicant Concentration) lies between 5.7 and 11.2 ug HCN/L. However, the authors went on to say that "When compared to the mean of the two controls, 5.7 ug/L HCN would appear to show a substantial reduction in eggs spawned per female, but due to the high variability in spawning in the two controls, further study would be required to reach this conclusion." Since that time other studies with brook trout have been conducted (Holcombe et al. 2000). The mean number of eggs spawned per female observed by Koenst et al. (1977) is within the range reported for these other studies, which supports the use of data from both controls in estimating the effect of cyanide on brook trout fecundity.

Again, in agreement with Gensemer et al.'s (2007) treatment of the same dataset, we collapsed the fecundity and egg viability endpoints into a single endpoint: "viable eggs per spawn" which is the product of (eggs per spawn) x (egg viability) at each treatment concentration. In the five-point segment of the data that we focus on, there was a substantive deviation from monotonicity at the 43.1 ug/L CN concentration. Therefore, once again we employed data-smoothing with a 3-point moving average to restore a monotonic progression of responses. Because the endpoint here is virtually the same as the endpoint for the fathead minnow dataset, other aspects of our treatment of the data for "building" a prediction model are the same as previously presented for the fathead minnow model. A summary of response data-smoothing and transformation is presented in Table 9 below.

TABLE 9. Brook trout input data for effects modeling.

Treatment (free CN ug/L)	Mean eggs/female	3-pt moving average of mean eggs/spawn	Proportion Viable	3-pt moving average of proportion viable	Smoothed mean viable/female[a]	SQRT transform
Control Mean	623	586[b]	0.935	0.923[b]	541	23.26
5.60	513	476	0.899	0.872	415	20.37
11.10	291	350	0.781	0.803	281	16.76
31.90	246	326	0.729	0.792	258	16.06
43.10	442	317	0.866	0.745	236	15.36
53.20	262	276	0.641	0.502	139	11.79
64.10	124	129	0	0.214	28	5.29
74.40	0	41[b]	0	0[b]	0	0

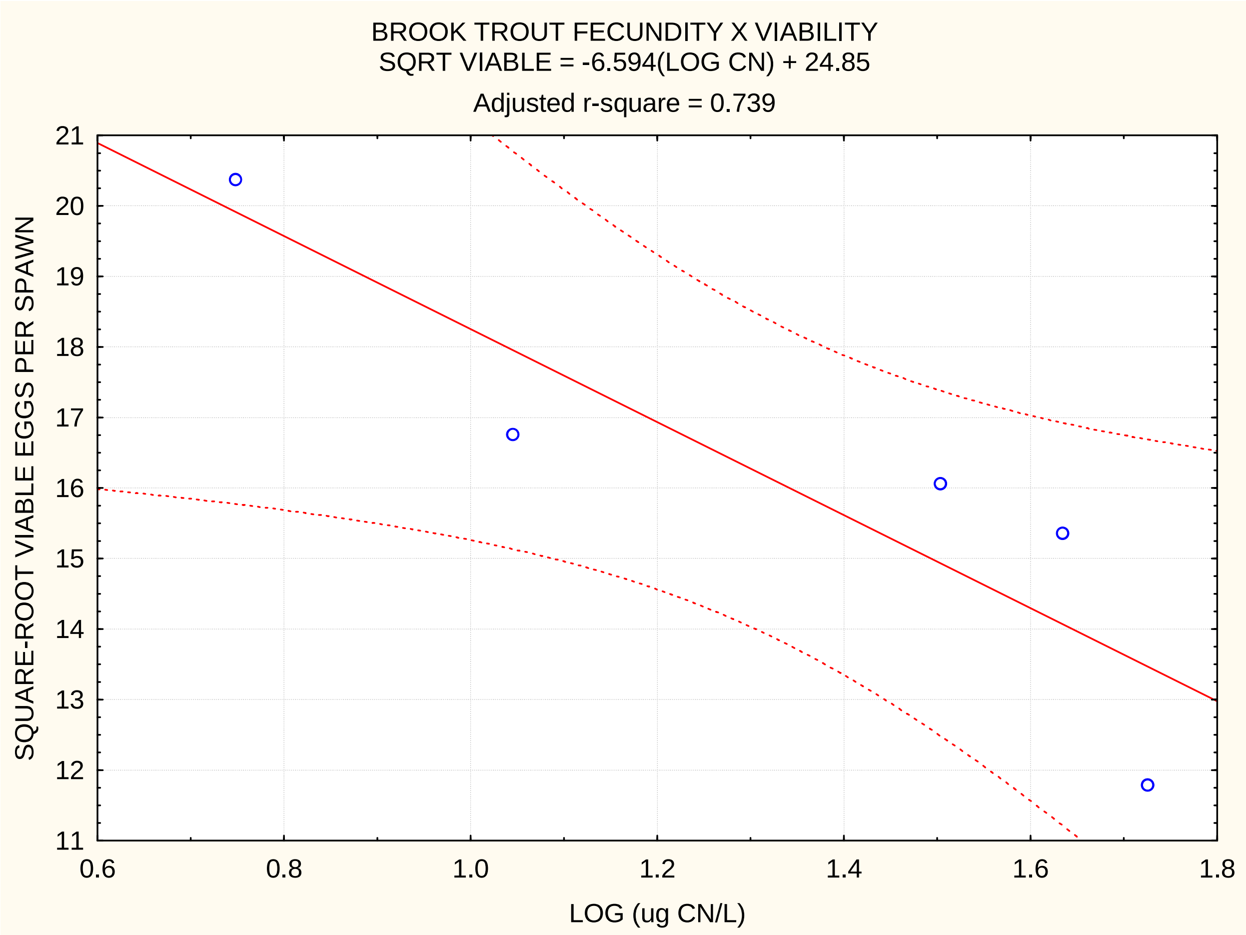
[a]Rounded to the nearest whole number.
[b]Based on double-weighted observed value; assuming any doses to the left of 0% response will be constant and any points to the right of 100% response will be constant.
[c]Final effects model based upon the shaded subset of data.

The resulting log-square root focal segment linear regression model does not show as strong a fit to the data as the fathead minnow model does, but still shows a reasonably good fit with an adjusted r-square of 0.739. The regression equation is:

Square-root (viable eggs per spawn) = -6.594(LOG CN) + 24.85

The regression plot is presented in Figure 4 and summary regression statistics are presented in Table 7. The regression was conducted using the multiple linear regression module of the *Statistica* software package (StatSoft 2006). Because we are dealing with small samples, i.e., five points in this case, we report the adjusted r-squared value which adjusts for the limited degrees of freedom in the model (StatSoft 2006).

<u>Figure 4.</u> Log-square root focal segment regression plot for brook trout fecundity x viability (= viable eggs per spawn).



<u>Prediction Model based on the Bluegill Dataset</u>

Kimball et al. (1978) examined bluegill juvenile survivorship in relation to a series of cyanide treatments (concentrations). The experimental structure, as well as the survivorship results, is summarized in Table 10. There were four control replicates, and two replicates each for eight cyanide treatments. The lowest five treatments produced intermediate effects responses and covered a range of concentrations from 4.9 to 40.6 ug/L CN; a span that closely corresponds to the $SSEC_x$ range we want to evaluate (Table 4).

Table 10. Survival of bluegills from fertilized egg to the 57-day juvenile state in various HCN concentrations (from Kimball et al. 1978; Table 3).

HCN (ug/L)	Mean HCN (ug/L)	Free cyanide as CN (ug/L)	Percent survival	Number of surviving juveniles *	Mean percent survival	Reduction in survival compared to controls
Control			37.5	75	23.3	
Control			20.0	40		
Control			10.0	20		
Control			25.5	51		
4.8	4.8	4.9	18.5	37	18.5	20.6%
5.2			lost			
8.9	9.1	9.4[N]	25.0	50	16.3	30.0%
9.2			7.5	15		
19.2	19.4	19.9[L]	3.0	6	2.8	88.0%
19.6			2.5	5		
28.5	29.1	29.9	2.5	5	2.5	89.3%
29.7			2.5	5		
38.7	39.5	40.6	3.0	6	3.8	83.7%
40.2			4.5	9		
49.3	49.3	50.7	13.5	27	13.5	42.1%
51.9			lost			
61.8	62.9	64.6	0.0	0	0.0	100.0%
64			0.0	0		
80.4	82.1	84.4	0.0	0	0.0	100.0%
83.8			0.0	0		

* Number of surviving juveniles was calculated by multiplying the reported percent survival times the starting number of fertilized eggs per treatment (200).

[N] NOEC

[L] LOEC

The bluegill dataset differs qualitatively from the fathead minnow and brook trout datasets because the response variable, juvenile survivorship, is a quantal (binary) rather than continuous variable. Quantal variables conform to a binomial distribution. Such data are typically analyzed via either probit transformation, as employed by Gensemer et al. (2007), or logit transformation of the proportions of responding and non-responding test subjects. Probits are normal equivalent deviates and logits are logistic equivalent deviates. These two transforms usually yield similar estimates of EC_{50} values, but differ appreciably in their EC estimates in the tails of the distributions.

Environment Canada (2005) recommends logistic methods over probits for "... mathematical simplicity and other good reasons." Logit = ln (p/1-p), where p is the proportion of effected test subjects (e.g., if juvenile survival were 30% for a particular treatment concentration, p would equal 0.3 and the logit transform would equal -0.8473).

The logit transform linearizes the sigmoidal logistic response curve (Environment Canada 2005, StatSoft 2006). Furthermore, in fitting the logit model, the control observations can be excluded, as they do not provide any information, unless a background parameter is included (OECD 2006).

Both Environment Canada (2005) and OECD (2006) note that it is common practice to correct the data for background response prior to analysis (for example via Abbott's correction), but that such pre-treatment of the data is unsound statistical practice that can result in substantive overestimation of EC_x values. The bias increases as the control effect being adjusted for increases. We fit a focal segment of the bluegill dataset to a log-logit regression using results that were not control-adjusted prior to analysis. Thus, our prediction model yields unbiased estimates of proportion effect that can be control-adjusted for reporting purposes after-the-fact. The dataset is reasonably monotonic until the highly anomalous result for the treatment at a concentration of 50.7 ug/L CN. Gensemer et al. (2007) censored that point as an outlier. Because our $SSEC_x$ range extended up to only 41.7 ug/L CN (Table 4) the 50.7 ug/L CN treatment did not fall within our focal segment of concern. The last three treatments in our focal segment produced results of greater than 84% effect which would place them in the nonlinear upper tail of the sigmoidal curve (Figure 2), but unlike a log-square root regression the logit transform will linearize points in the tails relative to intermediate effect points. Thus, for log-logit regression points that fall in tails do not have to be avoided in order to apply linear regression. The minor deviation from monotonicity in the last two points of our focal segment did not warrant data-smoothing. A summary of the logit transformed response data is presented in Table 11.

TABLE 11. Bluegill input data for effects modeling.

Treatment (free CN ug/L)	Mean surviving juveniles	Proportion Survival	Logit Proportion Survival
Control Mean	46.5	0.2325	-1.1942
4.9	37	0.1850	-1.4828
9.4	32.5	0.1630	-1.6361
19.9	5.5	0.0280	-3.5472
29.9	5	0.0250	-3.6636
40.6	7.5	0.0380	-3.2314
50.7	27	0.1350	-1.8575
64.6	0	0.0000	
84.4	0	0.0000	

[a]Final effects model based upon the shaded subset of data.

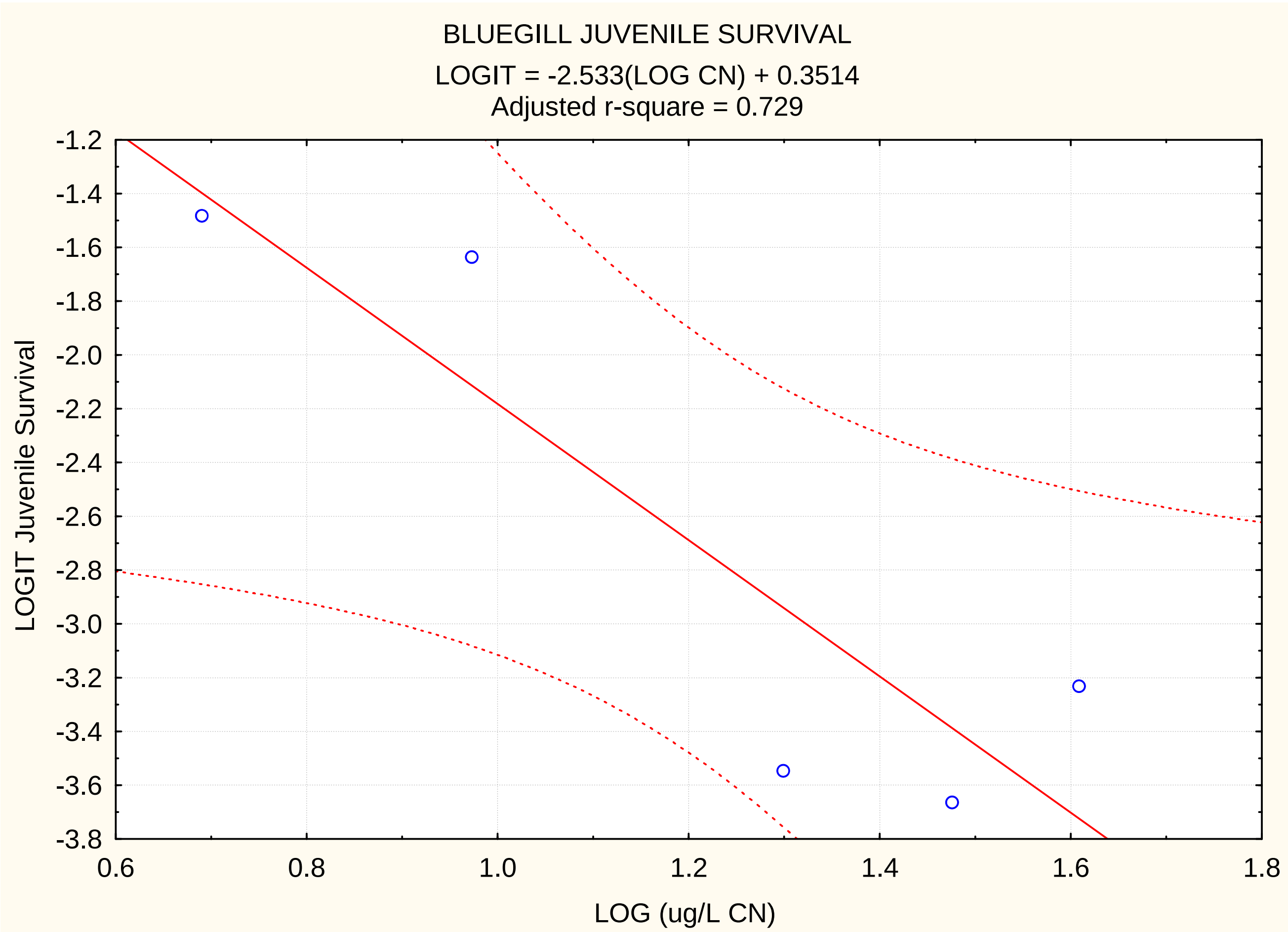
The resulting log-logit focal segment linear regression model does not show as strong a fit to the data as the fathead minnow model does, but with an adjusted r-square of 0.729

shows a reasonably good fit comparable to that achieved for the brook trout dataset. The regression equation is:

Logit (proportion juvenile survival) = -2.533 (LOG CN) + 0.3514

The regression plot is presented in Figure 5 and summary regression statistics are presented in Table 7. The regression was conducted using the multiple linear regression module of the *Statistica* software package (StatSoft 2006). Because we are dealing with small samples (five points in this case), we report the adjusted r-squared value which adjusts for the limited degrees of freedom in the model (StatSoft 2006).

FIGURE 5. Log-logit focal segment regression plot for bluegill juvenile survival.



<u>Prediction Results</u>

Effects predictions are generated by substituting LOG ($SSEC_x$) for LOG (CN) into the prediction regression equations. This was accomplished via the "predict dependent variable" algorithm in the multiple linear regression module of Statistica (StatSoft 2006). That algorithm also uses the estimated standard error of the regression coefficient to generate 95% confidence limits for the predicted point estimates (maximum likelihood estimates). For the fathead minnow and brook trout prediction regressions, the prediction and confidence limit output are in the form of square-roots of numbers of eggs. To convert those predictions to a percent effect, the predicted results were first squared and then scaled for percent change compared to the applicable smoothed control value according to the formula:

% Effect = [1- (predicted egg count / smoothed control value)] x 100

Any predicted egg counts exceeding the smoothed control value were automatically converted to 0% effect. For the bluegill prediction regression, the prediction and confidence limit output are in the form of logit transforms for proportions of juvenile survivorship. The logit transforms are back-transformed to proportions by the formula:

Proportion survival = $e^{(\text{logit})} / 1 + e^{(\text{logit})}$

The predicted survival proportions are scaled for percent change compared to the reported control value according to the formula:

% Effect = [1- (predicted proportion survival / mean control proportion survival)] x 100

Again, any predicted survivorship exceeding the observed mean control survivorship results in a percent effect prediction that is automatically converted to 0% effect. The raw input and output data for effects predictions are presented in Appendix E.

A summary of predicted effects and their estimated 95% confidence limits from each of the three prediction models for each of the 17 surrogate taxa from which listed evaluation species' LC_{50} values were derived are presented in Table 12. The effects estimates are presented in Table 13 for the listed evaluation species based on matching up the effects estimates for surrogate taxa in Table 12 with the listed species linked to each surrogate taxon.

The EC_{10} and EC_{20} concentrations for each of our three regression models were also estimated.

The fathead minnow regression yielded an estimated EC_{10} of 4.4 ug/L CN (95% CI = 2.6-6.2 ug/L CN) and an estimated EC_{20} of 5.5 ug/L CN (95% CI = 3.5-7.4). By comparison, Gensemer et al. (2007) estimated an EC_{20} of 6.0 ug/L CN from a log-probit analysis of the fathead minnow data, but did not report confidence limits for that estimate.

The brook trout regression yielded an estimated EC_{10} of 2.6 ug/L CN (95% CI = 0.0-8.4 ug/L CN) and an estimated EC_{20} of 4.1 ug/L CN (95% CI = 0.0-11.1). Gensemer et al. (2007) estimated an EC_{20} of 7.7 ug/L by linear interpolation of the brook trout data, and again did not report confidence limits for that estimate. It is important to note that, for their analysis, Gensemer et al. (2007) did not average the controls, as we did, but used the control which produced the lowest number of eggs (refer to the Table 8 footnote for more details).

The bluegill regression yielded an estimated EC_{10} of 4.6ug/L CN (95% CI = 0.0-10.5 ug/L CN) and an estimated EC_{20} of 5.3 ug/L CN (95% CI = 0.0-11.5). Gensemer et al. (2007) estimated an EC_{20} of 5.6 ug/L CN from a log-probit analysis of the bluegill data, and also estimated an EC_{20} of 8.9 ug/L CN for the bluegill data from EPA's TRAP program.

All of Gensemer et al.'s (2007) estimates fall within our 95% confidence limits, and in general show excellent agreement with our results even though Gensemer et al's methods differed from ours. This suggests that our results are not highly dependent on the particular statistical approach that we chose for our analysis.

Table 12. Estimated magnitude of effect of cyanide (at the CCC, 5.2 ug CN/L) on surrogate taxa for listed fish species (± 95% CL). The magnitude of effect was estimated using the regression model for each surrogate response species and SS EC_X value for each surrogate taxa (Table 1). For each surrogate taxa there were two estimates of effects on reproductive performance and one estimate of effects on early life stage survival.

	Surrogate species		
	Fathead Minnow	Brook Trout	Bluegill
Surrogate taxa used to estimate magnitude of effect on listed species	Reduction in the mean number of hatched eggs per spawn compared to controls	Reduction in the mean number of viable eggs per spawn compared to controls	Reduction in the number of surviving larvae/juveniles compared to controls
Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Cypriniformes (order)	39% (28%, 49%)	26% (0%, 54%)	44% (0%, 80%)
Family Catostomidae			
Xyrauchen texanus (species)	39% (28%, 49%)	26% (0%, 54%)	44% (0%, 80%)
Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Cyprinella monacha (species)	68% (63%, 72%)	42% (23%, 58%)	76% (50%, 89%)
Gila elegans (species)	57% (51%, 63%)	36% (12%, 56%)	66% (30%, 84%)
Notropis mekistocholas (species)	59% (53%, 65%)	37% (14%, 56%)	68% (34%, 85%)
Ptychocheilus lucius (species)	63% (57%, 68%)	39% (18%, 57%)	71% (41%, 86%)
Perciformes (order)	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)
Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
Etheostoma (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)

Surrogate taxa used to estimate magnitude of effect on listed species	Surrogate species		
	Fathead Minnow	Brook Trout	Bluegill
	Reduction in the mean number of hatched eggs per spawn compared to controls	Reduction in the mean number of viable eggs per spawn compared to controls	Reduction in the number of surviving larvae/juveniles compared to controls
Etheostoma fonticola (species)	81% (76%, 85%)	52% (37%, 64%)	86% (64%, 95%)
Order Salmoniformes, Family Salmonidae			
Oncorhynchus (genus)	60% (54%, 65%)	37% (15%, 57%)	69% (36%, 85)
Oncorhynchus apache (species)	87% (82%, 91%)	56% (42%, 68%)	90% (67%, 97%)
Oncorhynchus clarki henshawi (species)	80% (75%, 84%)	51% (36%, 63%)	85% (63%, 94%)
Salmo salar (species)	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)
Salvelinus (genus)	87% (83%, 92%)	57% (43%, 69%)	90% (68%, 97%)

Table 13. Estimated magnitude of effect of cyanide (at the CCC, 5.2 ug CN/L) on listed fish species (95% CL). There are two estimates for effects on fecundity and one estimate for effects on early life stage survival. Estimates are based on analyses using surrogate taxa (Table 12). Surrogate taxa were used to estimate LC_{50}'s for listed species Surrogate response species (fathead minnow, brook trout, bluegill) datasets were used to estimate magnitude of chronic effects.

Listed Species		Order/Family	Surrogate Taxa	Estimated reduction in fecundity and larvae/juvenile survival due to cyanide exposure (5.2 ug/L) based on surrogate species data sets.		
				Fathead Minnow (Reduction in the mean number of hatched eggs per spawn compared to controls)	Brook Trout (Reduction in the mean number of viable eggs per spawn compared to controls)	Bluegill (reduction in the number of surviving larvae/juveniles compared to controls)
Gulf sturgeon	*Acipenser oxyrinchus desotoi*	Acipenseriformes Acipenseridae	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Kootenai River white sturgeon	*Acipenser transmontanus*					
Pallid sturgeon	*Scaphirhynchus albus*					
Alabama sturgeon	*Scaphirhynchus suttkusi*					
Waccamaw silverside	*Menidia extensa*	Atheriniformes Atherinopsidae				
Modoc sucker	*Catostomus microps*	Cypriniformes Catostomidae	Cypriniformes (order)	39% (28%, 49%)	26% (0%, 54%)	44% (0%, 80%)
Santa Ana sucker	*Catostomus santaanae*					
Warner sucker	*Catostomus warnerensis*					
Shortnose sucker	*Chasmistes brevirostris*					
Cui ui	*Chasmistes cujus*					
June sucker	*Chasmistes liorus*					
Lost River sucker	*Deltistes luxatus*					
Razorback sucker	*Xyrauchen texanus*		*Xyrauchen texanus*	39% (28%, 49%)	26% (0%, 54%)	44% (0%, 80%)
Spotfin chub	*Cyprinella monacha*	Cypriniformes Cyprinidae	*Cyprinella monacha*	68% (63%, 72%)	42% (23%, 58%)	76% (50%, 89%)
Blue shiner	*Cyprinella caerulea*		Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Beautiful shiner	*Cyprinella formosa*					
Devils River minnow	*Dionda diaboli*					
Slender chub	*Erimystax cahni*					
Mohave tui chub	*Gila bicolor mohavensis*					
Owens tui chub	*Gila bicolor snyderi*					
Borax Lake chub	*Gila boraxobius*					
Humpback chub	*Gila cypha*					
Sonora chub	*Gila ditaenia*					
Gila chub	*Gila intermedia*					
Pahranagat roundtail chub	*Gila robusta jordani*					
Virgin River chub	*Gila robusta seminuda*					

Rio Grand silvery minnow	*Hybognathus amarus*					
Big Spring spinedace	*Lepidomeda mollispinis pratensis*					
Little Colorado spinedace	*Lepidomeda vittata*					
Spikedace	*Meda fulgida*					
Moapa dace	*Moapa coriacea*					
Palezone shiner	*Notropis albizonatus*					
Cahaba shiner	*Notropis cahabae*					
Arkansas River shiner	*Notropis girardi*					
Pecos bluntnose shiner	*Notropis simus pecosensis*					
Topeka shiner	*Notropis Topeka*					
Oregon chub	*Oregonichthys crameri*					
Blackside dace	*Phoxinus cumberlandensis*					
Woundfin	*Plagopterus agrentissimus*					
Ash Meadows speckled dace	*Rhinichthys osculus nevadensis*					
Kendall Warm Springs dace	*Rhinichthys osculus thermalis*					
Loach minnow	*Tiaroga cobitis*					
Bonytail chub	*Gila elegans*		*Gila elegans*	57% (51%, 63%)	36% (12%, 56%)	66% (30%, 84%)
Cape Fear shiner	*Notropis mekistocholas*		*Notropis mekistocholas*	59% (53%, 65%)	37% (14%, 56%)	68% (34%, 85%)
Colorado pikeminnow	*Ptychocheilus lucis*		*Ptychocheilus lucis*	63% (57%, 68%)	39% (18%, 57%)	71% (41%, 86%)
White River springfish	*Crenichthys baileyi baileyi*	Cyprinodontiformes Goodeidae	Actinopterygii (class**)**	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Hiko White River springfish	*Crenichthys baileyi grandis*					
Railroad Valley springfish	*Crenichthys nevadae*					
Big Bend gambusia	*Gambusia gaigei*	Cyprinodontiformes Poeciliidae				
San Marcos gambusia	*Gambusia georgei*					
Clear Creek gambusia	*Gambusia heterochir*					
Pecos gambusia	*Gambusia nobilis*					
Gila topminnow (including Yaqui)	*Poeciliopsis occidentalis*					
Unarmoned threespine stickleback	*Gasterosteus aculeatus williamsoni*	Gasterosteiformes Gasterosteidae				
Delta smelt	*Hypomesus transpacificus*	Osmeriformes Osmeridae				
Tidewater goby	*Eucyclogobius newberryi*	Perciformes Gobiidae	Perciformes	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)
Slackwater darter	*Etheostoma boschungi*	Perciformes Percidae	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
Vermilion darter	*Etheostoma chermocki*					

Relict darter	*Etheostoma chienense*					
Etowah darter	*Etheostoma etowahae*					
Niangua darter	*Etheostoma nianguae*					
Watercress darter	*Etheostoma nuchale*					
Okaloosa darter	*Etheostoma okaloosae*					
Duskytail darter	*Etheostoma percnurum*					
Bayou darter	*Etheostoma rubrum*					
Cherokee darter	*Etheostoma scotti*					
Maryland darter	*Etheostoma sellare*					
Bluemask darter	*Etheostoma sp.*					
Boulder darter	*Etheostoma wapiti*					
Fountain darter	*Etheostoma fonticola*		*Etheostoma fonticola* (species)	81% (76%, 85%)	52% (37%, 64%)	86% (64%, 95%)
Amber darter	*Percina antesella*		Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
Goldline darter	*Percina aurolineata*					
Conasauga logperch	*Percina jenkinsi*					
Leopard darter	*Percina pantherina*					
Roanoke logperch	*Percina rex*					
Snail darter	*Percina tanasi*					
Ozark cavefish	*Amblyopsis rosae*	Percopsiformes Amblyopsidae	Actinopterygii (class**)**	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Alabama cavefish	*Speoplatyrhinus poulsoni*					
Little Kern golden trout	*Oncorhynchus aguabonita whitei*	Salmoniformes Salmonidae	*Oncorhynchus* (genus)	60% (54%, 65%)	37% (15%, 57%)	69% (36%, 85)
Paiute cutthroat trout	*Oncorhynchus clarki seleniris*					
Greenback cutthroat trout	*Oncorhynchus clarki stomias*					
Gila trout	*Oncorhynchus gilae*					
Apache trout	*Oncorhynchus apache*		*Oncorhynchus apache* (species)	87% (82%, 91%)	56% (42%, 68%)	90% (67%, 97%)
Lahontan cutthroat trout	*Oncorhynchus clarki henshawi*		*Oncorhynchus clarki henshawi* (species)	80% (75%, 84%)	51% (36%, 63%)	85% (63%, 94%)
Atlantic salmon	*Salmo salar*		*Salmo salar*	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)
Atlantic salmon						
Bull trout	*Salvelinus confluentus*		*Salvelinus* (genus)	87% (83%, 92%)	57% (43%, 69%)	90% (68%, 97%)
Pygmy sculpin	*Cottus paulus*	Scorpaeniformes Cottidae	Actinopterygii (class**)**	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)

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Other Effects Estimates

The estimates of effects presented in Table 13 are based largely on ICE LCL (lower confidence limit) LC_{50} values for listed fish evaluation species. Those are the LC_{50} values that we accept as sufficiently accounting for the uncertainties inherent in relying on surrogate data and numerous other untested assumptions to estimate the sensitivity of listed species to cyanide. The Service, NMFS, and EPA agreed that using ICE LCL values was preferable to the practice of applying arbitrary uncertainty factors.

However, EPA has, at various times, questioned whether the use of ICE LCL values might not be overly conservative. Therefore, we also estimated effect levels using ICE MLE (maximum likelihood estimates) LC_{50} values for listed fish evaluation species (via revised $SSEC_x$ estimates). Those results are presented in Appendix F. Based on the fathead minnow prediction model, which was the strongest model, the median levels of effect predicted for the 15 ICE surrogate taxa were 51% and 65%, respectively, for ICE MLE and ICE LCL. The number of surrogate taxa with a predicted effect of 35% or greater was 11 and 14, respectively, for ICE MLE and ICE LCL. Those differences indicate only modest conservatism conferred by ICE LCL-based effects estimates as compared to ICE MLE-based estimates. Such modest differences would not have a decision-making impact. For both sets of results, unacceptably high levels of effect would overwhelmingly be the predominant prediction.

Empirical Test of Method Performance

Because only three concentration-response datasets are available, there is almost no basis for testing our method performance (i.e., there are no known directly measured “true” values for effects to our listed fish evaluation species at a concentration of 5.2 ug/L CN). However, because the fathead minnow and brook trout datasets focused on essentially the same response variable (number of hatchable/viable eggs produced per spawn) we can perform two tests of method performance. For each species, we can directly estimate a predicted effect level at 5.2 ug/L CN using the species-specific regressions. Those would be our estimates of the “true” effect level. Next, we can use our surrogate method and estimate an $SSEC_x$ for each species on the other species’ response curve and evaluate the predicted effect level for that $SSEC_x$ value and compare the surrogate estimate to the estimated “true” value. The results are as follows:

The directly estimated fathead minnow effect level at 5.2 ug/L CN is 18% with a 95% CI of 0%-34%. The fathead minnow $SSEC_x$ value on the brook trout response curve would be 3.2 ug/L CN, which yields an effects estimate of 15%. That is nearly identical to estimated “true” value and easily within the 95% CI for the “true value”.

The directly estimated brook trout effect level at 5.2 ug/L CN is 25% with a 95% CI of 0%-54%. The brook trout $SSEC_x$ value on the fathead minnow response curve would be 8.4 ug/L CN, which yields an effects estimate of 38%. Again, that is within the 95% CI for the “true” value, although our estimate of the “true” value is not very precise and therefore the 95% CI is fairly wide.

In summary, in both test cases, the estimated effect level derived from our surrogate methodology is not significantly different from the estimated "true" value in a statistical sense, but the second comparison has low statistical power. Further validation testing of this sort should be done as concentration-response datasets become available for more species using a comparable response variable, but it is reassuring that in these test cases our method yielded results that were nearly identical to the "true" value in one case and reasonably close to the "true" value in the other case.

Derivation of the Criterion Continuous Concentration (CCC)

Our analysis predicts that the listed fish evaluation species considered in this Biological Opinion would be highly affected by exposure to cyanide at the CCC. These results prompted us to better understand the level of protection that aquatic life criteria in general and the cyanide criterion in particular were intended to provide.

The objective of the Clean Water Act (CWA) is to "*restore and maintain the chemical, physical and biological integrity of the Nation's waters*" with an interim goal of "*water quality which provides for the protection and propagation of fish, shellfish and wildlife and provides for recreation in and on the water*", where attainable.

Section 304(a) of the CWA requires the EPA Administrator to publish "*criteria for water quality accurately reflecting the latest scientific knowledge on the kind and extent of all identifiable effects on the health and welfare including, but not limited to, plankton, fish shellfish, wildlife, plant life….*" including information "*on the factors necessary for the protection and propagation of shellfish, fish, and wildlife for classes and categories of receiving waters…*"

Water quality criteria are developed to protect different attributes or uses of water bodies, referred to as designated uses. Aquatic life water quality criteria are intended to protect waters where the designated use includes the protection and propagation of fish, shellfish and wildlife.

EPA's *Guidelines for Deriving Numeric National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Stephan et al. 1985) has been used to derive aquatic life water quality criteria, including the cyanide criterion, since the mid 1980's. This excerpt from the guidelines document describes, from an operational perspective, the intended purpose of national criteria and their limitations:

"*Because aquatic ecosystems can tolerate some stress and occasional adverse effects, protection of all species at all times and places is not deemed necessary. If acceptable data are available for a large number of appropriate taxa from an appropriate variety of taxonomic and functional groups, a reasonable level of protection will probably be provided if all except a small fraction of the taxa are protected, unless a commercially or recreationally important species is very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in* *comparison with the sets of data from which they were calculated. Use of 0.05 to calculate the Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is too high or too low or just right.* "

Based on this description it appears that National criteria are intended to protect ecosystems and ecosystem functions and not necessarily to protect all taxa or species within the ecosystem all of the time. It is expected that a small fraction (5%) of taxa or species may be adversely affected. The guidelines go on to say:

"*To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and other important species and (2) (a) fish and benthic invertebrates assemblages in rivers and streams, and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes reservoirs, estuaries, and oceans."*

Thus, the level of protection afforded to aquatic organisms should prevent *unacceptable* long-term and short term effects. The threshold for unacceptable long-term or chronic effects is estimated by the CCC. The guidelines indicate that some adverse effects may occur at the CCC but they should not rise to a level that is unacceptable:

"*However, it is important to note that this is a threshold of unacceptable effect, not a threshold of adverse effect. Some adverse effect, possibly even a small reduction in the survival, growth, or reproduction of a commercially or recreationally important species, will probably occur at, and possibly even below, the threshold. The Criterion Continuous Concentration (CCC) is intended to be a good estimate of this threshold of unacceptable effect.*"

EPAs Water Quality Standards Handbook (EPA 1994) defines the CCC as "*the EPA national water quality criteria recommendation for the highest instream concentration of a toxicant or an effluent to which organisms can be exposed indefinitely without causing unacceptable effect*".

The guidelines provide some guidance for determining what constitutes unacceptable levels of adverse effects. The following guidance is for monitoring programs designed to detect unacceptable levels of adverse effects in the field:

"*The amount of decrease in the number of taxa or number of individuals in an assemblage that should be considered unacceptable should take into account appropriate features of the body of water and its aquatic community. Because most monitoring programs can only detect decreases of more than 20 percent, any statistically significant decrease should usually be considered unacceptable. The insensitivity of most monitoring programs greatly limits their usefulness for studying the validity of criteria because unacceptable changes can occur and not be detected. Therefore, although* *limited field studies can sometimes demonstrate that criteria are underprotective, only high quality field studies can reliably demonstrate that criteria are not underprotective.*"

Here, the guidelines appear to suggest that because of the insensitivity of monitoring programs only relatively high levels of effect (>20%) may be detected as statistically significant, and suggest that lower, yet unacceptable, levels of effects may go undetected.

However, the National criteria are typically derived using chronic toxicity data from laboratory tests rather than field studies. Chronic data from individual tests are analyzed and a chronic value is computed according to the following guidance:

"*A chronic value may be obtained by calculating the geometric mean of the lower and upper chronic limits from chronic tests or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of adverse effect on any of the specified biological measurements, and (c) below which no tested concentration caused an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of adverse effect on one or more of the specified biological measurements, and (c) above which all tested concentrations also caused such an effect.*"

For most aquatic life criteria that have been derived thus far, including the cyanide criterion, chronic values have been obtained by calculating the geometric mean of the lower and upper chronic limits. In practice, the upper and lower chronic limits are often statistically determined by hypothesis testing. The lower limit is typically the No Observable Effect Concentration (NOEC), which is defined as the highest test concentration where the effects are not statistically significantly different from controls. The upper limit is typically the Lowest Observable Effect Concentration (LOEC), which is defined as the lowest test concentration where the effects are statistically significantly different from controls. The guidelines recommend that the magnitude of effect associated with the upper and lower chronic limits should be considered when determining values that appropriately estimate acceptable and unacceptable levels of adverse effect:

"*Because various authors have used a variety of terms and definitions to interpret and report results from chronic tests, reported results should be reviewed carefully. The amount of effect that is considered unacceptable is often based on a statistical hypothesis test, but might also be defined in terms of a specified percent reduction from the controls. A small percent reduction (e.g., 3%) might be considered acceptable even if it is statistically significantly different from the control, whereas, a large percent reduction (e.g., 30%) might be considered unacceptable even if it is not statistically significant.*"

Based on this guidance, the threshold for unacceptable adverse effects would be estimated by the chronic value. The magnitude of effect at the threshold would then be equivalent to the magnitude of effect at the chronic value. For chronic criteria derived using hypothesis tests, this would be the magnitude of effect occurring at a concentration equal to the geometric mean of the NOEC and LOEC, that is, somewhere between an acceptable and unacceptable level of adverse effect. The guidelines do not specify a level of adverse effect on which the threshold for unacceptability should be based. The only mention of a numeric value or range is provided in the guidance for selecting chronic limits (mentioned above) and suggests that this threshold may lie between 3% and 30%.

Thus, for a given species or test the magnitude of effect at the chronic value will depend on the magnitude of effect at the lower and upper chronic limits. We followed this approach for estimating the magnitude of effect occurring at the cyanide CCC. The freshwater cyanide CCC was derived based on chronic toxicity data for 4 species (Table 14): 3 fish (fathead minnow, brook trout, and bluegill) and 1 invertebrate (*Gammarus pseudolimnaeus*). Chronic values for each species were obtained by calculating the geometric mean of the lower and upper chronic limits. The magnitude of effect at the lower and upper chronic limits was calculated by comparing responses at the lower and upper limits to controls. For fathead minnow and brook trout these effects were expressed as reduction in the mean number of eggs spawned per female compared to controls; for the bluegill the effect was reduction in larvae/juvenile survival compared to controls; and for *G. pseudolimnaeus* the effect was a reduction in the mean number of eggs or young per gravid female relative to controls.

We then estimated the magnitude of effect at the chronic value by linear interpolation between lower and upper chronic limits (Table 14). Based on these calculations the magnitude of effect at the chronic values for the fathead minnow, brook trout, bluegill and *G. pseudolimnaeus* would be 52%, 32%, 54%, and 47%, respectively. According to the guidelines, if there were a sufficient number of chronic values (i.e., chronic values for species in 8 phylogenetic families) the chronic criterion could be computed directly from the distribution of chronic values. If there were fewer chronic values, as was the case for cyanide, the chronic criterion would be computed using Acute-Chronic Ratios (ACR). ACRs for the 4 freshwater species were reported in the cyanide criterion document and are shown in Table 14. The ACRs were calculated by dividing the species mean acute value (i.e., mean LC_{50} for the species) by the chronic value. For example, the ACR for fathead minnow (7.633) was computed by dividing 125.1 ug CN/L (the mean LC_{50} for the species) by 16.39 ug/ CN/L (the chronic value). Thus, the ACR is the ratio between the concentration of cyanide causing 50% lethality (following acute exposure) and the concentration following chronic exposure that causes a level of adverse effect that is at the threshold of unacceptability, i.e., 52% for fathead minnow. The guidelines require that, for criteria derivation, the geometric mean of individual species ACRs is used to obtain the Final ACR. For cyanide, the freshwater Final ACR was 8.562 (Table 14). We estimated the magnitude of chronic effects associated with the Final ACR to be about 45% (Table 14).

The Final ACR and the Final Acute Value (FAV) were then be used to derive the CCC. The guidelines describe how the FAV is computed. In short, the FAV is set equal to the 5th percentile estimate from the distribution of genus mean acute values. In other words, the FAV represents the genus with acute sensitivity (LC_{50}) in the *sensitive tail* of the distribution where, theoretically, approximately 5% of the genera would be more sensitive and about 95% of the genera would be less sensitive. Based on this analysis, the FAV for cyanide was determined to be 62.68 ug CN /L. The guidelines also include provisions for adjusting the FAV to protect commercially and recreationally important species:

"*However, in some cases, if the Species Mean Acute Value of a commercially or recreationally important species is lower than the calculated Final Acute Value, then that Species Mean Acute Value replaces the calculated Final Acute Value in order to provide protection for that important species.*"

For cyanide, the FAV was lowered from 62.68 ug/L to 44.73 ug/L because the Species Mean Acute Value for rainbow trout (44.73 ug/L) was below the calculated FAV. The cyanide criterion (5.2 ug/L) was then derived by division of the FAV (44.73 ug/L) by the

Table 14. Chronic toxicity data used by EPA to derive the freshwater chronic criterion for cyanide. Effects levels were calculated using data from the original papers.

Species	Chronic Limits[1]				Chronic Value[2]		LC_{50}[3] (ug CN/L)	ACR[3]
	Lower		Upper					
	(ug CN/L)	Effect	(ug CN/L)	Effect	(ug CN/L)	Effect		
Fathead Minnow	13.3	47%	20.2	58%	16.39	52%	125.1	7.633
Brook Trout	5.6	18%	11.0	53%	7.849	32%	83.14	10.59
Bluegill	9.3	30%	19.8	88%	13.57	54%	99.28	7.3
Gammarus	16	0%	21	100%	18.33	47%	167	9.111
Geometric mean						45%		8.562

[1] Lower and upper chronic limits were taken from the cyanide criteria document. For fathead minnow and bluegill these values were determined statistically (i.e., NOEC and LOEC identified via hypothesis tests). Effect levels were take from Tables 5, 8 and 10 in the Effects section of the BO and from Oseid and Smith 1979.
[2] Chronic values were taken from the cyanide criteria document. Effect levels associated with the chronic values were estimated by linear interpolation between the effects at the lower and upper chronic limits.
[3] Acute-Chronic Ratios were taken from the cyanide criteria document.

Final ACR (8.562). Thus the chronic criterion, 5.2 ug CN/L, was based on the concentration intended to protect rainbow trout from unacceptable adverse effects. Based on our estimate of the magnitude of effect associated with the Final ACR, we estimate the magnitude of adverse effects occurring to rainbow trout at the chronic criterion to be approximately 45%. This value is higher than we would have expected considering it is intended to represent the threshold for unacceptable adverse effects. However, the magnitude is in line with effects we predicted for the listed fish evaluation species, most of which were estimated to be as (or more) sensitive to cyanide as rainbow trout.

The same conclusion reached above, that NOEC/LOEC-based estimates of "chronic values" can correspond to $\geq$ 40% adverse effect, has also been reached by others. Decades ago Suter et al. (1987) reported that MATC's for fish fecundity, on average, corresponded to a 42% level of adverse effect (MATC = Maximum Acceptable Toxicant Concentration; a term for the geometric mean of the NOEC and LOEC from a given toxicity test and often assigned by EPA as the estimated "chronic value" from a test). Other response endpoints were found to correspond to average adverse effect levels of 12-35%. More recently, SETAC (Society for Environmental Toxicology and Chemistry) convened a panel of experts (Reiley et al. 2003) who concluded that "*...[toxicity] tests with high variability may result in an(sic) NOEC corresponding to a response greater than 40% different from the control.*" Moore and Caux (1997) statistically examined nearly 200 toxicity data sets and found that most NOEC's (76.9%) exceeded a 10% adverse effect level and most LOEC's (62.4%) exceeded a 30% effect level. Various other researchers have noted a variety of adverse effect levels for NOEC's, such that Crane and Newman (2000) were led in summary to conclude that "*…[NOEC] effect levels from individual tests ranged from nearly 0% to nearly 100%.*" For seven cyanide toxicity tests with sufficient data for comparison, Gensemer et al. (2007: Figure 3-7) found in all cases that the geometric mean of the NOEC and LOEC corresponded to an adverse effect level of $\geq$ 20% (how much greater was not reported).

Because of the highly variable and often substantive levels of effect associated with NOEC's, LOEC's, MATC's, and with the "chronic values" based on them, and for numerous other reasons, a strong professional consensus recommendation to avoid using NOEC/LOEC-based estimates for regulatory thresholds (when possible) has been expressed repeatedly. For example, there was an ISO (International Organization for Standardization) resolution (ISO TC147/SC5/WG10 Antalya 3) as well as an OECD (Organisation for Economic Co-operation and Development) workshop recommendation (OECD 1998) that the NOEC should be phased out from international standards (OECD 2006:14). Environment Canada (2005) notes, that there is a growing literature which points out many deficiencies of the NOEC approach (Suter et al. 1987, Miller et al. 1993, Pack 1993, Noppert et al. 1994, Chapman 1996, Chapman et al. 1996, Pack 1998, Suter 1996, Moore and Caux 1997, Bailer and Oris 1999, Andersen et al. 2000, Crane and Newman 2000, Crane and Godolphin 2000). Moving away from the NOEC/LOEC approach was also among the recommendations of the SETAC panel for improving the scientific basis of water-quality criteria (Reiley et al. 2003).

Accordingly, EPA has begun employing a regression approach for estimating "chronic values" whenever sufficient data are available to do so. For example, in the 1999 update for ammonia water quality criteria EPA used regression analyses to estimate 20% effect concentrations (EC_{20}'s) from individual toxicity tests and used those EC_{20}'s as estimates of "chronic values" (EPA 1999). Likewise, estimated EC_{20}'s have been the basis for estimating "chronic values" in recently proposed updates for copper and selenium water quality criteria (EPA 2003a, 2004). EPA's choice of the EC_{20} as a basis for estimating "chronic values" was justified from statistical considerations rather than from biological or demographic considerations:

"To make [chronic values] reflect a uniform level of effect, regression analysis was used here both to demonstrate that a significant concentration-effect relationship was present and to estimate [chronic values] with a consistent level of effect. Use of regression

analysis is provided for on page 39 of the 1985 Guidelines (U.S. EPA 1985b). The most precise estimates of effect concentrations can generally be made for 50 percent reduction (EC50); however, such a major reduction is not necessarily consistent with criteria providing adequate protection. In contrast, a concentration that caused a low level of reduction, such as an EC5 or EC10, is rarely statistically significantly different from the control treatment. As a compromise, the EC20 is used here as representing a low level of effect that is generally significantly different from the control treatment across the useful chronic datasets that are available for ammonia."

Pack (1993) asserted that most ecotoxicologists consider effects in the range of 5-20% to be biologically acceptable depending on the species involved and the type of effect. However, EPA appears to have chosen the top end of that range based more on the expected statistical power of toxicity tests than on a serious examination of the typical demographic sensitivity of biotic populations to a 20% adverse effect on survival, growth, or reproduction. Furthermore, 95% statistical confidence limits for most EC_{20} estimates are likely to extend well into adverse effect levels that would be of unquestionably serious demographic concern for most organisms. As evident from the above discussion, most chronic criteria derived by EPA, including for cyanide, are highly likely to be associated with $\geq$ 20% adverse effect level for species at the vulnerable end of species sensitivity distributions (such as the subset of ESA-listed species we are evaluating). Therefore, it should be no surprise that our estimated effect levels for such species at the current cyanide CCC of 5.2 ug/L are almost always higher than 20% and in some cases substantively higher.

Population Responses to Reductions in Fecundity and Juvenile Survival

Laboratory experiments have demonstrated that even closely related fish species can demonstrate great differences in sensitivity when exposed to the same chemical, as measured by differences in acute or chronic toxicity values. This variability in sensitivity has been related to differences in species' physiology and life history strategies. Similarly, population modeling and experimental studies have shown that variation in population-level responses to environmental toxicity can also be expected among species as a consequence of factors such as life history strategies, life stage affected, and density dependence. Studies have also demonstrated that chronic toxicity can lead to population decline and extirpation.

Under the ESA, in determining whether a proposed Federal action is likely to jeopardize the continued existence of a listed species under the ESA, we assess whether the proposed activity reasonably would be expected to appreciably reduce the likelihood of survival and recovery of a listed species by reducing its reproduction, numbers, or distribution. Two common metrics used in population modeling to assess effects of perturbations on populations are population growth rate and time to or probability of extinction.

Population growth rate is the change in a population size over a unit time period. Long-term reductions in population growth rate as low as 5% has been shown to significantly increase a population's likelihood of extinction (Snell and Serra 2000). Population growth rate can be positive when the population is increasing, negative when decreasing, or zero when the net difference between births, deaths, and migration is zero and the population is stable. For listed species, populations may exist in any of these states depending on its recovery status. Our analysis determines the relative predicted effects of the action to the population growth rate, regardless of its starting value.

Using known parameters of a species' life history, sensitivity analyses can be conducted to determine which parameters, when modified, will have the greatest impact on the species' population growth rate. Elasticity analysis is one type of sensitivity analysis that is commonly used in conservation biology to demonstrate the relative contributions to population growth rate made by life cycle transitions, based on vital rate statistics for survival, growth and fertility. While these types of analyses cannot predict absolute effects to population size, because they quantify the relative importance of an element to changes in population growth rate, they can help focus management decisions on those demographic parameters that exhibit the largest elasticity, and thus, the largest impact on population growth (de Kroon et al. 2000). However, elasticity analysis requires the development of a population model, for which adequate data are often scarce. Because this type of demographic data is often lacking for threatened and endangered species in particular, the need to develop generalized approaches for classifying population responses to perturbation for rare species has been recognized (Heppell et al. 2000, Dennis et al. 1991).

Several authors have examined the effect of life history strategies on the elasticities of various demographic measures. In evaluating demographic parameters of 50 mammal populations with different life history strategies, Heppell et al. (2000) found that phylogeny alone is often not a reliable indicator of which vital rates (survival, growth and fertility) will have the greatest impact on elasticity. Instead, the authors found that species that mature early and have high reproductive output had high fertility elasticities and low adult survival elasticities. Conversely, for those which mature late and have long lifespans, fecundity and early offspring survival are less important than survival of juveniles to maturity to changes in population growth rate. Calow et al. (1997) also found that the relative importance of juvenile fish survival can vary according to reproductive strategy. These authors concluded that reductions in juvenile survival would have the greatest impact on semelparous fish species, in which adults die after reproduction, a lesser impact on a moderately iteroparous population, in which adult postreproductive survival is intermediate, and the least impact on strongly iteroparous species, in which adult survival after reproduction is high. These assumptions held true for elasticity analysis of the green sturgeon, a fish species with life history patterns such as late-maturity and long-life that are common to other sturgeon (Heppell 2007). Juvenile survival had relatively lower elasticity values than adult and subadult survival, with compensation for the loss of adults requiring much larger increases in young-of-the-year survival than would be commensurate with the loss. However, other authors have found increased importance of juvenile survival for sturgeon, despite their lifespan (Gross et al. 2002, Paragamian and Hansen 2008). Gross et al. (2002) hypothesized that this difference was due to the vastly larger fecundity of sturgeon as compared to other long-lived species.

Vélez-Espino et al. (2006) argue the need for a broadscale summary of species' population dynamics to help guide the conservation biology of freshwater fishes, for which information on life history is often limited. Using information, on adult survival, juvenile survival, and fecundity, the authors performed elasticity analyses on 88 species of freshwater fish and found that they could be classified into 4 functional groups with regard to the sensitivity of their population growth rates:

1. species most sensitive to perturbations in adult survival
2. species most sensitive to perturbations to adult and juvenile survival
3. species most sensitive to perturbations to juvenile survival
4. species most sensitive to perturbations to juvenile survival and fecundity

These groups are characterized by decreased age at maturity, longevity, and reproductive lifespan as one moves from group 1 to group 4. Age at maturity, reproductive lifespan, fecundity, juvenile survivorship, and longevity were all correlated with adult survival and fecundity. However, the best predictors of elasticity patterns were longevity, which explained 93% of the variability in the elasticity of adult survival, and age at maturity, which explained 92% of the variability in the elasticity of fecundity. The authors also found that elasticities are highly conserved among genera within the same taxonomic family

Spromberg and Birge (2005) also found that life history strategies influence effects to populations. The five life history strategies they modeled encompassed differences in stage-specific survival, fecundity and hatch success, number of spawning events, and life-span. The authors found that regardless of strategy, changes in the number of young-of-the-year stage individuals had the greatest impact on population growth rate. However, the relative contribution of this parameter was greatest for life history strategies with multiple spawnings, high fecundity, and short lifespans as opposed to those with longer lifespan, which had increased elasticity of adult survival.

Spromberg and Meador (2005) linked toxicant effects on immune suppression, reproductive development, and growth reduction to demographic traits in Chinook salmon (*Oncorhynchus tshawytscha*) and modeled their influence on population growth rate. Overall, effects to first- and second-year survival had the greatest elasticities, with constant reductions to first year survival as low as 10% achieving population declines ranging from 35-78% compared to controls. Other studies have demonstrated the importance of first year survival in this species (Kareiva et al. 2000). Spromberg and Meador (2005) also found that models which incorporated effects to both survival and reproduction were additive, indicating the importance of evaluating the overall impact of all potential impacts to population growth.

Many listed species populations are limited by the amount of adequate habitat or resources and experience some degree of density dependence. Density-dependence at any life stage must be considered in elasticity analysis in order to yield appropriate results (Grant and Benton 2000, Hayashi et al. 2008). In a review of toxicant impacts on density-limited populations, Forbes et al. (2001) noted that the full range of interactions have been found between toxicant stress and density dependence, including less than additive, additive, and more than additive effects. Also, the type of effect may vary with increasing toxicant concentration from one that ameliorates density dependent effects at low toxicant concentrations to one that exacerbates density dependent effects at higher toxicant concentrations. Case studies which incorporate density-dependence into population modeling demonstrate this variability, with overall impacts to populations shown to be both lesser (Van Kirk and Hill 2007) and greater (Hayashi et al. 2008) than the level of effect that would be predicted from individual response depending on the situation. In time, density-dependant populations may rebound, stabilize at a lower absolute population number, or continue to decline until the population is extirpated (Forbes et al. 2001). Modeling exercises have demonstrated cases in which populations stabilize at new, lower equilibrium abundances in response to a constant impact (van Kirk and Hill 2007, Spromberg and Meador 2005).

A species' likelihood of persistence can also be estimated by modeling the species' time to extinction or probability of extinction. Population viability analysis (PVA) uses simulation modeling to identify threats to species and to assess the vulnerability of populations to these extinction risks. These models incorporate demographic parameters such as fecundity, survivorship, age structure, and population size, but can also incorporate effects to the environment such as habitat degradation and catastrophic events. As for the evaluation of population growth rate, sensitivity analysis is used to determine which factors have the greatest impact on population persistence, and many experts feel that parsing out these influential factors for management purposes is the best utilization of these models, as opposed to absolute predictions of population decline. Though PVA also requires a depth of demographic data that is often lacking for listed species, even PVAs with little data incorporated can be useful in comparative analyses of management considerations (Akçakaya and Sjögren-Gulve 2000).

Only a limited number of PVAs have been performed for listed aquatic species or other closely related species. A PVA for two darter species, the slackwater darter (*Etheostoma boschungi)* and the holiday darter (*E. brevirostrum)*, found that fertility made the largest relative contribution to population growth, with juvenile survivability a more influential contributor to fertility than egg production (Hartup 2005). This conclusion held true regardless if the species was a single or multiple-batch spawner. In modeling the contributions of population size, age structure, and migration rate on the leopard darter (*Percina pantherina*), migration had the greatest influence on persistence (Williams et al. 1999). Catastrophe also played a significant role in persistence of the species, especially considering that the species is short-lived, has only one reproductive opportunity, and is restricted to few isolated populations. A similar analysis of the freshwater rotifer *Brachionus calyciflorus* also found that reductions in growth rate resulted in substantially increased probability of extinction when coupled with catastrophic population reductions (Snell and Serra 2000). A PVA analysis for the gila trout (*Oncorynchus gilae*) revealed that the number of populations was the most influential of several life history parameters, and that the model was relatively insensitive to changes in population size and proportional abundance of age classes (Brown et al. 2001). The model was also sensitive to large change in fecundity, producing significant changes in the probability of extinction when halved.

There are few field studies of pollutant effects on populations. Kidd et al. (2007) studied the effects of 17α-ethynlestradiol (EE2) on fathead minnows in Canadian experimental lakes over a 7-year period. EE2, a synthetic estrogen found in birth control pills, was introduced at concentrations found in the receiving waters downstream from municipal wastewater dischargers. The fathead minnow, a short-lived species, was the first to show population collapse, but recovered once the pollutant stress was removed.

Summary of Population Responses to Reductions in Fecundity and Juvenile Survival

Modeling and experimental studies have shown that chronic toxicity to pollutants can lead to population decline and extirpation. Variation in population-level responses to environmental toxicity can be expected among species as a consequence of factors like species life history strategies, life stage affected, density dependence, and magnitude of toxicant stress. Although the degree varied among different life history strategies, fecundity and juvenile survival remained a highly influential demographic parameter throughout modeled scenarios, with adult survival taking on greater importance in long-lived species. These results must be coupled with other influences on the population status, such as the degree of density dependence and additional environmental perturbations such as catastrophes. Although population modeling often requires more demographic information than is available for threatened and endangered species, careful selection of surrogates and use of their data may allow for extrapolation from models for species with similar life histories.

Individual Species and Critical Habitat Accounts

Acipenseridae

GULF STURGEON
Acipenser oxyrinchus desotoi

Gulf sturgeon exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Because no data for cyanide toxicity to sturgeon exist, LC50 values for sturgeon were derived from the 5% SSD concentration for the class Actinoptergyii, which encompasses all known cyanide toxicity data for fish. From this data, we developed quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13).

Compared to control populations, we estimate Gulf sturgeon exposed to cyanide at the CCC could experience an approximate 48% reduction in the number of hatched eggs. We estimate that Gulf sturgeon exposed to cyanide at the CCC could experience an approximate 56% reduction in survival of young fish through the first year. Though no cyanide-specific data exist for this species, there are data from other chemicals that support the relative sensitivity of sturgeon to contaminants. Dwyer et al. (2005) tested the relative sensitivity of 18 fish and 1 amphibian species to five chemicals. Of these, the two sturgeon tested, the Atlantic sturgeon (*A. oxyrhynchus*) and the shortnose sturgeon (*A. brevirostrum*) ranked first and second, respectively, in overall sensitivity. For all five chemicals, sturgeon were as or more sensitive than rainbow trout, for which reductions in viable eggs spawned and juvenile survival were estimated at 52% and 61%, respectively (Table 12).

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

Sturgeon have naturally high adult survival. Several authors have suggested that the rate of survival may be so high that management at the levels of these age classes is unlikely to improvement their survival or increase population growth rate (Heppell 2007, Gross et al 2002) As such, recovery efforts are often based upon increasing survival in juvenile age classes. A population viability analysis of the Suwannee River population of the gulf sturgeon found that slight changes in egg-to-age-1 mortality would strongly influence the recruitment and subsequent population (Pine et al. 2001). Decreasing the estimated 99.96% mortality for this life stage just 0.05 percentage points resulted in a 10-fold increase in population size, with a 5-fold increase in the number of recruits.

Gross et al (2002) modeled population growth rates for three species of sturgeon that varied in life history traits such as size, lifespan, age to maturity, and migration. All three sturgeons showed similar elasticity profiles, and thus the authors concluded that general interpretation could be applied to sturgeon across species. In contrast to other elasticity profiles for long-lived species, elasticity in sturgeon was highest in individual young-of-the-year and juvenile age classes, dropped at the onset of maturity, and continued to decline for each successive adult age class. Fecundity had relatively low elasticity, as the effects of changes in fecundity are shared among all adult age classes of these long-lived species, and the value of changes to egg numbers is lessened by the high mortality of the young-of-the-year age class. The authors concluded that population growth rate will show little response to improvements in fecundity, but greater responses in survival at either the young-of-the-year or juvenile age classes. However, since survival of the juvenile and adult age classes is naturally high, improvements at these stages will have smaller effects to improving population growth rate than increases to survival of young-of-the-year, when natural mortality is greater. The authors note that among biologists and managers involved in sturgeon conservation, habitat improvement was regarded as the most important conservation undertaking for sturgeon. Results from this study indicate that restoration efforts should target the survival of age classes with high elasticity, specifically young-of-year and juvenile.

Paragamian and Hansen (2008) drew similar conclusions in modeling effects on population growth of the Kootenai River white sturgeon. The authors found that subadult and adult survival (>90%) was much higher than that of juveniles (40% in the first year), and recovery was most dependant on increasing first-year survival. The authors suggested that to have the largest effect on recovery, the managers should increase the current targeted recruitment rate.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Gulf sturgeon reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survival of young fish through the first year. Gulf sturgeon may also experience effects on growth, swimming performance, condition, and development. While sturgeon have developed a life history that allows them to cope with low survivorship to maturity and occasional hits to recruitment, these adaptation are unlikely to compensate for a constant reduction in both fecundity and early life stage survival. The reductions we estimate in survival of young fish through the first year in particular would substantially decrease recovery of this species. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed.
We would anticipate a consequent reduction in numbers of Gulf sturgeon. An effected Gulf sturgeon population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Gulf sturgeon are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the gulf sturgeon.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the gulf sturgeon include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of gulf sturgeon, and cause sturgeon to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of gulf sturgeon. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the gulf sturgeon.

KOOTENAI RIVER WHITE STURGEON
Acipenser transmontanus

Kootenai sturgeon exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience adverse effects on growth, swimming performance, condition, and development, as described above in the *Overview* section. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies are available for estimating the magnitude of adverse effects that could occur following Kootenai River white sturgeon exposure to cyanide at criterion concentrations. Because no data for cyanide toxicity to sturgeon exist, LC_{50} values for sturgeon were derived from the 5% SSD concentration for the class Actinoptergyii, which encompasses all known cyanide toxicity data for fish. From these data, we developed quantitative estimates of the effects on sturgeon fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate that Kootenai River white sturgeon that are exposed to cyanide at the CCC are likely to be subject to an approximately 48% reduction in the number of hatched eggs. We estimate that Kootenai River white sturgeon exposed to cyanide at the CCC are likely to experience an approximately 56% reduction in the survival of young fish through the first year.

Although no cyanide-specific data exist for this species, there are data from other chemicals that support the relative sensitivity of sturgeon to contaminants. Dwyer et al. (2005) tested the relative sensitivity of 18 fish and 1 amphibian species to five chemicals. Of these, the two sturgeon tested, the Atlantic sturgeon (*A. oxyrhynchus*) and the shortnose sturgeon (*A. brevirostrum*) ranked first and second, respectively, in overall sensitivity. For all five chemicals, sturgeon were as or more sensitive than rainbow trout, for which reductions in viable eggs spawned and juvenile survival were estimated at 52% and 61%, respectively (Table 12).

As noted previously, the young first-year fish that do survive are likely to experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

Sturgeon have naturally high adult survival. Several authors have suggested that the rate of survival may be so high that management at the levels of these age classes is unlikely to improve their survival or increase the population growth rate (Heppell 2007, Gross et al. 2002). For that reason, recovery efforts are often based upon increasing survival in juvenile age classes. *The Recovery Plan for the Kootenai River White Sturgeon* lists as a Priority 1 task (tasks that must be taken to prevent extinction or to prevent the species from declining irreversibly in the foreseeable future) restoration of ecosystem functions "to ensure habitat conditions necessary for successful white sturgeon reproduction and recruitment, i.e. survival of juveniles during their first year of life and beyond" (Service 1999). The reestablishment of natural recruitment to the Kootenai River population of the white sturgeon is listed in the recovery plan as a recovery objective.

Gross et al. (2002) modeled population growth rates for three species of sturgeon that varied in life history traits such as size, lifespan, age to maturity, and migration. All three sturgeon species showed similar elasticity profiles, and thus the authors concluded that general interpretation could be applied to sturgeon across species. In contrast to other elasticity profiles for long-lived species, elasticity in sturgeon was highest in individual young-of-the-year and juvenile age classes, dropped at the onset of maturity, and continued to decline for each successive adult age class. Fecundity had relatively low elasticity, as the effects of changes in fecundity are shared among all adult age classes of these long-lived species, and the value of changes to egg numbers is lessened by the high mortality of the young-of-the-year age class. The authors concluded that population growth rate will show little response to improvements in fecundity, but greater responses in survival at either the young-of-the-year or juvenile age classes. However, since survival of the juvenile and adult age classes is naturally high, improvements at these stages will have smaller effects to improving population growth rate than increases to survival of young-of-the-year, when natural mortality is greater. The authors note that among biologists and managers involved in sturgeon conservation, habitat improvement was regarded as the most important conservation undertaking for sturgeon. Results from this study indicate that restoration efforts should target the survival of age classes with high elasticity: the young-of-the-year and juveniles.

Paragamian and Hansen (2008) drew similar conclusions in modeling effects on population growth of the Kootenai River white sturgeon. They reported that subadult and adult survival (>90%) was much higher than that of juveniles (40% in the first year), and recovery was most dependant on increasing first-year survival. Paragamian and Hansen (2008) suggested that to have the largest effect on recovery, the recruitment rate should increase.

In summary, exposure to cyanide concentrations at the chronic criterion are likely to substantially reduce Kootenai River white sturgeon reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survival of young fish through the first year. Such exposure may also subject Kootenai

River white sturgeon to adverse effects on growth, swimming performance, condition, and development. Although sturgeon have developed a life history that allows them to cope with low survivorship to maturity and occasional hits to recruitment, these adaptations are not sufficient to cope with a constant reduction in both fecundity and early life stage survival likely to be caused by exposure to cyanide concentrations at the chronic criterion. The estimated reductions in the survival of young fish through the first year in particular would significantly decrease recovery of this species in the wild. Because of the high magnitude of adverse effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed by sturgeon exposure to cyanide concentrations at the chronic criterion, which is likely to cause a consequent reduction in the numbers of Kootenai River white sturgeon. Based upon the magnitude of adverse effects that are likely to occur and given the extremely endangered status of this species, we conclude that ultimately Kootenai River white sturgeon are likely to be extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion throughout the range of this species is likely to reduce the reproduction, numbers, and distribution of the Kootenai River white sturgeon at the rangewide scale.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Kootenai River white sturgeon include water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval and cyanide in waters to these levels is likely to adversely affect the quality of water in sturgeon critical habitat to the degree that it would impair individual reproduction and survival of Kootenai River white sturgeon, and cause sturgeon to experience adverse effects to growth, swimming performance, condition, and development. Cyanide concentrations at the chronic criterion within critical habitat are likely to create habitat conditions that reduce the number of hatched sturgeon eggs by as much as 48% and reduce the survival of young sturgeon through the first year by as much as 56%. Approval of the CCC and cyanide in waters to these levels is likely to adversely affect the quality of water in sturgeon critical habitat to the degree that it is likely to preclude the intended conservation function of that habitat.

PALLID STURGEON
Scaphirhynchus albus

Pallid sturgeon exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Because no data for cyanide toxicity to sturgeon exist, LC50 values for sturgeon were derived from the 5% SSD concentration for the class Actinoptergyii, which encompasses all known cyanide toxicity data for fish. From this data, we developed quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate pallid sturgeon exposed to cyanide at the CCC could experience an approximate 48% reduction in the number of hatched eggs. We estimate that pallid sturgeon exposed to cyanide at the CCC could experience an approximate 56% reduction in survival of young fish through the first year. Though no cyanide-specific data exist for this species, there are data from other chemicals that support the relative sensitivity of sturgeon to contaminants. Dwyer et al. (2005) tested the relative sensitivity of 18 fish and 1 amphibian species to five chemicals. Of these, the two sturgeon tested, the Atlantic sturgeon (*A. oxyrhynchus*) and the shortnose sturgeon (*A. brevirostrum*) ranked first and second, respectively, in overall sensitivity. For all five chemicals, sturgeon were as or more sensitive than rainbow trout, for which reductions in viable eggs spawned and juvenile survival were estimated at 52% and 61%, respectively (Table 12).

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

Sturgeon have naturally high adult survival. Several authors have suggested that the rate of survival may be so high that management at the levels of these age classes is unlikely to improve their survival or increase population growth rate (Heppell 2007, Gross et al 2002) As such, recovery efforts are often based upon increasing survival in juvenile age classes. The 2007 Five-Year Review for the pallid sturgeon found that natural recruitment is limited throughout the species' range (USFWS 2007). Wild populations in two of six Recovery Priority Management Areas (RPMAs) are comprised of old aged individuals, and three of six RPMAs are dependent on hatchery augmentation programs for recruitment. Addressing recruitment bottlenecks in the three upper Missouri River RPMAs was deemed critically important for the species to become self sustaining and be recovered in those reaches.

Gross et al (2002) modeled population growth rates for three species of sturgeon that varied in life history traits such as size, lifespan, age to maturity, and migration. All three sturgeons showed similar elasticity profiles, and thus the authors concluded that general interpretation could be applied to sturgeon across species. In contrast to other elasticity profiles for long-lived species, elasticity in sturgeon was highest in individual young-of-the-year and juvenile age classes, dropped at the onset of maturity, and continued to decline for each successive adult age class. Fecundity had relatively low elasticity, as the effects of changes in fecundity are shared among all adult age classes of these long-lived species, and the value of changes to egg numbers is lessened by the high mortality of the young-of-the-year age class. The authors concluded that population growth rate will show little response to improvements in fecundity, but greater responses in survival at either the young-of-the-year or juvenile age classes. However, since survival of the juvenile and adult age classes is naturally high, improvements at these stages will have smaller effects to improving population growth rate than increases to survival of young-of-the-year, when natural mortality is greater. The authors note that among biologists and managers involved in sturgeon conservation, habitat improvement was regarded as the most important conservation undertaking for sturgeon. Results from this study indicate that restoration efforts should target the survival of age classes with high elasticity, young-of-year and juvenile.

Paragamian and Hansen (2008) drew similar conclusions in modeling effects on population growth of the Kootenai River white sturgeon. The authors found that subadult and adult survival (>90%) was much higher than that of juveniles (40% in the first year), and recovery was most dependant on increasing first-year survival. The authors suggested that to have the largest effect on recovery, the managers should increase the current targeted recruitment rate.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the pallid sturgeon reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survival of young fish through the first year. Pallid sturgeon may also experience effects on growth, swimming performance, condition, and development. While sturgeon have developed a life history that allows them to cope with low survivorship to maturity and occasional hits to recruitment, these adaptation are not designed to withstand a constant reduction in both fecundity and early life stage survival. The reductions we estimate in survival of young fish through the first year in particular would significantly decrease recovery of this species. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed.
We would anticipate a consequent reduction in numbers of pallid sturgeon. An effected Pallid sturgeon population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Pallid sturgeon are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the pallid sturgeon.

ALABAMA STURGEON

Scaphirhynchus suttkusi

Alabama sturgeon exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects

Formal Draft Biological Opinion.

Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Because no data for cyanide toxicity to sturgeon exist, LC50 values for sturgeon were derived from the 5% SSD concentration for the class Actinoptergyii, which encompasses all known cyanide toxicity data for fish. From this data, we developed quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Alabama sturgeon exposed to cyanide at the CCC could experience an approximate 48% reduction in the number of hatched eggs. We estimate that Alabama sturgeon exposed to cyanide at the CCC could experience an approximate 56% reduction in survival of young fish through the first year. Though no cyanide-specific data exist for this species, there are data from other chemicals that support the relative sensitivity of sturgeon to contaminants. Dwyer et al. (2005) tested the relative sensitivity of 18 fish and 1 amphibian species to five chemicals. Of these, the two sturgeon tested, the Atlantic sturgeon (*A. oxyrhynchus*) and the shortnose sturgeon (*A. brevirostrum*) ranked first and second, respectively, in overall sensitivity. For all five chemicals, sturgeon were as or more sensitive than rainbow trout, for which reductions in viable eggs spawned and juvenile survival were estimated at 52% and 61%, respectively (Table 12).

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

Sturgeon have naturally high adult survival. Several authors have suggested that the rate of survival may be so high that management at the levels of these age classes is unlikely to improvement their survival or increase population growth rate (Heppell 2007, Gross et al 2002) As such, recovery efforts are often based upon increasing survival in juvenile age classes. The May 5, 2000 listing rule for the Alabama sturgeon (65 FR 26438) designated the primary threat to the immediate survival of the species to be its small population size and apparent inability to offset mortality rates with current reproduction and/or recruitment rates. Its small population also makes it vulnerable to natural or human-induced events (*e.g.,* droughts, floods, competition, variations in prey abundance, toxic spills), which may further depress recruitment.

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Gross et al (2002) modeled population growth rates for three species of sturgeon that varied in life history traits such as size, lifespan, age to maturity, and migration. All three sturgeons showed similar elasticity profiles, and thus the authors concluded that general interpretation could be applied to sturgeon across species. In contrast to other elasticity profiles for long-lived species, elasticity in sturgeon was highest in individual young-of-the-year and juvenile age classes, dropped at the onset of maturity, and continued to decline for each successive adult age class. Fecundity had relatively low elasticity, as the effects of changes in fecundity are shared among all adult age classes of these long-lived species, and the value of changes to egg numbers is lessened by the high mortality of the young-of-the-year age class. The authors concluded that population growth rate will show little response to improvements in fecundity, but greater responses in survival at either the young-of-the-year or juvenile age classes. However, since survival of the juvenile and adult age classes is naturally high, improvements at these stages will have smaller effects to improving population growth rate than increases to survival of young-of-the-year, when natural mortality is greater. The authors note that among biologists and managers involved in sturgeon conservation, habitat improvement was regarded as the most important conservation undertaking for sturgeon. Results from this study indicate that restoration efforts should target the survival of age classes with high elasticity, young-of-year and juvenile.

Paragamian and Hansen (2008) drew similar conclusions in modeling effects on population growth of the Kootenai River white sturgeon. The authors found that subadult and adult survival (>90%) was much higher than that of juveniles (40% in the first year), and recovery was most dependant on increasing first-year survival. The authors suggested that to have the largest effect on recovery, the managers should increase the current targeted recruitment rate.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Alabama sturgeon reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survival of young fish through the first year. Alabama sturgeon may also experience effects on growth, swimming performance, condition, and development. While sturgeon have developed a life history that allows them to cope with low survivorship to maturity and occasional hits to recruitment, these adaptation are not designed to withstand a constant reduction in both fecundity and early life stage survival. The reductions we estimate in survival of young fish through the first year in particular would significantly decrease recovery of this species. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. We would anticipate a consequent reduction in numbers of Alabama sturgeon. An effected Alabama sturgeon population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Alabama sturgeon are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the Alabama sturgeon.

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Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Alabama sturgeon include a flow regime and river system which allows for all life stages and processes of the species, a river channel with a stable sand and gravel bottom, as well as a rock wall and associated mussel beds, limestone outcrops and cut limestone banks, riverline spawning sites with substance suitable for embryo deposition and development, large sections of free flowing water for spawning migrations and the development of young, water of 32 degrees Celsius (90 degrees Fahrenheit) or less, dissolved oxygen at 5 milligrams per liter or more, and pH from 6.0 to 8.5.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Alabama sturgeon critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Alabama sturgeon, and cause Alabama sturgeon to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of Alabama sturgeon. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Alabama sturgeon's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Alabama sturgeon.

Amblyopsidae

OZARK CAVEFISH
Amblyopsis rosae

Ozark cavefish exposed to cyanide at the criterion continuous concentration (CCC) are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Ozark cavefish exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Ozark cavefish exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the first-year fish that do survive could experience reduced growth rates that would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, such as Ozark cavefish, than on species with greater adult survival. Ozark cavefish can live up to 7-10 years, but do not reach adulthood until 4 or more years of age. Furthermore, only about 20% of the female population breeds each year, producing 20-25 eggs. We anticipate the effects of cyanide on fecundity and juvenile survival could have a substantial population-level effect on Ozark cavefish. Individual Ozark cavefish females reproduce infrequently and produce few offspring. A reduction in potential fecundity and juvenile survival in the wild at the magnitude we estimate will add to the challenges to recovering the Ozark cavefish.

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Ozark cavefish 's potential recruitment substantially. The Ozark cavefish 's potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction and survival could be exacerbated further if reduced growth rates diminish survival through to adulthood.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Ozark cavefish's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Ozark cavefish may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. These potential effect could add substantially to the difficulties in reintroducing reproductively successful populations and attaining recovery. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Ozark cavefish are not likely to overcome the effects to their recruitment in waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could limit the reproduction, numbers, and distribution of the Ozark cavefish .

ALABAMA CAVEFISH

Formal Draft Biological Opinion.

Speoplatyrhinus poulsoni

Alabama cavefish exposed to cyanide at the criterion continuous concentration (CCC) are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Alabama cavefish exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Alabama cavefish exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the first-year fish that do survive could experience reduced growth rates that would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Alabama cavefish may live 5-10 years. Cavefish do not reproduce every year, numbers of reproductive females are few, and those that do spawn lay very few eggs. We anticipate the potential effects of cyanide on fecundity and juvenile survival would have a substantial population-level effect on Alabama cavefish. A reduction in potential fecundity and juvenile survival in the wild at the magnitude we estimate will add to the significant challenges to recovering the Alabama cavefish.

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Alabama cavefish's potential recruitment substantially. The Alabama cavefish 's potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival beyond the early life stages analyzed.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Alabama cavefish's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Alabama cavefish may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be inadequate to avoid population decline. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Alabama cavefish are not likely to overcome the effects to their recruitment where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could limit the reproduction, numbers, and distribution of the Alabama cavefish.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Alabama cavefish include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of Alabama cavefish, and cause fish to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 48% and the reduction in the survival of young fish as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of Alabama cavefish. Approval of the CCC would adversely affect the quality of water to the degree that normal individual and population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Alabama cavefish.

Atherinidae

WACCAMAW SILVERSIDE
Menidia extensa

Waccamaw silverside exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were

available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Waccamaw silversides exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Waccamaw silversides exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than species with greater adult survival. The Waccamaw silverside has a 1-year life cycle and depends upon successful reproduction each year for its survival. Almost all adults die soon after spawning. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on freshwater fish with similar longevity and reproductive lifespans to the Waccamaw silverside found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the Waccamaw silverside's reproductive performance would be reduced substantially. The Waccamaw silverside's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Waccamaw silverside's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Waccamaw silversides may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Waccamaw silverside population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Waccamaw silversides are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Waccamaw silverside.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Waccamaw silverside include high quality water which is clear, open, and has a neutral pH, and a clean sand substrate. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Waccamaw silverside critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Waccamaw silversides, and cause Waccamaw silversides to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of Waccamaw silverside. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Waccamaw silverside's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Waccamaw silverside.

Catostomidae

MODOC SUCKER
Catostomus microps

Modoc suckers exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Modoc suckers exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that Modoc suckers exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Modoc suckers typically lives up to 5 years, achieving sexual maturation in its third year, resulting in a reproductive life span of only one or two years. A comparison of 88 freshwater fish species found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on suckers with similar life-history traits to the Modoc sucker found population growth rate to be highly susceptible to perturbations in juvenile survival, accounting for about 60% of the total elasticity (Vélez-Espino et al., 2006). In combination, juvenile survival and fecundity in these species accounted for about 80% of the total elasticity of the population growth rate. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the Modoc sucker's reproductive performance would be reduced substantially. The reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Modoc sucker's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Modoc suckers may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Modoc sucker's population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Modoc suckers are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Modoc sucker.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Modoc sucker include intermittent and permanent-water creeks, and adjacent land areas that provide vegetation for cover and protection from soil erosion. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Modoc sucker critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Modoc suckers, and cause Modoc sucker to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 39% and the reduction in the survival of young fish through the first year as high as 344%. These effects are estimated to be of a magnitude great enough to reduce numbers of Modoc sucker. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Modoc sucker’s extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Modoc sucker.

SANTA ANA SUCKER

Catostomus santaanae

Santa Ana suckers exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Santa Ana suckers to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that Santa Ana suckers exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival, such as sturgeon. Santa Ana sucker females are short-lived (up to 3 years), mature early, and may have prolonged spawning periods. Fecundity is considered to be exceptionally high for a species of its small size. In a comparison of 88 freshwater fish species, longevity and age at maturity were found to be the best predictors of elasticity patterns Vélez-Espino et al., 2006). Elasticity analyses performed on freshwater fish with similar longevity, age at maturity, and reproductive lifespan as the Santa Ana sucker found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the Santa Ana sucker's reproductive performance would be reduced substantially. The Santa Ana sucker's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Santa Ana sucker's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Santa Ana suckers may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Santa Ana sucker population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Santa Ana suckers are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Santa Ana sucker.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Santa Ana sucker include the following: A functioning hydrological system that experiences peaks and ebbs in the water volume that reflects seasonal variation in precipitation throughout the year; A mosaic of loose sand, gravel, cobble, and boulder substrates in a series of riffles, runs, pools, and shallow sandy stream margins; Water depths greater than 3 cm (1.2 in) and bottom water velocities greater than 0.03 meter per second (0.01 feet per second); Non-turbid water or only seasonally turbid water; Water temperatures less than 30 [deg]C (86 [deg]F); and stream habitat that includes algae, aquatic emergent vegetation, macroinvertebrates, and riparian vegetation.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Santa Ana sucker critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Santa Ana suckers, and cause Santa Ana suckers to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 39% and the reduction in the survival of young fish through the first year as high as 44%. These effects are estimated to be of a magnitude great enough to reduce numbers of Santa Ana sucker. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Santa Ana sucker's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Santa Ana sucker.

WARNER SUCKER

Catostomus warnerensis

Warner suckers exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Warner suckers exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that Warner suckers exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through

the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). Warner suckers have been documented to live up to 17 years, though individuals residing in lakes are presumed to live longer than those found in rivers. Sexual maturity occurs at an age of 3 to 4 years. Spawning usually occurs in April and May in streams, in silt-free, gravel-bottomed, slow flowing sections of creeks. In years when access to stream spawning is limited by low flow or by physical in-stream blockages (such as beaver dams or diversion structures), suckers may attempt to spawn on gravel beds along the lake shorelines.

For species like the Warner sucker that exhibit intermediate longevity, moderately delayed reproduction, and multiple opportunities to spawn, population growth rate tends to be most sensitive to perturbation in juvenile survival (Vélez-Espino et al 2006). Elasticity analyses performed on suckers within the genus *Catostomus* that exhibit similar life-history traits to the Warner sucker found this pattern to hold true, with juvenile survival accounting for 50-60% of the total elasticity (Vélez-Espino et al., 2006). In combination, juvenile survival and fecundity accounted for 70 to 80% of the total elasticity of the population growth rate. A comparison of elasticities among closely related species found that elasticity values are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the Warner sucker's reproductive performance would be reduced substantially. The Warner sucker's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Warner sucker's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Warner suckers may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Warner sucker population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Warner suckers are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Warner sucker.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Warner sucker include the following: Streams should have clean, unpolluted flowing water and a stable riparian zone. The streams should support a variety of aquatic insects, crustaceans, and other small invertebrates for food.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Warner sucker critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Warner suckers, and cause Warner suckers to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 39% and the reduction in the survival of young fish through the first year as high as 44%. These effects are estimated to be of a magnitude great enough to reduce numbers of Warner sucker. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Warner sucker's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Warner sucker.

SHORTNOSE SUCKER

Chasmistes brevirostris

Shortnose sucker exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate shortnose suckers exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that shortnose suckers exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). The shortnose sucker can live more than 30 years, though a 2007 study from the Upper Klamath Lake indicated that the average life expectancy after entering the spawning population was only 3.6 years, suggesting that in some populations, adults may be dying before reproducing often enough for population replacement. Sexual maturity for shortnose suckers appears to occur between the ages of 4 and 6 years. Freshwater fish species with longevity and reproductive lifespan characteristics similar to those of the shortnose sucker were found to have population growth rates that were particularly susceptible to perturbations in juvenile survival, accounting for 40% to 50% of the total elasticity (Vélez-Espino et al., 2006). Recruitment for this species is historically low and continues to be below levels to sustain population growth. The 2007 5-Year Review for the shortnose sucker identified recruitment of young fish to the breeding population as a high priority goal for recovery.

We anticipate the shortnose sucker's reproductive performance and recruitment would be reduced substantially. The shortnose sucker's reproductive performance rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the shortnose sucker's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Shortnose suckers may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected shortnose sucker population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately shortnose suckers are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the shortnose sucker.

CUI-UI
Chasmistes cujus

Cui-ui exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate cui-uis exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that cui-uis exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). The cui-ui can live more than 40 years, reaching sexual maturity in 6 to 12 years. Females can produce from 25,000 to 186,000 eggs and larval survival is presumed to be extremely small. Freshwater fish species with longevity and reproductive lifespan characteristics similar to those of the Cui-ui were found to have population growth rates that were particularly susceptible to perturbations in juvenile survival, accounting for 40% to 50% of the total elasticity (Vélez-Espino et al., 2006). The Recovery Plan for the cui-ui stresses the need for increased survival, and consequent recruitment, of young.

We anticipate the cui-ui's reproductive performance and recruitment would be reduced substantially. The cui-ui's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the cui-ui's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Cui-uis may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected cui-ui population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately cui-uis are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the cui-ui.

JUNE SUCKER
Chasmistes lioris

June suckers exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate June suckers exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that June suckers exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

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The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). Freshwater fish species with longevity and reproductive lifespan characteristics similar to those of the June sucker were found to have population growth rates that were particularly susceptible to perturbations in juvenile survival, accounting for 40% to 50% of the total elasticity (Vélez-Espino et al., 2006). June suckers can live to be over 40 years old and reach sexual maturity at age 9. Survival rates for Red Butte refuge population were estimated as follows: age 1, 0.4225; age 2, 0.4625; age 3 0.8020, and adults, 0.9576 (Billman and Crowl, 2007). However, the wild population endemic to Utah Lake has continued to experience wide recruitment failure leaving it dominated by adult fish (Billman and Crowl, 2007). The June sucker Recovery Plan identifies the minimization of factors limiting recruitment as a priority in the recovery of the species.

We anticipate the June sucker's reproductive performance and subsequent recruitment would be further reduced. The June sucker's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the June sucker's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. June suckers may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected June sucker population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately June suckers are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the June sucker.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the June sucker include the following: One to three feet of high quality water constantly flowing over a clean, unsilted gravel substrate. Larval June suckers require shallow areas with low velocities connected to the main channel of the river.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect June sucker critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of June suckers, and cause June suckers to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 39% and the reduction in the survival of young fish through the first year as high as 44%. These effects are estimated to be of a magnitude great enough to reduce numbers of June sucker. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the June sucker's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the June sucker.

LOST RIVER SUCKER

Deltistes luxatus

Lost River sucker exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Lost River suckers exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that Lost River suckers exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). The Lost River sucker can live more than 40 years, though a 2007 study from the Upper Klamath Lake indicated that the average life expectancy after entering the spawning population was only 9 years, suggesting that in some populations, adults may be dying before reproducing often enough for population replacement. Sexual maturity for Lost River suckers occurs between the ages of 6 to 14 years, with most maturing at age 9. Freshwater fish species with longevity and reproductive lifespan characteristics similar to those of the Lost River sucker were found to have population growth rates that were particularly susceptible to perturbations in juvenile survival, accounting for 40% to 50% of the total elasticity (Vélez-Espino et al., 2006). A lack of recruitment has been reported for the river spawing population of the Lost River sucker, and the 2007 5-Year Review identified recruitment of young fish to the breeding population as a whole as a high priority goal for recovery due to large-scale die-offs of adults in the mid-1990's.

We anticipate the Lost River sucker's reproductive performance and recruitment would be reduced substantially. The Lost River sucker's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Lost River sucker's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Lost River suckers may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Lost River sucker population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Lost River suckers are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Lost River sucker.

RAZORBACK SUCKER

Xyrauchen texanus

Razorback suckers exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were

available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate razorback suckers exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 39%. We estimate that razorback suckers exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 44%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). The razorback sucker can live more than 40 years, and begin spawning at 3 to 4 years of age. Freshwater fish species with longevity and reproductive lifespan characteristics similar to those of the razorback sucker were found to have population growth rates that were particularly susceptible to perturbations in juvenile survival, accounting for 40% to 50% of the total elasticity (Vélez-Espino et al., 2006). Though the razorback sucker is a long-lived species, current populations have continued to experience persistent recruitment failure, resulting in the depletion and extirpation of numerous populations. Although razorback sucker in certain locations number in the thousands, low reproductive success, low survival of young, and little or no recruitment have contributed to high demographic uncertainty. Wild razorback sucker populations in many locations of the Colorado River Basin have become aged, senile, and perished from inadequate recruitment.

We anticipate the razorback sucker's reproductive performance and subsequent recruitment would be further reduced substantially. The razorback sucker's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the razorback sucker's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Razorback suckers may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected razorback sucker population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately razorback suckers are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the razorback sucker.

Cotidae

PYGMY SCULPIN
Cottus paulus

Pygmy sculpins exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate pygmy sculpins exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that pygmy sculpins exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Pygmy sculpin females are probably short-lived (1-3 years), mature early, and spawn year-round with a peaks in August and late winter. A comparison of 88 freshwater fish species found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the pygmy sculpin found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the pygmy sculpin's reproductive performance would be reduced substantially. The pygmy sculpin's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the pygmy sculpin's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Pygmy sculpins may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected pygmy sculpin population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately pygmy sculpins are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the pygmy sculpin.

Cyprinidae

BLUE SHINER
Cyprinella caerulea

Blue shiner exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were

available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table13). Compared to control populations, we estimate blue shiners exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that blue shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Blue shiner females are short-lived (about 3 years). Most spawning adults are aged 2 years and spawn from early May through late August.

We anticipate the blue shiner’s reproductive performance would be reduced substantially. The blue shiner’s reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the blue shiner’s reproductive performance by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Blue shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected blue shiner population’s decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately blue shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the blue shiner.

BEAUTIFUL SHINER
Cyprinella formosa

Beautiful shiner exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated beautiful shiner exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that beautiful shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like beautiful shiners, than on species with greater adult survival, such as sturgeon. Beautiful shiner females are short-lived (less than 3 years).

We anticipate the beautiful shiner's fertility would be reduced substantially. The beautiful shiner's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

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In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the beautiful shiner's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Beautiful shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected beautiful shiner population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately beautiful shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the beautiful shiner.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the beautiful shiner include permanently flowing small streams with riffles, or intermittent creeks with pools and riffles in the Rio Yaqui drainage. The waters must be clear and unpolluted, and free of exotic fishes. The beautiful shiner needs water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience adequate individual and population growth.

Approval of the CCC in State and Tribal water quality standards would authorized States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect beautiful shiner critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of delta smelt, and cause delta smelt to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of delta smelt. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the beautiful shiner's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the beautiful shiner.

DEVILS RIVER MINNOW
Dionda diaboli

Devils River minnow exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Devils River minnow exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Devils River minnows exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Devils River minnow females are short-lived (1-2 years), mature early, and probably spawn from January through August.

The Devils River minnow probably has a reproductive strategy similar to the smalleye shiner (*Notropis buccula*). Durham and Wilde (2009) studied the population dynamics of the smalleye shiner in the Brazos River, Texas. Smalleye shiner are members of a reproductive guild of cyprinids that broadcast spawn multiple batches of nonadhesive, semibuoyant ova throughout an extended reproductive season, and experience extremely high post-spawning mortality. Elasticity analysis and sensitivity simulations of the projection matrix indicated that age-0 survival and age-1 fecundity were the most influential parameters in the population dynamics of smalleye shiners. In combination, these two stages accounted for the majority (70%) of the total elasticity of the population growth rate.

We anticipate the Devils River minnow's reproductive performance would be reduced substantially. The Devils River minnow's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Devils River minnow's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Devils River minnows may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Devils River minnow population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Devils River minnows are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Devils River minnow.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Devils River minnow include the following: streams with slow to moderate water velocities (10 to 40 cm per second or 4 to 16 inches per second) and shallow to moderate water depths (10 cm to 1.5 m or 4 inches to 4.9 feet). These streams must be near vegetative structure, such as emergent or submerged vegetation or stream bank riparian vegetation that overhangs the water column. Gravel and cobble substrates with low or moderate amounts of fine sediment and low or moderate amounts of substrate embeddedness. Pool, riffle, run, and backwater components free of structures that would prevent fish movement up or downstream. High quality water from groundwater springs and seeps which is: between 17 and 29 degrees Celsius, has dissolved oxygen levels greater than 5 mg/l, a pH between 7 and 8.2, has less than 0.7 mS/cm conductivity and salinity of less than 1 part per thousand, has ammonia levels of less than 0.4 mg/l, and finally has no (or minimal amounts of) pollutants such as copper, arsenic, mercury, cadmium, human and animal waste, pesticides, fertilizers, suspended sediments, petroleum compounds, gasoline, and diesel fuel. Finally, the habitat must possess an adequate algae food base, and no nonnative aquatic species. By high quality water, we mean water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth sufficient for the critical habitat to serve its intended conservation function.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Devils River minnow critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Devils River minnows, and cause Devils River minnows to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Devils River minnow. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Devils River minnow's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Devils River minnow.

SPOTFIN CHUB
Cyprinella monarcha

Spotfin chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate spotfin chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 68%. We estimate that spotfin chub exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 76%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like spotfin chub, than on species with greater adult survival, such as sturgeon. Most spotfin chub start spawning after their second year and may not live beyond the age of three. Counts of mature ova numbered 157-791, but may greatly underestimate fecundity if this species spawns fractionally.

The spotfin chub shares a relatively similar life history with darters. Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to conduct population viability analyses for the two species.

Average slackwater darter fecundity was estimated as 92 and 197, respectively, for one-batch and two-batch fecundity. Based on estimates of adult survival, Hartup (2005) calculated the adult fertility rate would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the spotfin chub's potential recruitment substantially. The spotfin chub's potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the spotfin chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Spotfin chub may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected spotfin chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately spotfin chub are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the spotfin chub.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the spotfin chub include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of spotfin chubs, and cause chubs to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 68% and the reduction in the survival of young fish through the first year as high as 76%. These effects are estimated to be of a magnitude great enough to reduce numbers of spotfin chub. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the spotfin chub.

SLENDER CHUB
Erimystax cahni

Slender chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated slender chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that slender chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Slender chub females are short-lived (up to 3 years). A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the slender chub found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the slender chub's reproductive performance would be reduced substantially. The slender chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the slender chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Slender chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected slender chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately slender chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the slender chub.

Critical Habitat: Continued approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect slender chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of slender chub, and cause slender chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of slender chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the slender chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the slender chub.

MOJAVE TUI CHUB

Gila bicolor mohavensisi

Mojave tui chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Mojave tui chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Mojave tui chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Mojave tui chub females are short-lived (1-4 years) and highly fecund. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the slender chub found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the Mojave tui chub's reproductive performance would be reduced substantially. The Mojave tui chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Mojave tui chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Mojave tui chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Mojave tui chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Mojave tui chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Mojave tui chub.

OWENS TUI CHUB
Gila bicolor snyderi

Owens tui chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Owens tui chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Owens tui chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the Owens tui chub found that population growth rates for these species were most susceptible to perturbations in juvenile survival (Vélez-Espino et al., 2006). Juvenile survival accounted for about 58% of the total elasticity of the population growth rate in all species with these life-history traits. A comparison of elasticities among closely related species found that elasticity values are highly conserved among genera within the same taxonomic family. Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*).

We anticipate the Owens tui chub's reproductive performance would be reduced substantially. The Owens tui chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Owens tui chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Owens tui chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Owens tui chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Owens tui chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Owens tui chub.

Critical Habitat: Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Owens tui chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Owens tui chub, and cause Owens tui chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Owens tui chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Owens tui chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Owens tui chub.

BORAX LAKE CHUB
Gila boraxobius.

Borax Lake chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Borax Lake chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Borax Lake chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Borax Lake chub females are short-lived (mostly 1-2 years), mature early, and probably spawn twice per year. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the Moapa dace found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn. A comparison of elasticities among closely related species found that elasticity values are highly conserved among genera within the same taxonomic family. Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*).

We anticipate the Borax Lake chub's reproductive performance would be reduced substantially. The Borax Lake chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Borax Lake chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Borax Lake chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Borax Lake chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Borax Lake chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Borax Lake chub.

Critical Habitat: Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Borax Lake chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Borax Lake chub, and cause Borax Lake chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Borax Lake chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Borax Lake chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Borax Lake chub.

HUMPBACK CHUB

Gila boraxobius.

Humpback chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated humpback chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that humpback chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Little information is available for humpback chub. A comparison of 88 freshwater fish species, found that elasticities among closely related species are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*) and fecundity accounted for another 19% (Vélez-Espino et al., 2006).

We anticipate the humpback chub's reproductive performance would be reduced substantially. The humpback chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the humpback chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Humpback chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected humpback chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately humpback chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the humpback chub.

Critical Habitat: Continued approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect humpback chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of humpback chub, and cause humpback chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of humpback chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the humpback chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the humpback chub.

SONORA CHUB
Gila ditaenia.

Sonora chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Sonora chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Sonora chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Little information is available for Sonora chub. A comparison of 88 freshwater fish species, found that elasticities among closely related species are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*) and fecundity accounted for another 19% (Vélez-Espino et al., 2006).

We anticipate the Sonora chub's reproductive performance would be reduced substantially. The Sonora chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Sonora chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Sonora chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Sonora chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Sonora chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Sonora chub.

Critical Habitat: Continued approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Sonora chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Sonora chub, and cause Sonora chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Sonora chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Sonora chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Sonora chub.

BONYTAIL CHUB
Gila elegans

Bonytail chub exposed to cyanide at the criterion continuous concentration (CCC) are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate bonytail chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 57%. We estimate that bonytail chub exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 66%.

As noted previously, the first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival, such as bonytail. Bonytail may live for as many as 50 years and are very fecund. In hatcheries, females produce between 1,000 and 17,000 eggs and the survival rate of juveniles is 17-38%. Despite this reproductive potential, we anticipate the effects of cyanide on fecundity and juvenile survival would have a substantial population-level effect on bonytail. Although bonytail spawn many eggs, they do not guard their eggs and predation by non-native fish in the Colorado River basin is substantial, probably contributing to reproductive failure in the wild. A reduction in potential fecundity and juvenile survival in the wild at the magnitude we estimate will add to the significant challenges to recovering the bonytail.

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the bonytail chub's potential recruitment substantially. The bonytail chub's potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the bonytail chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Bonytail chub may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. These potential effects could add substantially to the difficulties in reintroducing reproductively successful populations and attaining recovery. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately bonytail chub are not likely to overcome the effects to their recruitment in waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could limit the reproduction, numbers, and distribution of the bonytail chub.

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Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the bonytail chub include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of bonytail chubs, and cause chubs to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 57% and the reduction in the survival of young fish through the first year as high as 66%. These effects are estimated to be of a magnitude great enough to reduce numbers of bonytail chubs. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the bonytail chub.

GILA CHUB
Gila intermedia

Gila chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Gila chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Gila chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Little information is available for Gila chub. A comparison of 88 freshwater fish species, found that elasticities among closely related species are highly conserved among genera within the same taxonomic family (Vélez -Espino et al., 2006). Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*) and fecundity accounted for another 19% (Vélez -Espino et al., 2006).

We anticipate the Gila chub's reproductive performance would be reduced substantially. The Gila chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Gila chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Gila chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Gila chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Gila chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Gila chub.

Critical Habitat: Continued approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Gila chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Gila chub, and cause Gila chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Gila chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Gila chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Gila chub.

YAQUI CHUB
Gila purpurea.

Yaqui chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Yaqui chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Yaqui chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Little information is available for Yaqui chub. A comparison of 88 freshwater fish species, found that elasticities among closely related species are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*) and fecundity accounted for another 19% (Vélez-Espino et al., 2006).

We anticipate the Yaqui chub's reproductive performance would be reduced substantially. The Yaqui chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Yaqui chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Yaqui chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Yaqui chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Yaqui chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Yaqui chub.

Critical Habitat: Continued approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Yaqui chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Yaqui chub, and cause Yaqui chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Yaqui chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Yaqui chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Yaqui chub.

PAHRANAGAT ROUNDTAIL CHUB

Gila robusta jordani.

Pahranagat roundtail chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Pahranagat roundtail chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Pahranagat roundtail chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Little information is available for Pahranagat roundtail chub. A comparison of 88 freshwater fish species, found that elasticities among closely related species are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*) and fecundity accounted for another 19% (Vélez-Espino et al., 2006).

We anticipate the Pahranagat roundtail chub's reproductive performance would be reduced substantially. The Pahranagat roundtail chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Pahranagat roundtail chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Pahranagat roundtail chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected

Pahranagat roundtail chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Pahranagat roundtail chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Pahranagat roundtail chub.

Critical Habitat: Continued approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Pahranagat roundtail chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Pahranagat roundtail chub, and cause Pahranagat roundtail chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Pahranagat roundtail chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Pahranagat roundtail chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Pahranagat roundtail chub.

VIRGIN RIVER CHUB

Gila robusta seminuda

Virgin River chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Virgin River chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Virgin River chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

Formal Draft Biological Opinion.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Little information is available for Virgin River chub. A comparison of 88 freshwater fish species, found that elasticities among closely related species are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). Juvenile survival accounted for about 59% of the total elasticity of the population growth rate for the Utah chub (*Gila atraria*) and fecundity accounted for another 19% (Vélez-Espino et al., 2006).

We anticipate the Virgin River chub's reproductive performance would be reduced substantially. The Virgin River chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Virgin River chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Virgin River chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Virgin River chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Virgin River chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Virgin River chub.

Critical Habitat: Continued approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Virgin River chub critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Virgin River chub, and cause Virgin River chub to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Virgin River chub. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Virgin River chub's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Virgin River chub.

RIO GRANDE SILVERY MINNOW

Hybognathus amarus

Rio Grande silvery minnow exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Rio Grande silvery minnow exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Rio Grande silvery minnows exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Rio Grande silvery minnow females are short-lived (less than

2 years). Norris et al. (2008) conducted a population viability analysis for the Rio Grande silvery minnow. The simulations indicated that reproductive output of those individuals just shy of one year old entering their first spawning season – hereafter referred to as Age 0 fish – is a primary factor that determines the extent of population growth from year to year. This reproductive rate, as defined in our analysis, includes both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the reproductive output of Age 0 fish would be reduced substantially. The Rio Grande silvery minnow's reproductive output would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Rio Grande silvery minnow's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Rio Grande silvery minnows may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Rio Grande silvery minnow population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Rio Grande silvery minnows are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Rio Grande silvery minnow.

The physical and biological features of critical habitat essential to the conservation of the Rio Grande silvery minnow include a hydrologic regime which provides sufficient flowing water with low to moderate currents that form the aquatic habitats which the minnow prefers, such as backwaters, shallow side channels, pools, eddies, and runs of varying depth and velocity. The minnow also requires substrates of sand or silt, and sufficient water quality (water of proper temperatures and conditions such as dissolved oxygen content and pH).

Critical Habitat: Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Rio Grande silvery minnow critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Rio Grande silvery minnows, and cause Rio Grande silvery minnows to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Rio Grande silvery minnows. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Rio Grande silvery minnow's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Rio Grande silvery minnows.

BIG SPRING SPINEDACE

Lepidomeda mollispinis pratensis

Big Spring spinedace exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 31). Compared to control populations, we estimated Big Spring spinedace exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Big Spring spinedaces exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Big Spring spinedace are sexually mature after about a year and live up to 4 years. Most spawning of the Big Spring spinedace occurs from March through June, with some continuing sporadically into July. The big spring spinedace is believed to broadcast spawn over gravel substrate. Females generally produce from 380-640 eggs during spawning, with older females sometimes producing two complements of ova in one breeding season. (BRRC 2001).

A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the Big Spring spinedace found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the Big Spring spinedace's reproductive performance would be reduced substantially. The Big Spring spinedace's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Big Spring spinedace's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Big Spring spinedaces may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Big Spring spinedace population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Big Spring spinedaces are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Big Spring spinedace.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Big Spring spinedace include the following: Clean, permanent, flowing, spring-fed stream habitat with deep pool areas and shallow marshy areas along the shore; and the absence of nonnative fishes.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Big Spring spinedace critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Big Spring spinedaces, and cause Big Spring spinedaces to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Big Spring spinedace. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Big Spring spinedace's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Big Spring spinedace.

LITTLE COLORADO SPINEDACE
Lepidomeda vitatta

Little Colorado spinedace exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Little Colorado spinedace exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Little Colorado spinedaces exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Little Colorado spinedace are believed to live 3 to 4 years and mature early. Spinedace are late-spring to early-summer spawners although some females have been found to contain mature eggs as late as October. Spawning occurs in slow current over cobbles. Females may spawn more than once per year, and fecundity estimates range from 650-1000 total eggs per female.

A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the Little Colorado spinedace found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the Little Colorado spinedace's reproductive performance would be reduced substantially. The Little Colorado spinedace's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Little Colorado spinedace's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Little Colorado spinedaces may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Little Colorado spinedace population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Little Colorado spinedaces are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Little Colorado spinedace.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Little Colorado spinedace include clean, permanently flowing water with pools and a fine gravel or silt-mud substrate. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Little Colorado spinedace critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Little Colorado spinedace, and cause Little Colorado spinedace to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Little Colorado spinedace. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Little Colorado spinedace's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Little Colorado spinedace.

SPIKEDACE
Meda fulgida

Spikedace exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated spikedace exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that spikedace exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Spikedace females are short-lived (1-2 years) and mature early. Spawning occurs in the spring between April and June and seems to be triggered by a combination of stream discharge and water temperature. Females may be fractional spawners, with elapsed periods of days to weeks between spawning. Fecundity is correlated to age and length, and has been found to be between 90 to 250 ova.

A comparison of 88 freshwater fish species, found that life history parameters such as age at maturity, reproductive lifespan, fecundity, juvenile survivorship, and longevity could be predictors of elasticity patterns (Vélez-Espino et al., 2006). The spikedace has a reproductive strategy similar to the smalleye shiner (*Notropis buccula*), a member of a reproductive guild of cyprinids that broadcast spawn multiple batches of nonadhesive, semibuoyant ova throughout an extended reproductive season, and experience extremely high post-spawning mortality. Durham and Wilde (2009) studied the population dynamics of the smalleye shiner in the Brazos River, Texas. Elasticity analysis and sensitivity simulations of the projection matrix indicated that age-0 survival and age-1 fecundity were the most influential parameters in the population dynamics of smalleye shiners. In combination, these two stages accounted for the majority (70%) of the total elasticity of the population growth rate. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the spikedace's reproductive performance would be reduced substantially. The spikedace's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the spikedace's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Spikedace may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected spikedace population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately spikedace are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the spikedace.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the spikedace include the following: living areas for each stage of the spikedace, a proper food base, a lack of non-native species, and high quality water, with the proper amount of dissolved oxygen, and no or minimal pollutant levels for pollutants such as copper, arsenic, mercury, and cadmium; human and animal waste products; pesticides; suspended sediments; and gasoline or diesel fuels.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect spikedace critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of spikedace, and cause spikedace to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of spikedace. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the spikedace's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the spikedace.

MOAPA DACE

Moapa coriacea

Moapa dace exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Moapa dace exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Moapa daces exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Moapa dace reach sexual maturity at 1 year of age and can live up to 4 years. Moapa dace apparently reproduce year-round, peaking in the spring, in water temperatures ranging from 28°C-32°C. Fecundity is related to fish size and egg counts have range from 60 to 772 depending on size. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the Moapa dace found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the Moapa dace's reproductive performance would be reduced substantially. The Moapa dace's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Moapa dace's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Moapa daces may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Moapa dace population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Moapa daces are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Moapa dace.

PALEZONE SHINER

Notropis albizonatus

Palezone shiner exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated palezone shiner exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that palezone shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Palezone shiner females are short-lived (3-4 years).

We anticipate the palezone shiner's reproductive performance would be reduced substantially. The palezone shiner's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the palezone shiner's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Palezone shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected palezone shiner population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately palezone shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration.

Formal Draft Biological Opinion.

Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the palezone shiner.

CAHABA SHINER
Notropis cahabae

Cahaba shiner exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Cahaba shiner exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Cahaba shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Cahaba shiner females are short-lived (probably 3- 4 years), mature early, and spawn from late May through June.

We anticipate the Cahaba shiner’s reproductive performance would be reduced substantially. The Cahaba shiner’s reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Cahaba shiner's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Cahaba shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Cahaba shiner population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Cahaba shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Cahaba shiner.

ARKANSAS RIVER SHINER

Notropis girardi

Arkansas River shiner exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Arkansas River shiner exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Arkansas River shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Arkansas River shiner females are short-lived (less than 3 years) and appear to experience extremely high mortality after spawning.

The Arkansas River minnow has a reproductive strategy similar to the smalleye shiner (*Notropis buccula*). Durham and Wilde (2009) studied the population dynamics of the smalleye shiner in the Brazos River, Texas. Smalleye shiner are members of a reproductive guild of cyprinids that broadcast spawn multiple batches of nonadhesive, semibuoyant ova throughout an extended reproductive season, and post-spawning mortality is extremely high. Elasticity analysis and sensitivity simulations of the projection matrix indicated that age-0 survival and age-1 fecundity were the most influential parameters in the population dynamics of smalleye shiners. In combination, these two stages accounted for the majority (70%) of the total elasticity of the population growth rate.

We anticipate the Arkansas River shiner's reproductive performance would be reduced substantially. The Arkansas River shiner's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Arkansas River shiner's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Arkansas River shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Arkansas River shiner population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Arkansas River shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Arkansas River shiner.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Arkansas River shiner include the following: —(i) a natural, unregulated hydrologic regime complete with episodes of flood and drought or, if flows are modified or regulated, a hydrologic regime characterized by the duration, magnitude, and frequency of flow events capable of forming and maintaining channel and instream habitat necessary for particular Arkansas River shiner life-stages in appropriate seasons; (ii) a complex, braided channel with pool, riffle (shallow area in a streambed causing ripples), run, and backwater components that provide a suitable variety of depths and current velocities in appropriate seasons; (iii) a suitable unimpounded stretch of flowing water of sufficient length to allow hatching and development of the larvae; (iv) a river bed of predominantly sand, with some patches of gravel and cobble; (v) water quality characterized by low concentrations of contaminants and natural, daily and seasonally variable temperature, turbidity, conductivity, dissolved oxygen, and pH; (vi) suitable reaches of aquatic habitat, as defined by primary constituent elements (i) through (v) above, and adjacent riparian habitat sufficient to support an abundant terrestrial, semiaquatic, and aquatic invertebrate food base; and (vii) few or no predatory or competitive non-native fish species present.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Arkansas River shiner critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Arkansas River shiners, and cause Arkansas River shiners to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Arkansas River shiner. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Arkansas River shiner's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Arkansas River shiner.

CAPE FEAR SHINER

Notropis mekistocholas

Cape Fear shiners exposed to cyanide at the criterion continuous concentration (CCC) are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Cape Fear shiners exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 59%. We estimate that Cape Fear shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 68%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.
The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Cape Fear shiners, than on species with greater adult survival, such as sturgeon. Cape Fear shiners live about two to three years. No information is presently available about this species' reproductive characteristics.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on Cape Fear shiner, we infer the Cape Fear shiner's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Cape Fear shiner's fertility substantially. The Cape Fear shiner's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

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In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Cape Fear shiner's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Cape Fear shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Cape Fear shiner population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Cape Fear shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Cape Fear shiner.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Cape Fear shiner include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of Cape Fear shiners, and cause shiners to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 59% and the reduction in the survival of young fish through the first year as high as 68%. These effects are estimated to be of a magnitude great enough to reduce numbers of Cape Fear shiners. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Cape Fear shiner.

PECOS BLUNTNOSE SHINER

Notropis simus pecosensis

Pecos bluntnose shiner exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Pecos bluntnose shiner exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Pecos bluntnose shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Pecos bluntnose shiner females are short-lived (up to 3 years).

The Pecos bluntnose shiner has a reproductive strategy similar to the smalleye shiner (*Notropis buccula*). Durham and Wilde (2009) studied the population dynamics of the smalleye shiner in the Brazos River, Texas. Smalleye shiner are members of a reproductive guild of cyprinids that broadcast spawn multiple batches of nonadhesive, semibuoyant ova throughout an extended reproductive season, and post-spawning mortality is extremely high. Elasticity analysis and sensitivity simulations of the projection matrix indicated that age-0 survival and age-1 fecundity were the most influential parameters in the population dynamics of smalleye shiners. In combination, these two stages accounted for the majority (70%) of the total elasticity of the population growth rate.
We anticipate the Pecos bluntnose shiner's reproductive performance would be reduced substantially. The Pecos bluntnose shiner's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Pecos bluntnose shiner's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Pecos bluntnose shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Pecos bluntnose shiner population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Pecos bluntnose shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Pecos bluntnose shiner.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Pecos bluntnose shiner include clean permanent water, a main river channel habitat with a sandy substrate, and a low velocity flow. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Pecos bluntnose shiner critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Pecos bluntnose shiners, and cause Pecos bluntnose shiners to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Pecos bluntnose shiner. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Pecos bluntnose shiner's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Pecos bluntnose shiner.

TOPEKA SHINER

Notropis Topeka

Topeka shiner exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Topeka shiner exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Topeka shiners exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Topeka shiners, than on species with greater adult survival, such as sturgeon. Topeka shiner females are short-lived (1-2 years), mature early, and spawn year-round with two peaks in August and late winter.

The Topeka shiner has a reproductive strategy similar to the smalleye shiner (*Notropis buccula*). Durham and Wilde (2009) studied the population dynamics of the smalleye shiner in the Brazos River, Texas. Smalleye shiner are members of a reproductive guild of cyprinids that broadcast spawn multiple batches of nonadhesive, semibuoyant ova throughout an extended reproductive season, and post-spawning mortality is extremely high. Elasticity analysis and sensitivity simulations of the projection matrix indicated that age-0 survival and age-1 fecundity were the most influential parameters in the population dynamics of smalleye shiners. In combination, these two stages accounted for the majority (70%) of the total elasticity of the population growth rate. Although Topeka shiners utilized a different spawning strategy than smalleye shiners, they are similar in that survival of post-spawning adults is extremely low. Consequently, we anticipate that age-0 survival and age-1 fecundity will similarly be most important to the Topeka shiner's growth rate.

We anticipate the Topeka shiner's reproductive performance would be reduced substantially. The Topeka shiner's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Topeka shiner's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Topeka shiners may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Topeka shiner population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Topeka shiners are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Topeka shiner.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Topeka shiner include the following: stream most often with permanent flow, but which become dry during intermittent periods. Side channel pools and oxbows which are either seasonally connected to a stream or maintained by groundwater inputs are also required. Streams and side channel pools with water quality (temperature, turbidity, conductivity, salinity, dissolved oxygen, pH, chemical contaminants, and other chemical characteristics) necessary for unimpaired behavior, growth, and viability of all life stages is essential. Living and spawning areas for adults are required, which must have water velocities of less than a half meter per second, and depths from 0.1 to 2 meters. Similarly, living areas for juveniles are required. These must have the same flow rate as areas for adults, but require greatly decreased depths, that of less than a quarter of a meter. Proper substrate for the Topeka shiner is sand, gravel, cobble, or silt, with proper amounts of fine sediment and substrate embeddedness. A proper food base of terrestrial, semiaquatic and aquatic invertebrates is necessary. A hydrologic regime capable of forming, maintaining, or restoring flow periodicity, channel morphology, fish community composition, off channel habitats, and habitat components essential for the fish. Finally, this fish requires few or no nonnative predatory or competitive species to be present in their habitat.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Topeka shiner critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Topeka shiners, and cause Topeka shiners to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Topeka shiner. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Topeka shiner's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Topeka shiner.

OREGON CHUB
Oregonichthys crameri

Oregon chub exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Oregon chub exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Oregon chubs exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Oregon chub females exhibit an intermediate life span, mature in their second year, and spawn from mid-May through August with peak activity in July. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan as the Oregon chub found that population growth rates for these species were highly susceptible to perturbations in juvenile survival. Juvenile survival accounted for about 58% of the total elasticity of the population growth rate in all species with these life-history traits. A comparison of elasticities among closely related species found that elasticity values are highly conserved among genera within the same taxonomic family.

We anticipate the Oregon chub's reproductive performance would be reduced substantially. The Oregon chub's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Oregon chub's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Oregon chubs may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Oregon chub population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Oregon chubs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Oregon chub.

BLACKSIDE DACE

Phoxinus cumberlandensis

Blackside dace exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated blackside dace exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that blackside daces exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Formal Draft Biological Opinion.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival. Blackside dace generally have a lifespan of 3 years, reach sexual maturity at age 1, and are broadcast spawners, spawning over clean, gravel-sized substrate from April to July. Average fecundity is about 1500. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns, and elasticity values are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). Sensitivity and elasticity analyses were performed for the southern redbelly dace, (*Phoxinus erythrogaster*), a closely-rated species with similar longevity, age at maturity, and reproductive lifespan (Stasiak 2007). Results indicated that population growth rate was most sensitive (>96%) to changes in first-year survival and most elastic to changes in first-year reproduction, followed by first-year survival. Overall fertility accounted for 70% of total elasticity. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the blackside dace's fertility would be reduced substantially. The blackside dace's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the blackside dace's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Blackside daces may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected blackside dace population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately blackside daces are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the blackside dace.

WOUNDFIN

Plagopterus argentissimus

Woundfin exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Woundfin exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Woundfin exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, than on species with greater adult survival. The generation time for the woundfin is predominately limited to 1 year, with most individuals reaching sexual maturity in the second year of a 3-year lifespan. Individuals must achieve sufficient growth prior to the spring spawning period in order to contribute to the next generation. In a comparison of 88 freshwater fish species, longevity and age at maturity were found to be the best predictors of elasticity patterns Vélez-Espino et al., 2006). Elasticity analyses performed on cyprinids with similar longevity, age at maturity, and reproductive lifespan to the woundfin found that population growth rates were most susceptible to perturbations in juvenile survival, followed by fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the woundfin's reproductive performance fertility would be reduced substantially. The woundfin's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the woundfin's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Woundfins may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected woundfin population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately woundfins are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the woundfin.

Critical Habitat: Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect woundfin critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of woundfins, and cause woundfins to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of woundfin. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the woundfin's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the woundfin.

COLORADO PIKEMINNOW
Ptychocheilus lucius

Colorado pikeminnows exposed to cyanide at the criterion continuous concentration (CCC) are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were

available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Colorado pikeminnows exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 63%. We estimate that Colorado pikeminnows exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 71%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

The Colorado pikeminnow is a long-lived fish (40+ years) that evolved in a variable system, with high adaptability to natural environmental variability and resilience to natural catastrophes. This evolution has become manifest as pulsed recruitment from periodic strong year classes, great longevity of adults, and low vulnerability of adults to environmental influences. Great longevity and stability of adults provides a “storage effect” for populations, into which periodic recruitment from strong year classes allows fish to become stored (Gilpin 1993). This is seen as a way that Colorado pikeminnow maintain long-term population viability and stability under environmental variation.

As stated in the recovery plan for this species, a critical aspect of recovery is increased frequency of strong year classes. The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Colorado pikeminnow’s potential recruitment substantially. The Colorado pikeminnow’s potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter. The reductions in the Colorado pikeminnow’s recruitment could diminish the frequency and could even preclude the occurrence of strong year classes.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Colorado pikeminnow’s reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Colorado pikeminnows may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Colorado pikeminnow population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Colorado pikeminnows are likely to become extirpated from waters where they are exposed to cyanide toxicity at the CCC. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Colorado pikeminnow.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Colorado pikeminnow include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of Colorado pikeminnow, and cause pikeminnows to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 683 and the reduction in the survival of young fish through the first year as high as 71%. These effects are estimated to be of a magnitude great enough to reduce numbers of Colorado pikeminnows. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Colorado pikeminnow.

ASH MEADOWS SPECKLED DACE

Rhinichthys osculus spp

Ash Meadows speckled dace exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Ash Meadows speckled dace exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Ash Meadows speckled daces exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, than on species with greater adult survival. Ash Meadows speckled dace females reach sexual maturity at 2 years, spawn primarily over the spring and summer, and are believed to live up to 4 years. Elasticity analyses performed on cyprinids with similar life-history traits, including the blacknose dace**,** *Rhinichthys atratulus***,** found that population growth rates for these species were most susceptible to perturbations in juvenile survival, followed by fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate. A comparison of elasticities among closely related species found that elasticity values are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the Ash Meadows speckled dace's fertility would be reduced substantially. The Ash Meadows speckled dace's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Ash Meadows speckled dace's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Ash Meadows speckled daces may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Ash Meadows speckled dace population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Ash Meadows speckled daces are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Ash Meadows speckled dace.

Critical Habitat: Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect Ash Meadows speckled dace critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Ash Meadows speckled daces, and cause Ash Meadows speckled daces to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of Ash Meadows speckled dace. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Ash Meadows speckled dace's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the Ash Meadows speckled dace.

KENDALL WARM SPRINGS DACE

Rhinichthys osculus thermalis

Kendall Warm Springs dace exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Kendall Warm Springs dace exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Kendall Warm Springs daces exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn. Kendall Warm Springs dace females reach sexual maturity at 2 years, are thought to spawn several times a year, and are likely to live between 3 and 5 years. Elasticity analyses performed on cyprinids with similar life-history traits, including the blacknose dace**,** *Rhinichthys atratulus***,** found that population growth rates for these species were most susceptible to perturbations in juvenile survival, followed by fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate. A comparison of elasticities among closely related species found that elasticity values are highly conserved among genera within the same taxonomic family (Vélez-Espino et al., 2006). These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn

We anticipate the Kendall Warm Springs dace's reproductive performance would be reduced substantially. The Kendall Warm Springs dace's reproductive performance rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Kendall Warm Springs dace's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Kendall Warm Springs daces may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Kendall Warm Springs dace population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Kendall Warm Springs daces are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Kendall Warm Springs dace.

LOACH MINNOW
Tiaroga cobitis

Loach minnow exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated Loach minnow exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 31%. We estimate that Loach minnows exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 33%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than species with greater adult survival. Loach minnow females are short-lived (1-2 years), and mature early. Elasticity analyses performed on cyprinids with similar life-history traits found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the Loach minnow's reproductive performance would be reduced substantially. The Loach minnow's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Loach minnow's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Loach minnows may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Loach minnow population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Loach minnows are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Loach minnow.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the loach minnow include the following: permanent flowing water with no or low levels of pollutants, which must include living areas for adult loach minnows with moderate to swift flow velocities (9 to 32 inches per second) in shallow water between approximately 1 and 30 inches (3 to 75 cm) in depth. The substrate of these areas should consist of gravel, cobble, or rubble. Furthermore, there must be living areas for juveniles as well. To support juveniles, areas must have moderate to swift flow velocities, from 1 to 34 inches per second (3 and 85 cm/second). Juveniles require the same depth and substrates as adults. It is the larval loach minnow which requires greatly different habitat. Loach minnow larvae require slow to moderate flow velocities, from 3 to 20 inches per second (9 to 50 cm/second) and shallow water with sand, gravel, and cobble substrates.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect loach minnow critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of loach minnows, and cause loach minnows to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. These effects are estimated to be of a magnitude great enough to reduce numbers of loach minnow. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the loach minnow's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the loach minnow.

Gasterosteidae

UNARMORED THREESPINE STICKLEBACK
Gasterosteus aculeatus williamsoni

Unarmored threespine stickleback exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated unarmored threespine stickleback exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Unarmored threespine stickleback s exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than species with greater adult survival. Unarmored threespine stickleback females are short-lived (about 1 year), mature early, and lay 50-300 eggs into nests guarded by males. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on freshwater fish with similar longevity and reproductive lifespans to the Unarmored threespine stickleback found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year’s spawn.

We anticipate the unarmored threespine stickleback's reproductive performance would be reduced substantially. The unarmored threespine stickleback's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the unarmored threespine stickleback's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Unarmored threespine stickleback s may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected unarmored threespine stickleback population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately unarmored threespine sticklebacks are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the unarmored threespine stickleback .

Gobiidae

TIDEWATER GOBY
Eucyclogobius newberryi

Tidewater goby exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimated tidewater goby exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 36%. We estimate that tidewater gobies exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through

the first year and that reduction could be as much as, but is not likely to be greater than, 40%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than species with greater adult survival. Tidewater goby females are short-lived (about 1 year), mature early, and can Reproduce all year. A comparison of 88 freshwater fish species, found that longevity and age at maturity were the best predictors of elasticity patterns (Vélez-Espino et al., 2006). Elasticity analyses performed on freshwater fish with similar longevity and reproductive lifespans to the tidewater goby found that population growth rates for these species were highly susceptible to perturbations in juvenile survival and fecundity (Vélez-Espino et al., 2006). In combination, these two stages accounted for over 90% of the total elasticity of the population growth rate in all species with these life-history traits. These reproductive parameters, as defined in our analysis, include both egg production and larval / juvenile survival rate to the next year's spawn.

We anticipate the tidewater goby's reproductive performance would be reduced substantially. The tidewater goby's reproductive performance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the tidewater goby's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Tidewater gobies may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected tidewater goby population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately tidewater gobies are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration.

Formal Draft Biological Opinion.

Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the tidewater goby.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the tidewater goby include the following: Persistent, shallow (in the range of about 0.1 to 2 m), still-to-slow-moving aquatic habitat most commonly ranging in salinity from less than 0.5 ppt to about 10 to 12 ppt; Substrates (e.g., sand, silt, mud) suitable for the construction of burrows for reproduction; Submerged and emergent aquatic vegetation, such as Potamogeton pectinatus and Ruppia maritima, that provides protection from predators; and Presence of a sandbar(s) across the mouth of a lagoon or estuary during the late spring, summer, and fall that closes or partially closes the lagoon or estuary, thereby providing relatively stable water levels and salinity.

Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect tidewater goby critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of tidewater gobies, and cause tidewater goby to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 36% and the reduction in the survival of young fish through the first year as high as 40%. These effects are estimated to be of a magnitude great enough to reduce numbers of tidewater goby. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the tidewater goby's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the tidewater goby.

Goodeidae

WHITE RIVER SPRINGFISH (ASH SPRING)
Crenichthys baileyi baileyi

White River springfish exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate White River springfish exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that White River springfish exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Springfish (genus *Crenichthys*) are uniquely adapted to survive in high temperatures (e.g. 84 to 97 F for Railroad Valley springfish, U.S. Fish and Wildlife Service 1996) and only occur in hot spring environments in Southern Nevada. Their thermal tolerance has enabled them to evolve in habitats that are otherwise uninhabitable for other fish species that are native to the region. Thus, historic populations have benefited from the lack of competition and predation by other fishes. Although life history information is limited, Springfish are believed to be relatively short lived (3 to 4 years), spawn infrequently (average two spawnings per year), and deposit few eggs per spawn (10 to 17; Kopec 1949). Such a reproductive strategy is unlike any other that was previously described in the *Population Response* section and would appear to be highly vulnerable to stressors which reduce fecundity or survival of larvae, juveniles or adults. The vulnerability of this strategy was evidenced by the decline in the Springfish populations which occurred following the introduction of nonnative fish species (Tuttle et al. 1990, U.S. Fish and Wildlife Service 1996 & 1998). These reductions were attributed in large part to predation by nonnative fishes on springfish larvae (U.S. Fish and Wildlife Service 1998). The effects of cyanide on fecundity, egg hatchability, and larval/juvenile survival could result in similar impacts on the springfish populations, as well as, worsen effects caused by nonnative fish predation.

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the White River springfish's potential recruitment substantially. The White River springfish's potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the White River springfish's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. White River springfish may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected White River springfish population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately White River springfish are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the White River springfish.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the White River springfish include the following: warm water springs and their outflows and surrounding areas that provide vegetation for cover and habitat for insects and other invertebrates on which the species feeds. By warm water springs and their outflows, we mean water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth sufficient for the critical habitat to serve its intended conservation function.

Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to the criterion concentration. This approval could adversely affect White River springfish critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of White River springfish, and cause White River springfish to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of White River springfish. Continued approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the White River springfish's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the White River springfish.

HIKO WHITE RIVER SPRINGFISH

Crenichthys baileyi grandis

Hiko White River springfish exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Hiko White River springfish exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Hiko White River springfish exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Springfish (genus *Crenichthys*) are uniquely adapted to survive in high temperatures (e.g. 84 to 97 F for Railroad Valley springfish, U.S. Fish and Wildlife Service 1996) and only occur in hot spring environments in Southern Nevada. Their thermal tolerance has enabled them to evolve in habitats that are otherwise uninhabitable for other fish species that are native to the region. Thus, historic populations have benefited from the lack of competition and predation by other fishes. Although life history information is limited, Springfish are believed to be relatively short lived (3 to 4 years), spawn infrequently (average two spawnings per year), and deposit few eggs per spawn (10 to 17; Kopec 1949). Such a reproductive strategy is unlike any other that was previously described in the *Population Response* section and would appear to be highly vulnerable to stressors which reduce fecundity or survival of larvae, juveniles or adults. The vulnerability of this strategy was evidenced by the decline in the Springfish populations which occurred following the introduction of nonnative fish species (Tuttle et al. 1990, U.S. Fish and Wildlife Service 1996 & 1998). These reductions were attributed in large part to predation by nonnative fishes on springfish larvae (U.S. Fish and Wildlife Service 1998). The effects of cyanide on fecundity, egg hatchability, and larval/juvenile survival could result in similar impacts on the springfish populations, as well as, worsen effects caused by nonnative fish predation.

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Hiko White River springfish's potential recruitment substantially. The Hiko White River springfish's potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Hiko White River springfish's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Hiko White River springfish may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Hiko White River springfish population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Hiko White River springfish are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Hiko White River springfish.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Hiko White River springfish include the following: warm water springs and their outflows and surrounding areas that provide vegetation for cover and habitat for insects and other invertebrates on which the species feeds. By warm water springs and their outflows, we mean water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth sufficient for the critical habitat to serve its intended conservation function.

Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to the criterion concentration. This approval could adversely affect Hiko White River springfish critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Hiko White River springfish, and cause Hiko White River springfish to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of Hiko White River springfish. Continued approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Hiko White River springfish's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Hiko White River springfish.

RAILROAD VALLEY SPRINGFISH

Crenichthys nevadae

Railroad Valley springfish exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Railroad Valley springfish exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Railroad Valley springfish exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Springfish (genus *Crenichthys*) are uniquely adapted to survive in high temperatures (e.g. 84 to 97 F for Railroad Valley springfish, U.S. Fish and Wildlife Service 1996) and only occur in hot spring environments in Southern Nevada. Their thermal tolerance has enabled them to evolve in habitats that are otherwise uninhabitable for other fish species that are native to the region. Thus, historic populations have benefited from the lack of competition and predation by other fishes. Although life history information is limited, Springfish are believed to be relatively short lived (3 to 4 years), spawn infrequently (average two spawnings per year), and deposit few eggs per spawn (10 to 17; Kopec 1949). Such a reproductive strategy is unlike any other that was previously described in the *Population Response* section and would appear to be highly vulnerable to stressors which reduce fecundity or survival of larvae, juveniles or adults. The vulnerability of this strategy was evidenced by the decline in the Springfish populations which occurred following the introduction of nonnative fish species (Tuttle et al. 1990, U.S. Fish and Wildlife Service 1996 & 1998). These reductions were attributed in large part to predation by nonnative fishes on springfish larvae (U.S. Fish and Wildlife Service 1998). The effects of cyanide on fecundity, egg hatchability, and larval/juvenile survival could result in similar impacts on the springfish populations, as well as, worsen effects caused by nonnative fish predation.

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Railroad Valley springfish's potential recruitment substantially. The Railroad Valley springfish's potential recruitment would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Railroad Valley springfish's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Railroad Valley springfish may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Railroad Valley springfish population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Railroad Valley springfish are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Railroad Valley springfish.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Railroad Valley springfish include the following: clear, unpolluted thermal spring waters ranging in temperature from 84 to 97 F in pools, flowing channels, and marshy areas with aquatic plants, insects and mollusks. By clear, unpolluted thermal spring waters, we mean water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth sufficient for the critical habitat to serve its intended conservation function.

Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to the criterion concentration. This approval could adversely affect Railroad Valley springfish critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of Railroad Valley springfish, and cause Railroad Valley springfish to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of Railroad Valley springfish. Continued approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the Railroad Valley springfish's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Railroad Valley springfish.

Osmeridae

DELTA SMELT
Hypomesus transpacificus

Delta smelt exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate delta smelt exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that delta smelt exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like delta smelt, than on species with greater adult survival, such as sturgeon. Delta smelt live only 1 year and die after spawning. Female smelt lay between 1,600 and 2,600 eggs in the spring. The number and hatchability of eggs together with the survival of larval and juvenile smelt are what drives annual population abundance in this annual fish species.

We anticipate the delta smelt's annual abundance would be reduced substantially. The delta smelt's annual abundance would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year to spawn, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the delta smelt's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish. Delta smelt may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected delta smelt population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur and the delta smelt's current population abundance, we conclude ultimately delta smelt are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the delta smelt.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the delta smelt include water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience adequate individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorized States and Tribes to continue to manage cyanide in waters to the criteria concentration. This approval could adversely affect delta smelt critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of delta smelt, and cause delta smelt to experience adverse effects to growth swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of delta smelt. Continued approval of the CCC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the delta smelt's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for the conservation of the delta smelt.

Percidae

SLACKWATER DARTER
Etheostoma boschungi

Slackwater darters exposed to cyanide at the CCC are likely to experience reduced survival, reproduction, growth, swimming performance, condition, and exhibit developmental abnormalities, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Few data are available to estimate the magnitude of effects that could occur. We developed a quantitative estimate of the effects on fecundity and juvenile survival from exposure to cyanide at the CCC (Table 13). Compared to control populations, we estimate slackwater darters exposed to cyanide at the CCC could experience a significant reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that slackwater darters exposed to cyanide at the CCC could experience a significant reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the juveniles that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and increase the interval between spawning events.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on shorter-lived fish, like darters, than on longer-lived species, such as sturgeon. Slackwater darter females are short-lived (up to 4 years) and reproduce in no more than three years. The slackwater darter has the potential to spawn multiple times per season, but its spawning period is short, about 1 month, and it is not known how many clutches are spawned (Hartup 2005).

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to conduct population viability analyses for the two species. Average slackwater darter fecundity was estimated as 92 and 197 eggs, respectively, for one-batch and two-batch fecundity. Based on estimates of adult survival, Hartup (2005) calculated the adult fertility rate would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable (the fertility rate was defined as the number of female offspring per female aged *i* per year). Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce Hartup's (2005) estimated slackwater darter fertility (0.896) substantially. Incorporating our estimates, the revised fertility rates would be reduced substantially, as follows: based upon (a) reductions in numbers of hatched eggs (0.31), (b) reductions in young fish surviving through the first year (0.23), and (c) the additive effect of reduced fecundity and survival (0.07). These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

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In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the slackwater darter's reproduction by reducing the number of eggs females spawn, reducing the hatchability of spawned eggs, and reducing the survival of young fish through the first year. Slackwater darters may also experience effects on growth, swimming performance, condition, and development. Based upon our estimates of the magnitude of effects, we would not expect a reduction in density-dependence, if any, to compensate for the reductions in fecundity and juvenile survivorship. We would anticipate a consequent reduction in numbers of slackwater darters. An effected slackwater darter population's growth rate could stabilize at a reduced absolute population number, or the population could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude slackwater darters could become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. The proposed chronic criterion could reduce the reproduction, numbers, and distribution of the slackwater darter.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the slackwater darter include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of slackwater darters, and cause darters to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 65% and the reduction in the survival of young fish through the first year as high as 74%. These effects are estimated to be of a magnitude great enough to reduce numbers of slackwater darters. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the slackwater darter.

VERMILION DARTER
Etheostoma chermocki

Vermilion darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate vermilion darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that vermilion darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon. Comprehensive studies of vermilion darter life history have not been completed, but it is believed that its life history attributes are similar to those of other snubnose darters, which reach sexual maturity at 1 year of age. Most darters are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on vermilion darters, we infer the vermilion darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the vermilion darter's fertility substantially. The vermilion darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the vermilion darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Vermilion darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected vermilion darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately vermilion darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the vermilion darter.

RELICT DARTER
Etheostoma chienense

Relict darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate relict darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that relict darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon. We have no information on the relict darter's lifespan, survival, or fecundity. We assume the relict darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on relict darters, we infer the relict darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the relict darter's fertility substantially. The relict darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the relict darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Relict darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected relict darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately relict darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the relict darter.

ETOWAH DARTER
Etheostoma etowahae

Etowah darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Etowah darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that Etowah darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon. We have no information on the Etowah darter's lifespan, survival, or fecundity. We assume the Etowah darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on Etowah darters, we infer the Etowah darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Etowah darter's fertility substantially. The Etowah darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Etowah darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Etowah darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Etowah darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Etowah darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Etowah darter.

FOUNTAIN DARTER

Etheostoma fonticola

Fountain darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column. Exposure of fountain darters to cyanide at the criterion maximum concentration (CMC) is likely to reduce survival.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate fountain darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 81%. We estimate that fountain darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 86%. In addition, we estimate that fountain darters exposed to cyanide at the CMC are likely to experience a substantial reduction in survival and that reduction could be as much as, but is not likely to be greater than, 58.2%. Effects of cyanide at the CMC on the survival of other fountain darter life stages is expected to be of lesser magnitude.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like fountain darters, than on species with greater adult survival, such as sturgeon. Fountain darter females are short-lived (1-2 years), mature early, and spawn year-round with two peaks in August and late winter. In the San Marcos River, mature ova were collected from individuals about 3.5 months old (Linam et al. 1993). Bonner et al. (1998) reported mean egg production (± 1 SD) is 760 (±310) per two breeding pairs during a 33-day period at 23 degrees C, under laboratory conditions. Annual fecundity is probably substantial.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The fountain darter is shorter-lived but more fecund than the slackwater darter and holiday darter. Nevertheless, we anticipate the fountain darter's fertility would be reduced substantially. The fountain darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the fountain darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Fountain darters may also experience effects on growth, swimming performance, condition, and development. Exposure to cyanide at the acute criterion could also substantially reduce the survivorship of juvenile fountain darters. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected fountain darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately fountain darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the acute and chronic criteria could reduce the reproduction, numbers, and distribution of the fountain darter.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the fountain darter include adequate flows, undisturbed substrate, aquatic vegetation including filamentous green algae, and water quality. Approval of the CCC and CMC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect fountain darter critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of fountain darters, and cause fountain darters to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high as 31% and the reduction in the survival of young fish through the first year as high as 33%. Reduction in survivorship of juvenile fish exposed at the CMC could be as high as 58.2% These effects are estimated to be of a magnitude great enough to reduce numbers of fountain darter. Continued approval of the CCC and CMC could adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the fountain darter's extirpation from critical habitat containing cyanide at the CCC and CMC. Impacts to water quality resulting from management of cyanide to the CCC and CMC would diminish the ability of critical habitat to provide for the conservation of the fountain darter.

NIANGUA DARTER

Etheostoma nianguae

Niangua darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Niangua darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that Niangua darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Niangua darters, than on species with greater adult survival, such as sturgeon. The Niangua darter reaches sexual maturity at 1 year of age and can live up to 4 years. Estimates of fecundity are based upon counts of mature ova from collected fish: the number of mature ova averaged 189.8 for four females of age-group I, 387.5 for two females of age-group II. A female of age-group IV had 748 mature eggs.

Niangua darter females are short-lived (up to 4 years) and reproduce in no more than three years. The Niangua darter spawns from March to June.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The Niangua darter's fecundity is somewhat greater than the slackwater darter's and holiday darter's fecundity and their lifespans are similar. The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Niangua darter's fertility substantially. The Niangua darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Niangua darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Niangua darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Niangua darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Niangua darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Niangua darter.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Niangua darter include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of Niangua darters, and cause darters to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 65% and the reduction in the survival of young fish through the first year as high as 74%. These effects are estimated to be of a magnitude great enough to reduce numbers of Niangua darters. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Niangua darter.

WATERCRESS DARTER
Etheostoma nuchale

Watercress darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate watercress darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that watercress darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon. We have no information on the watercress darter's lifespan, survival, or fecundity. We assume the relict darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on watercress darters, we infer the watercress darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the watercress darter's fertility substantially. The watercress darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the watercress darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Watercress darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected watercress darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately watercress darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the watercress darter.

OKALOOSA DARTER
Etheostoma okaloosae

Okaloosa darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Okaloosa darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that Okaloosa darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon. Fecundity of Okaloosa darters is very low - mean mature ova were only 29 (Ogilvie 1980).

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity 1analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The Okaloosa darter's fecundity is much less than the slackwater darter's and holiday darter's fecundity. The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Okaloosa darter's fertility substantially. The Okaloosa darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Okaloosa darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Okaloosa darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Okaloosa darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Okaloosa darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Okaloosa darter.

DUSKYTAIL DARTER

Etheostoma percnurum

Duskytail darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate duskytail darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that duskytail darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like duskytail darters, than on species with greater adult survival, such as sturgeon. Duskytail darter adult survival is very low. A very small percentage of the population survives to age 3. Duskytail darters can spawn as 1-year-olds. Spawning frequency ranged from 5-7 clutches per year, and fecundity from 135-189 (Layman 1991)

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The duskytail darter's fecundity and survival approximates or is somewhat less than the slackwater darter's and holiday darter's fecundity and survival. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the duskytail darter's fertility substantially. The duskytail darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the duskytail darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Duskytail darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected duskytail darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately duskytail darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the duskytail darter.

BAYOU DARTER

Etheostoma rubrum

Bayou darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate bayou darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that bayou darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like bayou darters, than on species with greater adult survival, such as sturgeon. Most Bayou darters start spawning after their first year and do not live beyond the age of three. Depending on the size of the female, clutches can range from 20-75 eggs. A single female likely spawns at least twice per reproductive season based on the size classes of ova.

Bayou darter females are short-lived (up to 3 years) and reproduce in no more than three years. The bayou darter spawns from March to June. Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The bayou darter's fecundity approximates or is slightly less than the slackwater and holiday darter's. The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the bayou darter's fertility substantially. The bayou darter's fertility would be diminished because of (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the bayou darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Bayou darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected bayou darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately bayou darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the bayou darter.

CHEROKEE DARTER
Etheostoma scotti

Cherokee darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Cherokee darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that Cherokee darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration.

Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Cherokee darters, than on species with greater adult survival, such as sturgeon. We have no information on the Cherokee darter's lifespan, survival, or fecundity. We assume the Cherokee darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on Cherokee darters, we infer the Cherokee darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Cherokee darter's fertility substantially. The Cherokee darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Cherokee darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Cherokee darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Cherokee darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Cherokee darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Cherokee darter.

MARYLAND DARTER
Etheostoma sellare

Maryland darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Maryland darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that Maryland darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon. We have no information on the Maryland darter’s lifespan, survival, or fecundity. We assume the Maryland darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on Maryland darters, we infer the Maryland darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Maryland darter's fertility substantially. The Maryland darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Maryland darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Maryland darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Maryland darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Maryland darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Maryland darter.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Maryland darter include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of Maryland darters, and cause darters to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 65% and the reduction in the survival of young fish through the first year as high as 74%. These effects are estimated to be of a magnitude great enough to reduce numbers of Maryland darters. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Maryland darter.

BLUEMASK DARTER
Etheostoma sp.

Bluemask darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate bluemask darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 65%. We estimate that bluemask darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon. We have no information on the bluemask darter's lifespan, survival, or fecundity. We assume the bluemask darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on bluemask darters, we infer the bluemask darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the bluemask darter's fertility substantially. The bluemask darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the bluemask darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Bluemask darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected bluemask darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately bluemask darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the bluemask darter.

BOULDER DARTER

Etheostoma wapiti

Boulder darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate boulder darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 59%. We estimate that boulder darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 74%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like boulder darters, than on species with greater adult survival, such as sturgeon. No life history studies have been conducted on this species. We assume the boulder darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years, and spawning occurs in spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

Since there are no data on boulder darters, we infer the boulder darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the boulder darter's fertility substantially. The boulder darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the boulder darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Boulder darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected boulder darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately boulder darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the boulder darter.

AMBER DARTER
Percina antesella

Amber darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate amber darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 63%. We estimate that amber darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 72%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species, like amber darters, that have fewer opportunities to spawn than on species with greater adult survival, such as sturgeon. Amber darter females are short-lived (approximately 3 years) and probably reproduce in only two years. The amber darter spawns from late fall to early spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

There are no data on the fecundity and survival of amber darters. We infer the amber darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the amber darter's fertility substantially. The amber darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the amber darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Amber darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected amber darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately amber darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the amber darter.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the amber darter include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of amber darters, and cause darters to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 63% and the reduction in the survival of young fish through the first year as high as 72%. These effects are estimated to be of a magnitude great enough to reduce numbers of amber darters. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the amber darter.

GOLDLINE DARTER
Percina aurolineata

Goldline darters exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate goldline darters exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 63%. We estimate that goldline darters exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 72%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like goldline darters, than on species with greater adult survival, such as sturgeon. The life history of the goldline darter is unknown. We assume the goldline darter is like other darters: females are short-lived (up to 4 years) and reproduce in no more than three years.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

There are no data on the fecundity and survival of goldline darters. We assume the goldline darter's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the amber darter's fertility substantially. The goldline darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the goldline darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Goldline darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected goldline darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately goldline darters are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the goldline darter.

CONASAUGA LOGPERCH

Percina jenkinsi

Conasauga logperch exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Conasauga logperch exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 63%. We estimate that Conasauga logperch exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 72%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Conasauga logperch, than on species with greater adult survival, such as sturgeon. Conasauga logperch females are short-lived (probably up to 4 years) and probably reproduce in no more than three years. The Conasauga logperch's spawning is probably limited to spring.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005). There are no data on the fecundity and survival of Conasauga logperch. We infer the Conasauga logperch's fecundity and survival are no greater than those estimated for the slackwater darter and holiday darter. Consequently, the reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the Conasauga logperch's fertility substantially. The Conasauga logperch's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Conasauga logperch's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Conasauga logperch may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Conasauga logperch population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Conasauga logperch are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Conasauga logperch.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Conasauga logperch include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of Conasauga logperch, and cause logperch to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 63% and the reduction in the survival of young fish through the first year as high as 72%. These effects are estimated to be of a magnitude great enough to reduce numbers of Conasauga logperch. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Conasauga logperch.

LEOPARD DARTER
Percina pantherina

Leopard darter exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate leopard darter exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 63%. We estimate that leopard darter exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through

the first year and that reduction could be as much as, but is not likely to be greater than, 72%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like leopard darter, than on species with greater adult survival, such as sturgeon. Leopard darter females are short-lived (up to 4 years) and reproduce in no more than three years. The leopard darter spawns from March to April.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

There is limited data on the fecundity of leopard darters and no information on survival. Robison's (1978) and Hartup's (2005) estimates of fecundity are not directly comparable because their methods differed. Nevertheless, the leopard darter's life history is similar to that of other darters, including the slackwater and holiday darter. The reductions we estimate in hatched eggs and survival of young fish through the first year would reduce the leopard darter's fertility substantially. The leopard darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the leopard darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Leopard darters may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected leopard darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately leopard darter are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the leopard darter.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the leopard darter include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of leopard darter, and cause darters to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 63% and the reduction in the survival of young fish through the first year as high as 72%. These effects are estimated to be of a magnitude great enough to reduce numbers of leopard darter. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the leopard darter.

ROANOKE LOGPERCH
Percina rex

Roanoke logperch exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were

available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Roanoke logperch exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 63%. We estimate that Roanoke logperch exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 72%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like most darters, than on species with greater adult survival, such as sturgeon. Roanoke logperch females live 5-6 years.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The Roanoke logperch is longer-lived and more fecund than the slackwater darter and holiday darter. Nevertheless, we anticipate the Roanoke logperch's fertility would be reduced substantially. The Roanoke logperch's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Roanoke logperch's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Roanoke logperch may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Roanoke logperch population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Roanoke logperch are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Roanoke logperch.

SNAIL DARTER
Percina tanasi

Snail darter exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate snail darter exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 63%. We estimate that snail darter exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 72%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration.

Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like snail darter, than on species with greater adult survival, such as sturgeon. Snail darter females are short-lived (up to 4 years) and reproduce in no more than three years. The snail darter spawns in early February through April and average fecundity is about 600 eggs.

Hartup (2005) conducted field research on the slackwater darter and holiday darter (*E. brevirostrum*) and developed species-specific estimates for fecundity, adult survival, and population sizes, then used these data to perform population viability analyses for the two species. Average slackwater darter annual fecundity was estimated as 92 and 197 eggs spawned, respectively, for one-batch and two-batch fecundity. Two-batch fecundity for the holiday darter was averaged 175 eggs spawned. Based on estimates of adult survival, Hartup (2005) estimated the adult fertility rate (number of female offspring surviving to year 1) would need to average 0.896 for the slackwater darter population to be stable and 0.818 for the holiday darter population to be stable. Assuming the slackwater darter population was stable, Hartup estimated the average proportion of spawned eggs needing to survive through the first year as 0.019 or 0.009, for one- and two-batch fecundity, respectively. For the holiday darter, the two-batch estimate was also 0.009. An elasticity analysis for the slackwater darter and holiday darter (*E. brevirostrum*) identified fertilities (defined as fecundity plus juvenile survival) as having the largest relative contribution to population growth rates for each species, as opposed to adult survival (Hartup 2005).

The snail darter has a similar lifespan but is more fecund than the slackwater darter and holiday darter. We anticipate the snail darter's fertility would be reduced substantially, but less so than the slackwater or holiday darter's fertility. The snail darter's fertility rates would be reduced based upon (a) reductions in numbers of hatched eggs, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the snail darter's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. snail darter may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected snail darter population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately snail darter are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the snail darter.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the snail darter include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of snail darter, and cause darters to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 63% and the reduction in the survival of young fish through the first year as high as 72%. These effects are estimated to be of a magnitude great enough to reduce numbers of snail darter. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the snail darter.

Poeciliidae

BIG BEND GAMBUSIA
Gambusia gaigei

Big Bend gambusia exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity and survival of young first-year fish (Table 13). Compared to control populations, we estimate Big Bend gambusia exposed to cyanide at the CCC could experience a substantial reduction in the number of young produced and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Big Bend gambusia exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Big Bend gambusia is a live-bearing fish, that is short lived (life expectancy 2 years; U.S. Fish and Wildlife Service 2009), has a prolonged reproductive period (5 to 8 months), and females may produce 50 or more young in the peak season. Such a life history strategy would appear to be highly sensitive to reductions in fecundity and survival of young fish. An elasticity analysis for other short-lived fish species indicates that population growth is, in fact, most influenced by changes in fecundity and juvenile survival (Vélez-Espino et al. 2006). Although gambusia are viviparous, we believe that our estimates of effects on Big Bend gambusia fecundity, which were based on studies with oviparous fish, are applicable. Koyo et al. (2000) described the dynamics of oocyte (egg) and embryonic development in *Gambusia affinis*, the mosquitofish. The process of oocyte development, fertilization, and "hatching" in viviparous and oviparous fishes are comparable, except that fertilization and "hatching" occur internally in viviparous species. Thus, the effects of cyanide on oocyte development described in the *Chronic Toxicity to Fish* section are likely to occur in Gambusia, with similar outcomes. In studies with oviparous fish, only the largest and most mature oocytes were spawned (ovulation) and capable of being fertilized. Similarly, in the viviparous gambusia only the largest and most mature oocytes are capable of being fertilized in the follicle. Thus reductions in the number of mature oocytes, by cyanide, would reduce the number of eggs capable of being fertilized, the number of embryos that are "hatched" (internally), and the number of live young that are "born". We would also expect predicted effects on juvenile survival to be the same for live-bearing species.

The reductions we estimate in the number of young produced and survival of young fish through the first year would reduce the Big Bend gambusia's potential recruitment substantially. The Big Bend gambusia's potential recruitment would be diminished because of (a) reductions in numbers of young produced, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the young that are produced in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Big Bend gambusia's reproduction by reducing the number of young produced by females, and reducing the survivorship of young fish in their first year. Big Bend gambusia may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Big Bend gambusia population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Big Bend gambusia are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Big Bend gambusia.

Formal Draft Biological Opinion.

SAN MARCOS GAMBUSIA
Gambusia georgei

San Marcos gambusia exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity and survival of young first-year fish (Table 13). Compared to control populations, we estimate San Marcos gambusia exposed to cyanide at the CCC could experience a substantial reduction in the number of young produced and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that San Marcos gambusia exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Little is known about the reproductive capabilities of San Marcos gambusia except that it is a live-bearing fish and two individuals kept in laboratory aquaria produced 12, 30, and 60 young although the largest clutch appeared to have been aborted and did not survive (Edwards et al. 1980). However, more is known about the closely related Big Bend gambusia (*Gambusia gaigei*) which we will use to provide insight into the reproductive traits of San Marcos gambusia. Big Bend gambusia is short lived (life expectancy 2 years; U.S. Fish and Wildlife Service 2009), has a prolonged reproductive period (5 to 8 months), and females may produce 50 or more young in the peak season. Such a life history strategy would appear to be highly sensitive to reductions in fecundity and survival of young fish. An elasticity analysis for other short-lived fish species indicates that population growth is, in fact, most influenced by changes in fecundity and juvenile survival (Vélez-Espino et al. 2006). Although gambusia are viviparous, we believe that our estimates of effects on San Marcos gambusia fecundity, which were based on studies with oviparous fish, are applicable. Koyo et al. (2000) described the dynamics of oocyte (egg) and embryonic development in *Gambusia affinis*, the mosquitofish. The process of oocyte development, fertilization, and “hatching” in viviparous and oviparous fishes are comparable, except that fertilization and “hatching” occur internally in viviparous species. Thus, the effects of cyanide on oocyte development described in the *Chronic Toxicity to Fish* section are likely to occur in Gambusia, with similar outcomes. In studies with oviparous fish, only the largest and most mature oocytes were spawned (ovulation) and capable of being fertilized. Similarly, in the viviparous gambusia only the largest and most mature oocytes are capable of being fertilized in the follicle. Thus reductions in the number of mature oocytes, by cyanide, would reduce the number of eggs capable of being fertilized, the number of embryos that are "hatched" (internally), and the number of live young that are "born". We would also expect predicted effects on juvenile survival to be the same for live-bearing species.

The reductions we estimate in the number of young produced and survival of young fish through the first year would reduce the San Marcos gambusia's potential recruitment substantially. The San Marcos gambusia's potential recruitment would be diminished because of (a) reductions in numbers of young produced, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the young that are produced in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the San Marcos gambusia's reproduction by reducing the number of young produced by females, and reducing the survivorship of young fish in their first year. San Marcos gambusia may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected San Marcos gambusia population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately San Marcos gambusia are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the San Marcos gambusia.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the San Marcos gambusia were not identified in the final rule designating critical habitat. We describe them here to include water of sufficient quality for the species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth sufficient for the critical habitat to serve its intended conservation function.

Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to the criterion concentration. This approval could adversely affect San Marcos gambusia critical habitat by diminishing the quality of water to the degree that it would impair individual reproduction and survival of San Marcos gambusia, and cause San Marcos gambusia to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of young produced could be as high 48% and the reduction in the survival of young fish through the first year as high as 56%. These effects are estimated to be of a magnitude great enough to reduce numbers of San Marcos gambusia. Continued approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in the San Marcos gambusia's extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the San Marcos gambusia.

CLEAR CREEK GAMBUSIA
Gambusia heterochir

Clear Creek gambusia exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity and survival of young first-year fish (Table 13). Compared to control populations, we estimate Clear Creek gambusia exposed to cyanide at the CCC could experience a substantial reduction in the number of young produced and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Clear Creek gambusia exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The Clear Creek gambusia is viviparous and females can produce several broods per year. In Clear Creek, females are reproductive for 7 months (March-September) and all stream reaches inhabited by Clear Creek gambusia have pregnant females during the midsummer reproductive period. In the lab (at 25 C), females produced up to 50 young every 42 days. However, in the cooler waters of Clear Creek (20 C) the estimated interbrood interval is 60 days. It's not clear how long Clear Creek gambusia live, but the closely related Big Bend gambusia (*Gambusia gaigei*) has a life expectancy of 2 years (U.S. Fish and Wildlife Service 2009). If the Clear Creek gambusia has similar longevity, their life history strategy (short-lived with extended reproductive periods and relatively low numbers of young per brood) would appear to be highly sensitive to reductions in fecundity and survival of young fish. An elasticity analysis for other short-lived fish species indicates that population growth is, in fact, most influenced by changes in fecundity and juvenile survival (Vélez-Espino et al. 2006). Although gambusia are viviparous, we believe that our estimates of effects on Clear Creek gambusia fecundity, which were based on studies with oviparous fish, are applicable. Koyo et al. (2000) described the dynamics of oocyte (egg) and embryonic development in *Gambusia affinis*, the mosquitofish. The process of oocyte development, fertilization, and "hatching" in viviparous and oviparous fishes are comparable, except that fertilization and "hatching" occur internally in viviparous species. Thus, the effects of cyanide on oocyte development described in the *Chronic Toxicity to Fish* section are likely to occur in Gambusia, with similar outcomes. In studies with oviparous fish, only the largest and most mature oocytes were spawned (ovulation) and capable of being fertilized. Similarly, in the viviparous gambusia only the largest and most mature oocytes are capable of being fertilized in the follicle. Thus reductions in the number of mature oocytes, by cyanide, would reduce the number of eggs capable of being fertilized, the number of embryos that are "hatched" (internally), and the number of live young that are "born". We would also expect predicted effects on juvenile survival to be the same for live-bearing species.

The reductions we estimate in the number of young produced and survival of young fish through the first year would reduce the Clear Creek gambusia's potential recruitment substantially. The Clear Creek gambusia's potential recruitment would be diminished because of (a) reductions in numbers of young produced, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the young that are produced in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Clear Creek gambusia's reproduction by reducing the number of young produced by females, and reducing the survivorship of young fish in their first year. Clear Creek gambusia may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Clear Creek gambusia population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Clear Creek gambusia are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Clear Creek gambusia.

PECOS GAMBUSIA

Gambusia nobilis

Pecos gambusia exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity and survival of young first-year fish (Table 13). Compared to control populations, we estimate Pecos gambusia exposed to cyanide at the CCC could experience a substantial reduction in the number of young produced and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Pecos gambusia exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Pecos gambusia bear live young. Females produce up to 40 young per brood during spring and summer. The interbrood interval for Pecos gambusia is unknown, but other species in the Poeciliidae family typically bear young every 1-2 months while reproductive. It's not clear how long Pecos gambusia live, but the closely related Big Bend gambusia (*Gambusia gaigei*) has a life expectancy of 2 years (U.S. Fish and Wildlife Service 2009). If the Pecos gambusia has similar longevity, their life history strategy (short-lived with extended reproductive periods and relatively low numbers of young per brood) would appear to be highly sensitive to reductions in fecundity and survival of young fish. An elasticity analysis for other short-lived fish species indicates that population growth is, in fact, most influenced by changes in fecundity and juvenile survival (Vélez-Espino et al. 2006). Although gambusia are viviparous, we believe that our estimates of effects on Pecos gambusia fecundity, which were based on studies with oviparous fish, are applicable. Koyo et al. (2000) described the dynamics of oocyte (egg) and embryonic development in *Gambusia affinis*, the mosquitofish. The process of oocyte development, fertilization, and "hatching" in viviparous and oviparous fishes are comparable, except that fertilization and "hatching" occur internally in viviparous species. Thus, the effects of cyanide on oocyte development described in the *Chronic Toxicity to Fish* section are likely to occur in Gambusia, with similar outcomes. In studies with oviparous fish, only the largest and most mature oocytes were spawned (ovulation) and capable of being fertilized. Similarly, in the viviparous gambusia only the largest and most mature oocytes are capable of being fertilized in the follicle. Thus reductions in the number of mature oocytes, by cyanide, would reduce the number of eggs capable of being fertilized, the number of embryos that are "hatched" (internally), and the number of live young that are "born". We would also expect predicted effects on juvenile survival to be the same for live-bearing species.

The reductions we estimate in the number of young produced and survival of young fish through the first year would reduce the Pecos gambusia's potential recruitment substantially. The Pecos gambusia's potential recruitment would be diminished because of (a) reductions in numbers of young produced, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the young that are produced in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Pecos gambusia's reproduction by reducing the number of young produced by females, and reducing the survivorship of young fish in their first year. Pecos gambusia may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Pecos gambusia population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Pecos gambusia are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Pecos gambusia.

GILA TOPMINNOW (including YAQUI TOPMINNOW)
Poeciliopsis occidentalis

Gila topminnow exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity and survival of young first-year fish (Table 13). Compared to control populations, we estimate Gila topminnow exposed to cyanide at the CCC could experience a substantial reduction in the number of young produced and that reduction could be as much as, but is not likely to be greater than, 48%. We estimate that Gila topminnow exposed to cyanide at the CCC could experience a substantial reduction in the survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 56%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

Topminnow bear live young and two broods are carried simultaneously (one further along in development than the other). Brood size is from 1 to 20, and gestation time is 24-28 days. Breeding season is from January to August, with some populations capable of breeding all year if temperatures and food availability are suitable. Life span is approximately 1 year; however, this varies with season of birth and fluctuations in environmental conditions in the habitat. Such a life history strategy (short-lived with extended reproductive periods and relatively low numbers of young per brood) would appear to be highly sensitive to reductions in fecundity and survival of young fish. An elasticity analysis for other short-lived fish species indicates that population growth is, in fact, most influenced by changes in fecundity and juvenile survival (Vélez-Espino et al. 2006). Although topminnows are viviparous, we believe that our estimates of effects on Gila topminnow fecundity, which were based on studies with oviparous fish, are applicable. Koyo et al. (2000) described the dynamics of oocyte (egg) and embryonic development in another live-baring species in the Poeciliidae family, *Gambusia affinis* (the mosquitofish). The process of oocyte development, fertilization, and "hatching" in viviparous and oviparous fishes are comparable, except that fertilization and "hatching" occur internally in viviparous species. Thus, the effects of cyanide on oocyte development described in the *Chronic Toxicity to Fish* section are likely to occur in Gambusia, with similar outcomes. In studies with oviparous fish, only the largest and most mature oocytes were spawned (ovulation) and capable of being fertilized. Similarly, in the viviparous gambusia only the largest and most mature oocytes are capable of being fertilized in the follicle. Thus reductions in the number of mature oocytes, by cyanide, would reduce the number of eggs capable of being fertilized, the number of embryos that are "hatched" (internally), and the number of live young that are "born". We would also expect predicted effects on juvenile survival to be the same for live-bearing species.

The reductions we estimate in the number of young produced and survival of young fish through the first year would reduce the Gila topminnow's potential recruitment substantially. The Gila topminnow's potential recruitment would be diminished because of (a) reductions in numbers of young produced, (b) reductions in young fish surviving through the first year, and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the young that are produced in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rates diminish survival through the first winter. These reductions in reproduction could be exacerbated further if reduced growth rates delay maturity or lengthen the time needed between batched spawning events.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Gila topminnow's reproduction by reducing the number of young produced by females, and reducing the survivorship of young fish in their first year. Gila topminnow may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Gila topminnow population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Gila topminnow are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Gila topminnow.

Salmonidae

BULL TROUT

Salvelinus confluentus

Bull trout exposed to cyanide at the criterion continuous concentration (CCC) are likely to experience reduced survival and reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the *Overview* section. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column. Exposure of bull trout to cyanide at the criterion maximum concentration (CMC) is likely to reduce survival.

Relatively few studies are available for estimating the magnitude of effects to the bull trout that could occur following exposure to cyanide at criterion concentrations. However, data are available to develop quantitative estimates of the effects of such exposure on bull trout fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate that bull trout exposed to cyanide at the CCC are likely to experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 87%. We estimate that bull trout exposed to cyanide at the CCC are likely to experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 90%. In addition, we estimate that juvenile bull trout exposed to cyanide at the CMC are likely to experience a substantial reduction in survival and that reduction could be as much as, but is not likely to be greater than, 99.9%. Effects of cyanide at the CMC on the survival of other bull trout life stages is expected to be of lesser magnitude.

Although these effects are based on modeled estimates, direct toxicity tests with other chemicals indicate that bull trout can be very sensitive to the adverse effects of water pollutants. Several studies have compared the sensitivity of bull trout to rainbow trout. Among the species considered in the cyanide criteria document (and in the BE), the rainbow trout was the most sensitive and, thus, was the species on which the acute and chronic cyanide criteria were based. Bull trout have been found to be less sensitive than rainbow trout to some metals (cadmium and zinc; Hansen et al. 2002a), but as sensitive to other metals (copper; Hansen et al. 2002b) and as sensitive or more sensitive to some herbicides (Fairchild et al. 2006). Dioxin, like cyanide, is a potent reproductive toxin. Cook et al. (2000) reported that, among fish that have been tested, bull trout was the most sensitive to dioxin. The bull trout was three times more sensitive than the lake trout (the next most sensitive species) and more sensitive than the brook trout or the rainbow trout.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

Rieman and McIntyre (1993) characterized the demographic and habitat requirements needed for bull trout conservation. As part of their evaluation they considered the consequences of habitat disturbances in terms of their potential impacts on reproduction, survival and extinction risk. Using a population model, they simulated four types of bull trout populations that differ in growth and maturation rates in order to capture the variation exhibited in natural populations, e.g. resident versus migratory life forms. For each simulated population they varied the survival from egg to age one to determine the level of mortality each population could sustain without collapsing. They next calculated the level of survival from egg to fry stage needed to attain those minimum, egg-to-age-one, survival rates. The required egg-to-fry survival rates were highest for slow growth/late maturity populations (0.25 – 0.49) and lowest for the fast growth populations (0.03 - 0.05). Rieman and McIntyre (1993) reported that egg-to-fry survival rates of 0.25 to 0.50 may approach the highest values possible in many streams. For slow growth populations, the egg-to-fry survival rates would have to be at or near the highest possible rates in order to sustain the population. Thus, reductions in egg-to-fry survival caused by exposure to cyanide would result in an egg-to-age-one survival rate that would not sustain the population. For fast growth populations, the egg-to-fry survival rates are lower than the highest possible rates, so these populations may be able to absorb additional mortality at this life stage. However, the large reduction in egg-to-fry survival caused by cyanide (90%), coupled with reductions in the number of eggs spawned, would likely reduce the egg-to-age-one survival rate below the minimum required to sustain the population.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the bull trout's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year by 87 to 90 percent. Bull trout may also experience effects on growth, swimming performance, condition, and development. Exposure to cyanide at the acute criterion could also substantially reduce the survivorship of juvenile bull trout. Because of the high magnitude of these effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An affected bull trout population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect that is likely to occur at the chronic criterion level, we conclude that ultimately bull trout are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the acute and chronic criteria at the rangewide scale is likely to reduce the reproduction, numbers, and distribution of the bull trout at the rangewide scale.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the bull trout include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC and CMC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of the bull trout, and cause adverse effects to bull trout growth, swimming performance, condition, and development. We estimate that implementation of the proposed action could reduce water quality conditions in critical habitat to the extent that a reduction in the number of hatched eggs could be as high 87% and a reduction in the survival of young fish through the first year could be as high as 90%. Reduction in survivorship of juvenile fish exposed at the CMC could be as high as 99.9%. These effects are estimated to be of a magnitude great enough to reduce numbers of bull trout using areas of critical habitat that support breeding and rearing areas for the bull trout. Approval of the CCC and CMC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation of affected populations from critical habitat containing cyanide at the CCC and CMC. Impacts to water quality resulting from management of cyanide to the CCC and CMC would diminish the ability of critical habitat to provide for conservation of the bull trout.

LITTLE KERN GOLDEN TROUT

Oncorhynchus aquabonita whitei

Little Kern golden trout exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Little Kern golden trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 60%. We estimate that Little Kern golden trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 69%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like darters, than on species with greater adult survival, such as sturgeon.

Little Kern golden trout appear to be intermediate. Females reach reproductive maturity in 3-4 years and live up to 9 years. While their fecundity appears relatively low, females contain between 41 and 65 eggs per year, they appear to reproduce each year after reaching maturity. Nevertheless, we expect that reductions in fecundity and survival of young fish through the first year would have a substantial population-level effect on Little Kern golden trout.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Little Kern golden trout's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Little Kern golden trout may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Little Kern golden trout population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Little Kern golden trout are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the Little Kern golden trout.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the Little Kern golden trout include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of the Little Kern golden trout, and cause trout to experience adverse effects to growth, swimming performance, condition, and development. We estimate the reduction in the number of hatched eggs could be as high 60% and the reduction in the survival of young fish through the first year as high as 69%. These effects are estimated to be of a magnitude great enough to reduce numbers of Little Kern golden trout. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the Little Kern golden trout.

APACHE TROUT

Oncorhynchus apache

Apache trout exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column. Exposure of Apache trout to cyanide at the criterion maximum concentration (CMC) is likely to reduce survival.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Apache trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 87%. We estimate that Apache trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 90%. In addition, we estimate that juvenile Apache trout exposed to cyanide at the CMC are likely to experience a substantial reduction in survival and that reduction could be as much as, but is not likely to be greater than, >99.9%. Effects of cyanide at the CMC on the survival of other Apache trout life stages is expected to be of lesser magnitude.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Apache trout, than on species with greater adult survival, such as sturgeon.

We expect that reductions in fecundity and survival of young fish through the first year would have a greater population-level effect on Apache trout than on longer-lived species, because the majority of Apache trout females spawn only twice in their lifetime. It is estimated that female Apache trout produce between 72 and 1,083 eggs per female, depending on size class. If we reduce the number of hatched eggs by 87% and reduce the survival of fish in their first year by 90% then few fish will survive to the adult stage. If we consider the effects additive, then only 0.2 fish will survive to the adult stage. The combined effect could be less than additive (i.e. between 0.2 and 0.6) if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. The reductions we estimate in hatched eggs and juvenile survivorship could reduce, by an order of magnitude, the number of individuals surviving to adulthood. These reductions in reproduction could be exacerbated further if reductions in juvenile growth rates result in smaller females reproducing in a given year. As we noted in the Status/Baseline section, the number of eggs female Gila trout produce is proportional to their mass.

Apache trout are considered a subspecies of Gila trout and share a very similar life history and the same threats. Brown et al. (2001) performed a population viability analysis for the Gila trout to explore potential management strategies. A base model was constructed to be used as a benchmark for comparison of the effects of different management strategies. Fecundity was estimated from the overall mean count of ova from field-stripped fish. Among their findings, the model was sensitive to large changes in fecundity. Halving fecundity significantly increased the probability of extinction as compared to the base model. We would anticipate a similar response pattern for Apache trout. Exposure to cyanide at the chronic criterion affects not only fecundity, but also hatchability, and juvenile survival of Apache trout. The combined effects could be much greater than what was analyzed by Brown et al. (2001).

Brown et al. (2001) identified catastrophic events as having a much larger influence on the viability of Gila trout than population size, fecundity, or population structure. This conclusion applied to the relative importance of each variable separately and not in combination. Any combination of variables would pose a greater risk to continued viability than any one risk alone. The reductions in reproduction and survival we estimate could occur would diminish the ability of the Apache trout's populations to recover from a catastrophic event, such as a forest fire or drought, and would increase the risk of extirpation of an effected population. Cyanide toxicity could result in the extant population persisting at a reduced population size prior to a catastrophic event. If the local population initially survives the catastrophic event, reductions in reproduction and survival could lengthen the time to recover and leave the population more vulnerable to extirpation.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Apache trout's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Apache trout may also experience effects on growth, swimming performance, condition, and development. Exposure to cyanide at the acute criterion could also substantially reduce the survivorship of juvenile Apache trout. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Apache trout population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Apache trout are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the acute and chronic criteria could reduce the reproduction, numbers, and distribution of the Apache trout.

LAHONTAN CUTTHROAT TROUT
Oncorhynchus clarkii henshawi

Lahontan cutthroat trout exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the *Overview* section. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column. Exposure of Lahontan cutthroat trout to cyanide at the criterion maximum concentration (CMC) is likely to reduce survival.

Relatively few studies are available for estimating the magnitude of effects that could occur following exposure of this species to cyanide at criterion concentrations. However, data are available to develop quantitative estimates of the effects of such exposure on Lahontan cutthroat trout fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate that Lahontan cutthroat trout exposed to cyanide at the CCC are likely to experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 80%. We estimate that Lahontan cutthroat trout exposed to cyanide at the CCC are likely to experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 85%. In addition, we estimate that juvenile Lahontan cutthroat trout exposed to cyanide at the CMC are likely to experience a substantial reduction in survival and that reduction could be as much as, but is not likely to be greater than, 43%. Effects of cyanide at the CMC on the survival of other life stages of Lahontan cutthroat trout is expected to be of lesser magnitude.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also reduce productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section above on *Population Responses to Reductions in Fecundity and Juvenile Survival*, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Lahontan cutthroat trout where the majority of females die after their first spawning, than on species with greater adult survival, such as sturgeon.

In summary, exposure to cyanide concentrations at the chronic criterion are likely to substantially reduce the Lahontan cutthroat trout's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Lahontan cutthroat trout may also experience adverse effects on growth, swimming performance, condition, and development. Exposure to cyanide at the acute criterion could also substantially reduce the survivorship of juvenile Lahontan cutthroat trout. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An affected Lahontan cutthroat trout population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect that is likely to occur at the criterion concentration, we conclude that ultimately Lahontan cutthroat trout are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the acute and chronic criteria at the rangewide scale is likely to reduce the reproduction, numbers, and distribution of the Lahontan cutthroat trout at the rangewide scale.

PAIUTE CUTTHROAT TROUT

Oncorhynchus clarkii seleniris

Paiute cutthroat trout exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Paiute cutthroat trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 60%. We estimate that Paiute cutthroat trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 69%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Paiute cutthroat trout, than on species with greater adult survival, such as sturgeon.

We expect that reductions in fecundity and survival of young fish through the first year would have a greater population-level effect on Paiute cutthroat trout than on longer-lived species, because female Paiute cutthroat trout spawn few times in their lifetime.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Paiute cutthroat trout's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Paiute cutthroat trout may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Paiute cutthroat trout population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Paiute cutthroat trout are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the Paiute cutthroat trout.

GREENBACK CUTTHROAT MOUNTAIN TROUT

Oncorhynchus clarki stomias

Greenback cutthroat trout exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Greenback cutthroat trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 60%. We estimate that Greenback cutthroat trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 69%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn than on species with greater adult survival, such as sturgeon.

The reductions we estimate in hatched eggs and survival of young fish through the first year could reduce substantially the number of individuals surviving to adulthood. These reductions in reproduction could be exacerbated further if reductions in juvenile growth rates result in smaller females reproducing in a given year. The number of eggs female greenback trout produce is proportional to their mass.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Greenback cutthroat trout's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Greenback cutthroat trout may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Greenback cutthroat trout population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Greenback cutthroat trout are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the Greenback cutthroat trout.

GILA TROUT

Oncorhynchus gilae

Gila trout exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Gila trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 60%. We estimate that Gila trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 69%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Gila trout, than on species with greater adult survival, such as sturgeon.

We expect that reductions in fecundity and survival of young fish through the first year would have a greater population-level effect on Gila trout than on longer-lived species, because the majority of Gila trout females spawn only twice in their lifetime. Brown et al. (2001) estimated that female fish produced between 62 and 197 eggs per spawn, depending on size class. In the Gila Trout Recovery Plan (Service 2003) the Service estimated that for every 100 eggs that hatch about half will survive to the juvenile life stage. Of those approximately 50 fish, only about 6 will survive to the subadult stage and of those 6 subadults, only 2 will survive to the adult life stage. This estimate provides a good reference for characterizing the effects of chronic cyanide toxicity. If we simply reduce the number of hatched eggs by 60% and assume survivorship remains the same for other transitions, then only 0.7 fish will survive to the adult stage. If we reduce the survival of fish in their first year by 69% and assume survivorship remains the same for other transitions, then only 0.6 fish will survive to the adult stage. If we consider the effects additive, then only 0.2 fish will survive to the adult stage. The combined effect could be less than additive (i.e. between 0.2 and 0.6) if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. The reductions we estimate in hatched eggs and juvenile survivorship could reduce, by an order of magnitude, the number of individuals surviving to adulthood. These reductions in reproduction could be exacerbated further if reductions in juvenile growth rates result in smaller females reproducing in a given year. As we noted in the Status/Baseline section, the number of eggs female Gila trout produce is proportional to their mass.

Brown et al. (2001) performed a population viability analysis for the Gila trout to explore potential management strategies. A base model was constructed to be used as a benchmark for comparison of the effects of different management strategies. Fecundity was estimated from the overall mean count of ova from field-stripped fish. Among their findings, the model was sensitive to large changes in fecundity. Halving fecundity significantly increased the probability of extinction as compared to the base model. Exposure to cyanide at the chronic criterion affects not only fecundity, but also hatchability, and juvenile survival. The combined effects could be much greater than what was analyzed by Brown et al. (2001).

Brown et al. (2001) identified catastrophic events as having a much larger influence on the viability of Gila trout than population size, fecundity, or population structure. This conclusion applied to the relative importance of each variable separately and not in combination. Any combination of variables would pose a greater risk to continued viability than any one risk alone. The reductions in reproduction and survival we estimate could occur would diminish the ability of the Gila trout's populations to recover from a catastrophic event, such as a forest fire or drought, and would increase the risk of extirpation of an effected population. Cyanide toxicity could result in the extant population persisting at a reduced population size prior to a catastrophic event. If the local population initially survives the catastrophic event, reductions in reproduction and survival could lengthen the time to recover and leave the population more vulnerable to extirpation. Brown et al. (2001) found the Gila trout's extinction risk was sensitive to the number of populations. For example, increasing the number of Gila trout populations in the model from 10 to 16 significantly reduced risk of extinction by 15%.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Gila trout's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Gila trout may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Gila trout population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Gila trout are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the Gila trout.

ATLANTIC SALMON
Salmo salar
Gulf of Maine Distinct Population Segment

Atlantic salmon exposed to cyanide at the chronic criterion concentration are likely to experience reduced survival, reproduction, and may also experience effects on growth, swimming performance, condition, and development, as described above in the Effects Overview. The effects of chronic toxicity could be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. However, data were available to develop quantitative estimates of the effects on fecundity, hatching success, and survival of young first-year fish (Table 13). Compared to control populations, we estimate Atlantic salmon exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than, 36%. We estimate that Atlantic salmon exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than, 41%.

As noted previously, the young first-year fish that do survive could experience reduced growth rates which would increase their vulnerability to a host of potential stressors, including temperature, flow, and inter- and intraspecific competition for food and cover. It could also delay reproductive maturity and productivity.

The reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the chronic criterion concentration. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section on Population Level Effects, we noted that reductions in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to spawn, like Atlantic salmon, than on species with greater adult survival, such as sturgeon.

Atlantic salmon typically live about 5 years, spending 1-3 years as freshwater smolts prior to migrating to the ocean as adults where they will develop in about 2-3 years into mature salmon and then return to their natal freshwater rivers to spawn. We expect that reductions in fecundity and survival of young fish through the first year would have a greater population-level effect on Atlantic salmon than on longer-lived species, because the majority of Atlantic salmon females spawn only once in their lifetime. Females deposit 7,000-8,000 eggs per spawn. Studies in Maine indicate less than 10% of the eggs spawned will survive to emerge as feeding fry. The Gulf of Maine DPS has declined to critically low levels. Adult returns, juvenile abundance estimates and survival have continued to decline since the listing. In 2004, total adult returns to the eight rivers still supporting wild Atlantic salmon populations within the DPS were estimated to range from 60 to 113 individuals. No adults were documented in three of the eight rivers. Declining smolt production has also been documented in recent years, despite fry stocking. For example, from 1996 through 1999, annual smolt production in the Narraguagus River was estimated to average about 3,000 fish. Smolt production declined significantly in 2000 and for the past three years has averaged only about 1,500 fish per year. Overwinter survival in the Narraguagus River since 1997 has only averaged about 12%, approximately half of the survival rate of previous years and significantly less than the 30% previously accepted for the region. These estimates provide a good reference for characterizing the effects of chronic cyanide toxicity.

Based on a minimum of 7000 eggs per spawn, if we simply reduce the number of hatched eggs by 36% assuming overwinter survival is at the previously accepted level of 30%, then only 134 smolts (as opposed to 210) will survive through the first year. If we reduce the smolt survivorship by 41% then only 124 fish will survive through the first year. If we consider the effects additive then only 79 smolts will survive through the first year. The combined effect could be less than additive (i.e. between 79 and 134) if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. If we consider that in some rivers survival is as low as 12%, the effects of cyanide at the CCC could result in as little as 32 smolts per spawn surviving through the first year.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Atlantic salmon's reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and reducing the survivorship of young fish in their first year. Atlantic salmon may also experience effects on growth, swimming performance, condition, and development. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Atlantic salmon population's decline could stabilize at a reduced absolute population number or could continue to decline until it is extirpated. Based upon the magnitude of effect we anticipate could occur, we conclude ultimately Atlantic salmon are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion likely reduces the reproduction, numbers, and distribution of the Atlantic salmon.

7.2 Effects to Invertebrates

Direct effects to invertebrates are anticipated for two species within the genus Gammaras, the Illinois cave amphipod (*Gammarus acherondytes*), and Noel's amphipod (*Gammarus desperatus*). No direct effects are anticipated for other invertebrate species (see Appendix B for screening methodology).

ILLINOIS CAVE AMPHIPOD
Gammarus acherondytes

NOEL'S AMPHIPOD
Gammarus desperatus

EC_A values for the Illinois cave amphipod and Noel's amphipod were derived using EPA's Interspecies Correlation Estimate (ICE) model for the genus *Gammaras* with *Daphnia magna* as the surrogate species (EPA 2003b). The lower 95% confidence interval of the LC_{50} value was divided by the cyanide-specific value of 1.21 to derive the acute EC_A (24.5 ug/L) and by the invertebrate ACR to derive the chronic EC_A (3.92 ug/L). The proposed chronic criterion (5.2 ug/L) exceeds the chronic EC_A for these species.. On that basis, exposure of these amphipods to cyanide concentrations at these criteria is likely to cause adverse effects.

Two, acute 96-hour LC_{50} values were found in the literature for species within the genus *Gammarus*: *G. fasciatus* (LC_{50} value = 903 ug/L) and *G. pseudolimnaeus* (142.9 ug/L) (Ewell et al 1986, Smith et al.1978). Smith et al. (1978) also derived a lethal threshold concentration at which the first signs of lethality appear of 74 ug/L for *G. pseudolimnaeus*. Although the measured LC_{50} values are an order of magnitude greater than that derived using the lower 95% confidence interval of the ICE estimate (29.6 ug/L), it is not unusual for species that are closely related taxonomically to have widely variable sensitivities to particular toxicants. For cyanide, numerous LC_{50} values for species within the family Cyprinidae were measured and the results varied by a factor of 18 between the most and least sensitive species. The measured LC_{50} values for *Gammarus* represent the only two such estimates known for this group, a large genus comprised of over 200 species.

In the only study in which chronic toxicity was tested in freshwater amphipods (Smith et al.1979, Oseid and Smith 1979), *G. pseudolimnaeus* was found to be 6 to 25 times more sensitive to cyanide than the isopod species *Asellus communis* for acute and chronic endpoints. When *G. pseudolimnaeus* was housed alone, overall mass (free individuals plus eggs and young) in tanks containing cyanide concentrations of 16 ug/L and 32 ug/L and above was significantly lower than controls. Smith et al. (1979) and Oseid and Smith (1979) did not believe that reductions in the 16 ug/L tank were due to cyanide exposure, but provided no alternate explanation for the decline. Effects of cyanide exposure were heightened for *G. pseudolimnaeus* when exposed concurrently with *A. communis* in the same test tank. While the aggressive and competitive *G. pseudolimnaeus* greatly out-competed *A. communis* in tanks containing the control or 4 ug/L treatment, a significant shift took place in tanks with concentrations of 9 ug/L or greater cyanide. When exposed to cyanide in tanks containing *A. communis*, total mass of *G. pseudolimnaeus* was reduced 63% compared to controls at 9 ug/L and 97% at 21 ug/L, the next highest concentration tested. This impact occurred at concentrations lower than when *G. pseudolimnaeus* was exposed alone. Smith et al. (1979) and Oseid and Smith (1979) speculated that decreased survival of *G. pseudolimnaeus* may have been caused by predation by *A. communis.* Thus, cyanide not only exerts direct effects on *G. pseudolimnaeus*, but can shift the competitive advantage to more tolerant species in mixed communities. Smith et al. (1979) and Oseid and Smith (1979) concluded that where a mixed community exists, Gammarus is likely to be excluded in the presence of cyanide pollution. No effects were seen at cyanide concentrations of 4 ug/L and below. The differential sensitivity reported by Smith et al. (1979) and Oseid and Smith (1979) was supported by Ewell et al. (1986), who did not look at chronic effects, but found that acute sensitivity of *G. fasciatus* was nearly twice that of *A. intermedius.*

These results may be applicable to the listed amphipods, as interspecific interactions with other amphipod and isopod species are believed to be important factors in the biology of Gammerids. The Illinois cave amphipod was regularly recorded in caves containing several species of amphipod and isopod species, as well as other invertebrates and fish (Webb et al. 1998). Noel's amphipod is part of a complex of Gammerus species that co-occur together in the Pecos River Basin of New Mexico and Texas. Resource partitioning according to substrate and water depth has been examined for these species, but is not fully understood. While fungi and detritus have been suggested as important food sources for amphipods and isopods, it is also recognized that these species can be predators on other species and even exhibit cannibalistic behaviors in high density situations.

Both the chronic EC_A and the chronic data available indicate that cyanide at criteria concentrations may result in the loss of individuals of the Illinois cave amphipod and Noel's amphipod. Effects of cyanide appear to be exasperated when amphipods are exposed in situations of resource competition or possibly predation, when a population may experiences a loss of mass that is greater than 0% and less than 63%. The Illinois cave amphipod and Noel's amphipod are known to occur in systems that contain other invertebrate species that may occupy a similar niche and exhibit more tolerance to cyanide, making these two species more susceptible to the effects of cyanide under these conditions. Because both of these species exist in populations that are geographically isolated from one another, the ability of these amphipods to recolonize habitat is limited. For these reasons, exposure of these two species to cyanide concentrations at or near the proposed chronic criterion value is likely to contribute to the elimination of a population unit. The loss of a population unit for either amphipod species would substantially reduce its reproduction, numbers, or distribution at the rangewide scale.

7.3 Effects to Mussels

Listed mussels are not anticipated to experience direct effects of cyanide at criteria concentrations (see Appendix B for screening metholodoly and discussion of direct effects to museels), but were screened in based on indirect effects to host fish species For most mussel species, transformation on a host fish is a required element of their life cycle that cannot be bypassed. Host fish availability and density have been found to be significant factors influencing mussel persistence in particular habitats (Haag and Warren 1998). Contact between glochidia and suitable host fishes is a low-probability event even in healthy populations (Neves et al. 1997), and despite the large number of glochidia produced by an individual mussel, infestation rates tend to be very low (Haag and Warren 1999, Layzer et al. 2003). Host fish generalists, whose glochidia can transform on multiple species, release glochidia in large mucous webs that entangle fish indiscriminately. Host fish specialists, relying on one or few species to transform, tend to employ specialized lures that mimic prey items of host fish (Strayer et al. 2004). This strategy likely reduces the probability of infestation in unsuitable fish hosts, which can result in immune system incompatibility, and discharge of glochidia. Glochidia that fail to infest a suitable host or that have been sloughed off by an unsuitable host will not transform to the adult stage and will survive only as long as their energy reserves last, from a few days to up to two weeks. The odds of an individual glochidium infesting a host and completing transformation have been estimated at 4 in 100,000. Given those odds, any reduction of host fish species populations caused by exposure to cyanide at either the acute or chronic criterion levels is likely to cause an adverse effect to the listed mussel species.

In addition, mussel glochidia infested on fish are completely parasitic and are dependent on the host for oxygen, nutrition, and overall survival. Once contact is made with a suitable host, successful glochidia encyst on the gills, fins, or skin for a period of several weeks. Any mortality to host fish during this period will necessarily result in mortality to infested glochidia. Thus, the host fish become surrogates for the listed mussels during the parasitic stage.

Estimating Acute and Chronic EC_A Values for Host Fish Species

In estimating risks to glochidia host species, the Service derived acute and chronic assessment effects concentrations (EC_A) estimates according to the prioritization described in Figures 2 and 4 of the 2004 Draft Methodology for Conducting Biological Evaluations of Aquatic Life Criteria (Table 15). Although host fish species identified for listed mussels were themselves not listed species, the host fish species is an obligate part of the mussel lifecycle to which it has a parasitic dependence while attached, and is treated with the same conservatism as a listed species.

1. When acute toxicity data were available for a host species, the species mean acute value from Table 1 of the BE was used to derive the acute EC_A, and, when appropriate, the chronic EC_A. However, when only one LC_{50} was available for a species, the lower 95% confidence interval of the LC_{50}, when available, was used to derive the EC_A.
2. For yellow perch (*Perca flavescens*), the LC_{50} reported in Table 1 of the BE could not be reproduced from the original source, so an acute EC_A value was calculated from the mean of all 96-hour LC_{50} values (converted to free CN) reported in Smith et al. (1978).
3. For fish with no species-specific data, ICE models were derived at the lowest taxonomic level with adequate data (EPA 2003b). The lower 95% confidence interval of the predicted LC_{50} was used to determine EC_A values.
4. For species for which no ICE models were available, the 5^{th} percentile LC_{50} from the appropriate SSD was used in lieu of the mean LC_{50}

All acute EC_A values were calculated by division of the LC_{50} by the cyanide-specific 1.21 factor derived by the Service.

Table 15. Surrogate taxa used to estimate LC_{50} values for evaluation of to host fish effects

Surrogate taxa used to estimate host fish LC_{50}	LC_{50} (ug CN/L)	Acute EC_A (ug/L)	Chronic EC_A (ug/L)
Actinopterygii (class)	66.5[1]	54.93	2.86
Cypriniformes (order)	84.5[1]	69.88	3.64
Cyprinidae (family)	101.7[2]	84.07	4.38
Pimephales promelas (species)	138.4[3]	114.38	5.96
Cyprinodontiformes			
Poecilia reticulate (species)	187.8[3]	155.21	8.09
Perciformes (order)	90.8[1]	75.04	3.91
Centrarchidae (family)	73.2[2]	60.46	3.15
Lepomis (genus)	89.5[2]	73.99	3.85
Lepomis cyanellus (species)	126.2[2]	104.03	5.43
Lepomis macrochirus (species)	126.1[3]	104.21	5.43
Micropterus (genus)			
Micropterus. salmoides (species)	95.7[4]	79.09	4.12
Pomoxis (genus)			
Pomoxis nigromaculatus (species)	84.5[4]	70.66	3.64
Percidae (family)	42.3[2]	34.97	1.82
Etheostoma (genus)	40.0[2]	33.07	1.72
Perca (genus)			
Perca flavescens (species)	93.3[5]	77.11	4.02
Salmoniformes			
Salmo salar (species)	90[3]	74.38	3.88
Salmo trutta (species)	54.9[2]	45.38	2.36
Siluriformes (order)			
Ictaluridae (family)	182.8[2]	151.06	7.87
Ictalurus punctatus (species)	190.3[2]	83.83	8.20

[1] LC_{50} based on 5[th] percentile estimate from species sensitivity distribution (SSD), Table 2 – Cyanide BE.
[2] LC_{50} estimate based on lower bound of the 95% CI from ICE model (Appendix D)
[3] LC_{50} based on measured value from the Cyanide BE (Table 1);
[4] LC_{50} based on lower bound of the 95% CI of the measured value from the Cyanide BE (Table 1);
[5] LC_{50} based on mean measured value from Smith et al 1978

Effects to Listed Mussel Species

Table 16 identifies host fish species for all currently listed mussel species, with estimates of their acute and chronic EC_A values, and predicted maximum effects to fecundity and juvenile survival. We used listed species accounts, NatureServe, recent FWS Recovery Plans and 5-Year Reviews, the open literature, and consultation with species experts to update and expand the list of known hosts for these species.

Two of the listed mussels, the fat pocketbook (*Potamilus capax)* and the scaleshell mussel (*Leptodea leptodon)*, have an identified obligate relationship with a single host, the freshwater drum (*Aplodinotus grunniens)*. The remaining listed mussels either lack a known obligate relationship with a host fish or host fish relationships are unknown. Of the 300 identified North American mussel species, there have been no host fish species identified for a significant percentage, and it is likely that the complement of host fish remains incomplete for many other species. Individual mussel species have been found to have up to 25 fish species known to serve as suitable hosts, though host fish specificity to one species or a group of species related by taxonomy or food guild can be common in mussels. To assess effects to these species, we looked at the range of all fish species that have been identified as hosts for listed mussels (Table 17). We then totaled the number of species identified as hosts in each family, as well as the frequency that each family was represented as a host fish for a listed mussel species, as identified in Table 15. Fish within families that may be sensitive to cyanide at concentrations below acute or chronic criteria, accounted for 96% of the species diversity (Table 18), and 98% of all fish identified as hosts for listed mussels (Table 19). Extrapolating these results to fish with no known hosts or a potentially incomplete list of hosts, we can assume that any mussel is likely to have at least one host fish for which adverse effects cannot be ruled out at exposure to cyanide at criteria concentrations Therefore, for mussels lacking a known obligate relationship with a host fish, in the absence of information to the contrary, it was assumed that the species was likely to have at least one host fish that is sensitive to cyanide at the criteria concentrations, whether one is currently identified or not. On that basis, these mussel species are likely to be adversely affected at cyanide criteria concentrations due to potential reductions in host fish abundance.

Table 16. Cyanide sensitivity of fish species that serve as hosts for glochidia of listed mussels.

Listed Mussel	Host Fish	Acute EC_A (ug/L)	Chronic EC_A (ug/L)	Source/surrogate taxa for LC_{50} values (Table 15)	Estimated reduction in fecundity and larvae/juvenile survival of host fish due based on surrogate species data sets (Appndix F)		
					Fathead Minnow (Reduction in the mean number of eggs spawned)	Brook Trout (Reduction in the mean number of eggs spawned)	Bluegill (reduction in larvae/juvenile survival)
MUSSLES WITH OBLIGATE HOST FISH							
Fat Pocketbook *Potamilus capax*	Freshwater drum	75.04	3.91	Perciformes (order)	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)
Scaleshell Mussel *Leptodea leptodon*	Freshwater drum	75.04	3.91	Perciformes (order)	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)
MUSSLES WITH NO KNOWN OBLIGATE HOST FISH							
Cumberland Elktoe *Alasmidonta atropurpurea*	Rainbow darter Redline darter Fantail darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Banded sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Rock bass	60.46	3.15	*Centrarchidae* (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Northern hogsucker	69.88	3.64	Cypriniformes (order)	39% (28%, 49%)	26% (0%, 54%)	44% (0%, 80%)
	Longear sunfish	73.99	3.85	*Lepomis* (genus)	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)
	Whitetail shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Dwarf Wedgemussel *Alasmidonta heterodon*	Tesselated darter Johnny darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Roanoke darter	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Mottled sculpin Slimy sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Atlantic salmon	74.38	3.88	*Salmo salar* (species)	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)
Appalachian Elktoe *Alasmidonta raveneliana*	Banded sculpin Mottled sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Fat Threeridge *Amblema neislerii*	Blackbanded darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Redear sunfish	73.99	3.85	*Lepomis* (genus)	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)

	Largemouth bass	79.09	4.12	*Micropterus salmoides* (species)	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
	Weed shiner	84.04	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
	Bluegill	104.21	5.43	*Lepomis macrochirus* (species)	---	---	---
Ouachita Rock Pocketbook *Arkansia wheeleri*	White crappie	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Warmouth Smallmouth bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Black crappie	70.66	3.64	*Pomoxis nigromaculatus* (species)	39% (24%, 49%)	26% (0%, 54%)	44% (0%, 80%)
	River carpsucker	69.88	3.64	Cypriniformes (order)	39% (28%, 49%)	26% (0%, 54%)	44% (0%, 80%)
	Longear sunfish Orangespotted sunfish	73.99	3.85	*Lepomis* (genus)	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)
	Freshwater drum	75.04	3.91	Perciformes (order)	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)
	Largemouth bass	79.67	4.12	*Micropterus salmoides* (species)	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
	Dusky shiner Bleeding shiner Golden shiner Emerald shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
	Green sunfish	104.03	5.43	*Lepomis cyanellus* (species)	---	---	---
	Bluegill	104.21	5.43	*Lepomis macrochirus* (species)	---	---	---
Birdwing Pearlymussel *Conradilla caelata*	Greenside darter Tennessee snubnose darter Banded darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
Fanshell *Cyprogenia stegaria*	Banded darter Greenside darter Tennessee snubnose darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Blotchside logperch Logperch Tangerine darter	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Mottled sculpin Banded sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Dromedary perlymussel *Dromus dromas*	Fantail darter Banded darter Tangerine darter Greenside darter Tennessee snubnose darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)

	Gilt darter Channel darter Logperch Blotchside logperch	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Black sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Chipola Slabshell *Elliptio chipolaensis*	Bluegill	104.21	5.43	*Lepomis macrochirus* (species)	---	---	---
Tar River Spinymussel *Elliptio steinstansana*	Bluehead chub Satinfin shiner White shiner Pinewoods shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Purple Bankclimber *Elliptoideus sloatianus*	Blackbanded darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Eastern mosquitofish	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Greater Jumprock	69.88	3.64	Cypriniformes (order)	39% (28%, 49%)	26% (0%, 54%)	44% (0%, 80%)
	Guppy	155.21	8.09	*Poecilia reticulate* (species)	---	---	---
Cumberlandian Combshell *Epioblasma brevidens*	Wounded darter Redline darter Bluebreast darter Snubnose darter Greenside darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Logperch	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Banded sculpin Mottled sculpin Black sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Oyster Mussel *Epioblasma capsaeformis*	Wounded darter Redline darter Bluebreast darter Greenside darter Fantail darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Dusky darter	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Banded sculpin Black sculpin Mottled sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Curtis Pearly Mussel	Rainbow darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)

Epioblasma florentina curtisii	Banded sculpin Mottled sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Yellow Blossom (Pearlymussel) *Epioblasma florentina florentina*	Not known						
Tan Riffleshell *Epioblasma florentina walkeri*	Fantail darter Greenside darter Redline darter Snubnose darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Banded sculpin Mottled sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Upland Combshell *Epioblasma metastriata*	Not known						
Catspaw (Purple Cat's Paw Pearlymussel) *Epioblasma obliquata obliquata*	Blackside darter Logperch	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Mottled sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Rock bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Stonecat	151.06	7.87	Ictaluridae (family)	---	---	---
White Catspaw Pearlymussel *Epioblasma obliquata perobliqua*	Not known						
Southern Acornshell *Epioblasma othcaloogensis*	Not known						
Southern Combshell *Epioblasma penita*	Not known						
Tubercled Blossom *Epioblasma torulosa torulosa*	Not known						
Turgid Blossom *Epioblasma turgidula*	Not known						
Green Blossom *Epioblasma torulosa gubernaculums*	Not known						
Northern Riffleshell *Epioblasma turulosa rangiana*	Banded darter Bluebreast darter Johhny darter Iowa darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Brown trout	45.38	2.36	*Salmo trutta* (species)	55% (24%, 61%)	34% (9%, 56%)	63% (23%, 84%)

	Banded sculpin Mottled sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Shiny Pigtoe *Fusconaia cor*	Whitetail shiner Common shiner Warpaint shiner Telescope shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Fine-rayed Pigtoe *Fusconaia cuneolus*	Mottled sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	River chub Central stoneroller Telescope shiner Tennessee shiner Whitetail shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
	Fathead minnow	114.38	5.96	*Pimephales promelas* (species)	---	---	---
Cracking Pearlymussel *Hemistena lata*	Not known						
Pink Mucket *Lampsilis abrupta*	Walleye Sauger	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Spotted bass Smallmouth bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Freshwater drum	75.04	3.91	Perciformes (order)	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)
	Largemouth bass	79.67	4.12	*Micropterus salmoides* (species)	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
Fine-lined Pocketbook *Lampsilis altilis*	Redeye bass Spotted bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Largemouth bass	79.09	4.12	*Micropterus salmoides* (species)	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
	Green sunfish	104.03	5.43	*Lepomis cyanellus* (species)	---	---	---
Higgins Eye *Lampsilis higginsii*	Sauger Walleye	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Smallmouth bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Black crappie	70.66	3.64	*Pomoxis nigromaculatus* (species)	39% (24%, 49%)	26% (0%, 54%)	44% (0%, 80%)
	Freshwater drum	75.04	3.91	Perciformes (order)	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)
	Largemouth bass	79.09	4.12	*Micropterus salmoides*	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
	Yellow perch		4.02	*Perca flavescens* (species)	35% (24%, 46%)	23% (0%, 53%)	38% (0%, 79%)

Orangenacre Mucket *Lampsilis perovalis*	Chain pickerel	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Redeye bass Spotted bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Largemouth bass	79.67	4.12	*Micropterus salmoides* (species)	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
Arkansas Fatmucket *Lampsilis powelli*	Spotted bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Largemouth bass	79.09	4.12	*Micropterus salmoides* (species)	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
Speckled Pocketbook *Lampsilis streckeri*	Warmouth Spotted bass Smallmouth bass Shadow bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Longear sunfish	73.99	3.85	*Lepomis* (genus)	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)
	Green sunfish	104.03	5.43	*Lepomis cyanellus* (species)	---	---	---
	Bluegill	104.21	5.43	*Lepomis macrochirus* (species)	---	---	---
Shinyrayed pocketbook *Lampsilis subangulata*	Eastern mosquitofish	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Spotted bass	60.46	3.15	Centrarchidae (family)	44% (35%, 53%)	28% (0%, 54%)	52% (0%, 81%)
	Largemouth bass	79.09	4.12	*Micropterus salmoides* (species)	34% (21%, 45%)	23% (0%, 53%)	38% (0%, 79%)
	Bluegill	104.21	5.43	*Lepomis macrochirus* (species)	---	---	---
	Guppy	155.21	8.09	*Poecilia reticulate* (species)	---	---	---
Alabama Lampmussel *Lampsilis virescens*	Not known						
Carolina Heelsplitter *Lasmigon decorate*	Not known						
Louisiana Pearlshell *Margaritifera hembeli*	Brown madtom	151.06	7.87	Ictaluridae (family)	---	---	---
	Blackspotted topminnow	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Striped shiner Redfin shiner Golden shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Alabama Moccasinshell *Medionidus acutissimus*	Tuskaloosa darter Redfin darter Blackbanded darter Southern sand darter Johnny darter Speckled darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)

	Saddleback darter Naked sand darter Logperch	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Blackspotted topminnow	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Coosa Moccasinshell *Medionidus parvulus*	Blackbanded darter	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
Gulf Moccasinshell *Medionidus penicillatus*	Blackbanded darter Brown darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Eastern mosquitofish	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Guppy	155.21	8.09	*Poecilia reticulate* (species)	---	---	---
Ochlockonee Moccasinshell *Medionidus simpsonianus*	Not known						
Ring Pink *Obovaria retusa*	Not known						
Littlewing Pearlymussel *Pegis fibula*	Redline darter Greenside darter Emerald darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Banded sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
White Wartyback *Plethobasus cicatricosus*	Not known						
Orangefoot Pimpleback *Plethobasus cooperianus*	Not known						
Clubshell *Pleurobema clava*	Blackside darter Logperch	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
	Striped shiner Central stoneroller	84.07	4.38	*Cyprinidae* (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
James Spinymussel *Pleurobema collina*	Fantail darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Pumpkinseed	73.99	3.85	*Lepomis* (genus)	36% (24%, 47%)	24% (0%, 54%)	41% (0%, 79%)

	Bluehead chub Rosyside dace Satinfin shiner Rosefin shiner Blacknose dace Central stoneroller Mountain redbelly dace Swallowtail shiner Common shiner	84.07	4.38	Cyprinidae *(*family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Black Clubshell *Pleurobema curtum*	Not known						
Southern Clubshell *Pleurobema decisum*	Blacktail shiner Alabama shiner Tricolor shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Dark Pigtoe *Pleurobema furvum*	Blackspotted topminnow	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Largescale stoneroller Alabama shiner Blacktail shiner Creek chub	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Southern Pigtoe *Pleurobema georgianum*	Alabama shiner Blacktail shiner Tricolor shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Cumberland Pigtoe *Pleurobema gibberum*	Telescope shiner Striped shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Flat Pigtoe *Pleurobema marshalli*	Not known						
Ovate Clubshell *Pleurobema perovatum*	Not known						
Rough Pigtoe *Pleurobema plenum*	Not known						
Oval pigtoe *Pleurobema pyriforme*	Eastern mosquitofish	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
	Sailfin shiner	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
	Guppy	155.21	8.09	*Poecilia reticulate* (species)	---	---	---
Heavy Pigtoe *Pleurobema taitianum*	Not known						
Alabama Heelsplitter *Potamilus inflatus*	Freshwater drum	75.04	3.91	Perciformes (order)	36% (24%, 47%)	24% (0%, 53%)	40% (0%, 79%)

Triangular Kidneyshell *Ptychobranchus greeni*	Warrior darter Tuskaloosa darter Blackbanded darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Logperch	34.97	1.82	Percidae (family)	63% (58%, 68%)	39% (18%, 57%)	72% (43%, 87%)
Rough rabbitsfoot *Quadrula cylindrical strigillata*	Whitetail shiner Spotfin shiner Bigeye chub	84.07	4.38	Cyprinidae (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Winged Mapleleaf *Quadrula fragosa*	Blue catfish	151.06	7.87	Ictaluridae (family)	---	---	---
	Channel catfish	83.83	8.20	*Ictalurus punctatus* (species)	---	---	---
Cumberland Monkeyface *Quadrula intermedia*	Streamline chub Blotched chub	84.07	4.38	*Cyprinidae* (family)	31% (18%, 44%)	22% (0%, 53%)	33% (0%, 78%)
Appalachian Monkeyface *Quadrula sparsa*	Not known						
Stirrupshell *Quadrula stapes*	Not known						
Pale Lilliput *Toxolasma cylindrellus*	Not known						
Purple Bean *Villosa perpurpurea*	Fantail darter Greenside darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)
	Black sculpin Mottled sculpin Banded sculpin	54.93	2.86	Actinopterygii (class)	48% (39%, 56%)	30% (1%, 55%)	56% (3%, 82%)
Cumberland Bean *Villosa trabalis*	Fantail darter Striped darter	33.07	1.72	*Etheostoma* (genus)	65% (60%, 70%)	40% (20%, 58%)	74% (46%, 88%)

Table 17. Fish species identified as hosts of listed mussels.

Family	Common Name	Genus species
Catostomidae		
	Northern hogsucker	*Hypentelium nigricans*
	Greater jumprock	*Moxostoma lachneri*
Centrarchidae		
	Shadow bass	*Ambloplites ariommus*
	Rock bass	*Ambloplites rupestris*
	Warmouth	*Chaenobryttus gulosus*
	Green sunfish	*Lepomis cyanellus*
	Bluegill	*Lepomis macrochirus*
	Longear sunfish	*Lepomis megalotis*
	Redear sunfish	*Lepomis microlophus*
	Redeye bass	*Micropterus coosae*
	Smallmouth bass	*Micropterus dolomieu*
	Spotted bass	*Micropterus punctulatus*
	Largemouth bass	*Micropterus salmoides*
	Black crappie	*Pomoxis nigromaculatus*
Cottidae		
	Black sculpin	*Cottus baileyi*
	Mottled sculpin	*Cottus bairdii*
	Banded sculpin	*Cottus carolinae*
	Slimy sculpin	*Cottus cognatus*
Cyprinidae		
	Central stoneroller	*Campostoma anomalum*
	Largescale stoneroller	*Campostoma oligolepis*
	Rosyside dace	*Clinostomus funduloides*
	Satinfin shiner	*Cyprinella analostana*
	Alabama shiner	*Cyprinella callistia*
	Whitetail shiner	*Cyprinella galactura*
	Spotfin shiner	*Cyprinella spiloptera*
	Tricolor shiner	*Cyprinella trichroistia*
	Blacktail shiner	*Cyprinella venusta*
	Streamline chub	*Erimystax dissimilis*
	Blotched chub	*Erimystax insignis*
	Bigeye chub	*Hybopsis amblops*
	Striped shiner	*Luxilus chrysocephalus*
	Common shiner	*Luxilus cornutus*
	Rosefin shiner	*Lythrurus ardens*
	Pinewoods shiner	*Lythrurus matutinus*
	Redfin shiner	*Lythrurus umbratilis*
	Bluehead chub	*Nocomis leptocephalus*
	River chub	*Nocomis micropogon*

	Golden shiner	*Notemigonus crysoleucas*
	White shiner	*Notropis albeolus*
	Warpaint shiner	*Notropis coccogenis*
	Tennessee shiner	*Notropis leuciodus*
	Swallowtail shiner	*Notropis procne*
	Telescope shiner	*Notropis telescopus*
	Weed shiner	*Notropis texanus*
	Mountain redbelly	*Phoxinus oreas*
	Sailfin shiner	*Pteronotropis hypselopterus*
	Blacknose dace	*Rhinichthys atratulus*
	Creek chub	*Semotilus atromaculatus*
Esocidae		
	Chain pickerel	*Esox niger*
Fundulidae		
	Blackspotted topminnow	*Fundulus olivaceus*
Ictaluridae		
	Blue catfish	*Ictalurus furcatus*
	Channel catfish	*Ictalurus punctatus*
	Stonecat	*Noturus flavus*
	Brown madtom	*Noturus phaeus*
Percidae		
	Naked sand darter	*Ammocrypta beanii*
	Emerald darter	*Etheostoma baileyi*
	Warrior darter	*Etheostoma bellator*
	Greenside darter	*Etheostoma blennioides*
	Rainbow darter	*Etheostoma caeruleum*
	Bluebreast darter	*Etheostoma camurum*
	Tuskaloosa darter	*Etheostoma douglasi*
	Brown darter	*Etheostoma edwini*
	Fantail darter	*Etheostoma flabellare*
	Southern sand darter	*Etheostoma meridianum*
	Johnny darter	*Etheostoma nigrum*
	Tesselated darter	*Etheostoma olmstedi*
	Redline darter	*Etheostoma rufilineatum*
	Tennessee snubnose darter	*Etheostoma simoterum*
	Snubnose darter	*Etheostoma simoterum*
	Speckled darter	*Etheostoma stigmaeum*
	Striped darter	*Etheostoma virgatum*
	Wounded darter	*Etheostoma vulneratum*
	Redfin darter	*Etheostoma whipplei*
	Banded darter	*Etheostoma zonale*
	Yellow perch	*Perca flavescens*
	Tangerine darter	*Percina aurantiaca*
	Blotchside logperch	*Percina burtoni*
	Logperch	*Percina caprodes*

	Channel darter	*Percina copelandi*
	Gilt dater	*Percina evides*
	Blackside darter	*Percina maculata*
	Blackbanded darter	*Percina nigrofasciata*
	Roanoke darter	*Percina roanoka*
	Dusky darter	*Percina sciera*
	Saddleback darter	*Percina vigil*
	Fathead minnow	*Pimephales promelas*
	Sauger	*Sander canadensis*
	Walleye	*Sander vitreus*
Poecilidae		
	Eastern mosquitofish	*Gambusia holbrooki*
	Guppy	*Poecilia reticulata*
Salmonidae		
	Atlantic salmon	*Salmo salar*
	Brown trout	*Salmo trutta*
Sciaenidae		
	Freshwater drum	*Aplodinotus grunniens*

Table 18. Species diversity of host fish for glochidia of listed mussels.

Host fish by species		
Family	**# species**	**% all species**
Percidae* (darters, perch)	35	34
Cyprinidae* (shiners, chub, dace, stonerollers)	34	33
Centrarchidae* (bass, bluegill, sunfish)	15	15
Cottidae* (sculpin)	4	4
Ictaluridae (catfish)	4	4
Catostomidae* (suckers)	3	3
Poecilidae* (mosquitofish, guppies)	2	2
Salmonidae* (salmon, trout)	2	2
Sciaenidae* (drum)	1	1
Esocidae* (pickerel)	1	1
Fundulidae* (topminnows)	1	1
TOTAL	**102**	**100**

*Sensitive to cyanide concentrations below EPA's proposed Aquatic Life Criteria.

Table 19. Frequency of occurrence of host fish for glochidia of listed mussels.

Host fish by frequency of occurrence		
Family	**# occurrences**	**% all occurrences**
Percidae* (darters, perch)	82	36%
Cyprinidae* (shiners, chub, dace, stonerollers)	51	22%
Centrarchidae* (bass, bluegill, sunfish)	42	18%
Cottidae* (sculpin)	26	11%
Poecilidae* (mosquitofish, guppies)	8	4%
Sciaenidae* (drum)	6	3%
Ictaluridae (catfish)	4	2%
Catostomidae* (suckers)	3	1%
Fundulidae* (topminnows)	3	1%
Salmonidae* (salmon, trout)	2	1%
Esocidae* (pickerel)	1	<1%
TOTAL	**228**	**100%**

*Sensitive to cyanide concentrations below EPA's proposed Aquatic Life Criteria

Effects to Mussel Populations

There are no host fish for listed mussel species currently identified for which acute effects are anticipated at the criteria values. For species where hosts have not yet been identified, the probability of acute effects to host fish appears to be small. The greatest effect to mussel populations due to host fish effects is anticipated to result from declines in host fish abundance due to adverse effects to their fecundity and juvenile survival caused by exposure to cyanide at chronic criterion levels.

For effects to host fish caused by exposure to cyanide at chronic criterion levels, estimates were derived using methodologies for listed fish (Appendix F). Based on those calculations, host fish species for which adverse effects are anticipated at the chronic criterion may experience a reduction in eggs hatched that could be as much as, but not likely greater than, 31% to 63% compared to unexposed control populations (Table 16). Additionally, reductions in juvenile survival are expected at magnitudes that could be as high as 33% to 72% of control populations. Given the importance of fecundity and juvenile survival on overall effects to population abundance, this magnitude of adverse effect to the reproduction of host fish species is likely to translate into a decreased abundance of affected host fish species.

For listed mussels, any perturbation that limits fertilization rates and survivability of the glochidia, decreases host fish abundance, or decreases host fish community composition is detrimental to mussel population viability and, ultimately, the species as a whole (Downing et al. 1993, Neves 1993, Neves et al. 1997). Densities of host-specialist mussels, particularly those lacking elaborate host-attracting mechanisms, have been correlated to densities of host fish present in two drainage basins of Alabama (Haag and Warren 1998). No correlation was found for host-generalists or host-specialists with attractant lures. These data suggest that mussel species that showed a positive correlation may exhibit a density-dependence with host fish, limited by their abundance. It has been hypothesized that a gradual underlying decline of host fish abundance may play a major role in the steady decline of endangered mussel populations.

Although adult survival is typically the most influential life stage in population growth models of long-lived species like mussels, modeled effects to changes in reproduction can also have a significant influence on population growth. In simulation modeling performed for the three-ridge mussel (*Amblema plicata)*, deterministic methods using life history tables estimated that a 20% drop in the average number of young produced by females would negatively impact the population growth rate, resulting in a yearly population decline of 4.3% (Hart et al. 2004). Simulations incorporating environmental stochasticity predicted a 98% percent decline in the number of individuals after 100 years.

To determine the effects of exposure to chronic criteria concentrations of cyanide on listed mussel populations, species were grouped into one of the following four categories according to the number and diversity of fish species that have been identified as suitable hosts, and the estimated effects on those host species at criteria concentrations:

Category 1. Host-fish obligates, where the host fish is sensitive to cyanide exposure at criteria concentrations;

Category 2. Host-fish generalists or specialists, where most or all of known hosts are sensitive to cyanide exposure at criteria concentrations;

Category 3. No host fish identified; and

Category 4. Few fish hosts identified. While those which are currently known may be either sensitive or insensitive to cyanide at criteria concentrations, there are no data to indicate whether these fish species represent obligate hosts, a significant portion of the species assemblage of host fish, or a minor portion.

Category 1:

The mussel species listed below are host fish obligates. Using the methods described herein, the obligate host fish for these species, the freshwater drum, may experience a reduction in eggs hatched that could be as much as, but not likely greater than, 36% compared to unexposed control populations (Table 16). Additionally, reductions in juvenile survival are expected at magnitudes that could be as high as 40% of control populations. Given the importance of fecundity and juvenile survival on overall effects to population abundance, this magnitude of adverse effect to the reproduction of host fish species is likely to translate into a decreased abundance of the freshwater drum. If the abundance of an obligate host fish species decreases as a result of cyanide exposure, increased glochidia mortality is likely to occur as a result of their inability to attach to a suitable host. Since attachment of glochidia to a suitable host is a rare and necessary event in the mussel reproductive cycle, reductions in host fish abundance are likely to cause adverse impacts to mussel reproduction and population numbers. Since the oblighate host fish identified for the following mussel species are likely to exhibit population declines at cyanide criteria concentrations, these mussel species are likely to be subject to reduced reproduction, numbers, and distribution:

Scaleshell Mussel	*Leptodea leptodon*
Fat Pocketbook	*Potamilus capax*

Category 2

The mussel species listed below are either host fish specialists or generalists. Using the methods described herein, most of the identified host fish species for these mussels are likely to be subject to reduced levels of reproduction and juvenile survival as a result of exposure to cyanide at criteria concentrations (Table 16). Some host fish species identified for these mussels may not experience adverse effects to reproduction and juvenile survival at these concentrations (Table 16). There may also be host fish for these mussels which have yet to be identified that will be either be sensitive to or tolerant of cyanide exposure at criteria concentrations. If the abundance of any host fish species decreases as a result of cyanide exposure, increased glochidia mortality is likely to occur as a result of their inability to attach to a suitable host or the host dies during infestation. Since attachment of glochidia to a suitable host is a rare and necessary event in the mussel reproductive cycle, reductions in host fish abundance or the death of individual host fish that are infested by glochidia are likely to cause adverse impacts to mussel reproduction and population numbers. Since the majority of fish hosts identified for the following mussel species are likely to exhibit population declines at cyanide criteria concentrations, these mussel species are likely to be subject to reduced reproduction, numbers, and distribution:

Cumberland Elktoe	*Alasmidonta atropurpurea*
Dwarf Wedgemussel	*Alasmidonta heterodon*
Fat Threeridge	*Amblema neislerii*
Ouachita Rock Pocketbook	*Arkansia wheeleri*
Birdwing Pearlymussel	*Conradilla caelata*
Fanshell	*Cyprogenia stegaria*
Dromedary Pearlymussel	*Dromus dromas*
Tar Spinymussel	*Elliptio steinstansana*
Purple Bankclimber	*Elliptoideus sloatianus*
Cumberland Combshell	*Epioblasma brevidens*
Oyster Mussel	*Epioblasma capsaeformis*
Curtis Pearlymussel	*Epioblasma florentina curtisii*
Tan Riffleshell	*Epioblasma florentina walkeri*
Catspaw (Purple cat's paw pearlymussel)	*Epioblasma obliquata obliquata*
Northern Riffleshell	*Epioblasma turulosa rangiana*
Shiny Pigtoe	*Fusconaia cor*
Fine-rayed Pigtoe	*Fusconaia cuneolus*
Pink Mucket	*Lampsilis abrupta*
Fine-lined Pocketbook	*Lampsilis altilis*
Higgins Eye	*Lampsilis higginsii*
Orangenacre Mucket	*Lampsilis perovalis*

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Speckled Pocketbook	*Lampsilis streckeri*
Shinyrayed pocketbook	*Lampsilis subangulata*
Louisiana Pearlshell	*Margaritifera hembeli*
Alabama Moccasinshell	*Medionidus acutissimus*
Gulf Moccasinshell	*Medionidus penicillatus*
Littlewing Pearlymussel	*Pegis fibula*
Clubshell	*Pleurobema clava*
James Spinymussel	*Pleurobema collina*
Southern Clubshell	*Pleurobema decisum*
Dark Pigtoe	*Pleurobema furvum*
Southern Pigtoe	*Pleurobema georgianum*
Oval Pigtoe	*Pleurobema pyriforme*
Triangular Kidneyshell	*Ptychobranchus greeni*
Rough Rabbitsfoot	*Quadrula cylindrical strigillata*
Purple Bean	*Villosa perpurpurea*

Category 3

No fish hosts have been identified for 23 of the listed mussel species considered in this biological opinion. While host fish are a necessary factor in the reproduction of freshwater mussels, there has not been adequate study to identify which fish can serve for hosts for a significant portion of North American mussels, including those which are threatened and endanagered. In cases of uncertainty, it is Service policy to error on the side of listed species. Therefore, for purposes of this analysis, we are assuming that the mussel species in this category are either host fish specialists or generalists but that all of the host fish species are sensitive to cyanide exposure at criteria concentrations to an extent that their populations are likely to be reduced. This effect is likely to cause increased glochidia mortality due to their inability to attach to a suitable host or death of the host. Since attachment of glochidia to a suitable host is a rare and necessary event in the mussel reproductive cycle, reductions in host fish abundance or death of individual host fish that are infested by glochidia are likely to cause declines in the reproduction, numbers, and distribution of the following mussel species:

Yellow Blossom	*Epioblasma florentina florentina*
Upland Combshell	*Epioblasma metastriata*
White Catspaw Pearlymussel	*Epioblasma obliquata perobliqua*
Southern Acornshell	*Epioblasma othcaloogensis*
Southern Combshell	*Epioblasma penita*
Tubercled Blossom	*Epioblasma torulosa torulosa*
Turgid Blossom	*Epioblasma turgidula*
Green Blossom	*Epioblasma torulosa gubernaculums*
Cracking Pearlymussel	*Hemistena lata*
Alabama Lampmussel	*Lampsilis virescens*
Carolina Heelsplitter	*Lasmigon decorate*
Ochlockonee Moccasinshell	*Medionidus simpsonianus*
Ring Pink	*Obovaria retusa*
White Wartyback	*Plethobasus cicatricosus*

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Orangefoot Pimpleback	*Plethobasus cooperianus*
Black Clubshell	*Pleurobema curtum*
Flat Pigtoe	*Pleurobema marshalli*
Ovate Clubshell	*Pleurobema perovatum*
Rough Pigtoe	*Pleurobema plenum*
Heavy Pigtoe	*Pleurobema taitianum*
Appalachian Monkeyface	*Quadrula sparsa*
Stirrupshell	*Quadrula stapes*
Pale Lilliput	*Toxolasma cylindrellus*

Category 4:

For several species, few host fish have been identified. While those which are currently known may be either sensitive or insensitive to cyanide at criteria concentrations, there are no data to indicate whether these fish represent obligate hosts, a significant portion of the species assemblage of host fish, or a minor portion. As noted above, in cases of uncertainty, it is Service policy to error on the side of listed species. Therefore, for purposes of this analysis, we are assuming that the mussel species in this category are either host fish specialists or generalists but that many or all of the host fish species are sensitive to cyanide exposure at criteria concentrations to an extent that their populations are likely to be reduced. This effect is likely to cause increased glochidia mortality due to their inability to attach to a suitable host or death of the host. Since attachment of glochidia to a suitable host is a rare and necessary event in the mussel reproductive cycle, reductions in host fish abundance or death of individual host fish that are infested by glochidia are likely to cause declines in the reproduction, numbers, and distribution of the following mussel species:

Appalachian Elktoe	*Alasmidonta raveneliana*
Chipola Slabshell	*Elliptio chipolaensis*
Arkanasas Fatmucket	*Lampsilis powelli*
Coosa Moccasinshell	*Medionidus parvulus*
Cumberland Pigtoe	*Pleurobema gibberum*
Alabama Heelsplitter	*Potamilus inflatus*
Winged Mapleleaf	*Quadrula fragosa*
Cumberland Monkeyface	*Quadrula intermedia*
Cumberland Bean	*Villosa trabalis*

Effects to Critical Habitat for Listed Mussels

<u>Category 1 Species</u>:
No critical habitat has been designated for species in this category.

<u>Category 2 Species</u>:
The physical and biological features of critical habitat essential to the conservation of mussels include water of sufficient quality for normal behavior, growth, and survival of all life stages of the mussel and its host fish. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This

approval is likely to adversely affect the quality of water within critical habitat for the following mussel species to the degree that it would impair individual reproduction and survival of host fish, and cause host fish to experience adverse effects to growth, swimming performance, condition, and development (Table 16). For those reasons, we conclude that management of cyanide to the CCC level in water within critical habitat for the following mussel species is likely to degrade or preclude the proper function of the primary constituent elements of critical habitat that support water quality for normal behavior, growth, and survival of all life stages of the mussel and its host fish:

Cumberland Elktoe	*Alasmidonta atropurpurea*
Fat Threeridge	*Amblema neislerii*
Purple Bankclimber	*Elliptoideus sloatianus*
Cumberlandian Combshell	*Epioblasma brevidens*
Oyster Mussel	*Epioblasma capsaeformis*
Fine-lined Pocketbook	*Lampsilis altilis*
Orangenacre Mucket	*Lampsilis perovalis*
Shinyrayed pocketbook	*Lampsilis subangulata*
Alabama Moccasinshell	*Medionidus acutissimus*
Gulf Moccasinshell	*Medionidus penicillatus*
Southern Clubshell	*Pleurobema decisum*
Dark Pigtoe	*Pleurobema furvum*
Southern Pigtoe	*Pleurobema georgianum*
Oval Pigtoe	*Pleurobema pyriforme*
Triangular Kidneyshell	*Ptychobranchus greeni*
Rough Rabbitsfoot	*Quadrula cylindrical strigillata*
Purple Bean	*Villosa perpurpurea*

<u>Category 3 Species</u>:
The physical and biological features of critical habitat essential to the conservation of mussels include water of sufficient quality for normal behavior, growth, and survival of all life stages of the mussel and its host fish. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval is likely to adversely affect the quality of water within critical habitat for the following mussel species to the degree that it would impair individual reproduction and survival of potential host fish, and cause host fish to experience adverse effects to growth, swimming performance, condition, and development (Table 16). For those reasons, we conclude that management of cyanide to the CCC level in water within critical habitat for the following mussel species is likely to degrade or preclude the proper function of the primary constituent elements of critical habitat that support water quality for normal behavior, growth, and survival of all life stages of the mussel and its host fish:

Upland Combshell	*Epioblasma metastriata*
Southern Acornshell	*Epioblasma othcaloogensis*
Carolina Heelsplitter	*Lasmigon decorate*
Ochlockonee Moccasinshell	*Medionidus simpsonianus*
Ovate Clubshell	*Pleurobema perovatum*

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Category 4 Species:
The physical and biological features of critical habitat essential to the conservation of mussels include water of sufficient quality for normal behavior, growth, and survival of all life stages of the mussel and its host fish. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval is likely to adversely affect the quality of water within critical habitat for the following mussel species to the degree that it would impair individual reproduction and survival of known and/or potential host fish, and cause host fish to experience adverse effects to growth, swimming performance, condition, and development (Table 16). For those reasons, we conclude that management of cyanide to the CCC level in water within critical habitat for the following mussel species is likely to degrade or preclude the proper function of the primary constituent elements of critical habitat that support water quality for normal behavior, growth, and survival of all life stages of the mussel and its host fish:

Appalachian Elktoe	*Alasmidonta raveneliana*
Chipola Slabshell	*Elliptio chipolaensis*
Coosa Moccasinshell	*Medionidus parvulus*

7.4 Effects to Amphibians

Our assessment of the sensitivity of listed amphibian species to cyanide was based on multiple lines of evidence. First, we evaluated the available information on cyanide-induced effects on amphibians. We then reviewed the approach EPA used in their Biological Evaluation to assess the sensitivity of listed amphibians to cyanide and the protectiveness of the cyanide criteria. Next, we examined additional toxicity information for amphibians, not used by EPA, and constructed regression models for predicting the acute sensitivities of amphibian genera to cyanide. Finally, we compared the predicted sensitivity of amphibians with that of rainbow trout; the most sensitive freshwater species (based on measured cyanide LC_{50}s) and the species that was used to set the acute and chronic cyanide criteria. Taken together, these data provided the basis for our effects determination.

The scientific literature for cyanide toxicity to amphibians is limited and somewhat dated. Early investigators studied the effects of cyanide on amphibian development. These experiments were generally focused on early embryogenesis including oviposited and fertilized egg morphogenesis and post gastrulation development. Repressive effects of cyanide on embryonic respiration and development were documented by several authors (Spiegelman and Moog, 1943, Lovtrup and Pigon, 1958, Nakatsuji, 1974). Others used sub-lethal exposure concentrations of cyanide as a mechanism to arrest or retard development in order to test various hypotheses regarding metabolism or physiology (Spiegelman and Steinbach, 1945; Ornstein and Gregg, 1952). Although these historical studies are important for understanding the physiological actions of cyanide on amphibians, they do not provide the traditional quantitative measures of acute and chronic toxicity (i.e. LC_{50}s, NOECs, EC_xs) that have been used in water quality criteria development.

Because cyanide-specific toxicity data (LC_{50}s) for amphibians were not available, EPA based their effects analysis on the relative sensitivity of amphibians to other pollutants (EPA 2007). They examined the rank order of amphibian LC_{50}s for seven water pollutants using data sets from ambient water quality criteria documents (Table 20). The 7 data sets included LC_{50}s for 9 amphibian species (in total), although 4 of the data sets contained LC_{50}s for only 1 amphibian species and the other 3 data sets contained data for 2 species. So among these seven criteria documents, the amphibian class was represented by no more than one or two species at a time. With so few species used to characterize the sensitivity of an entire class there is considerable uncertainty as to whether the most sensitive amphibian species are adequately represented, especially considering the large interspecies variability in cyanide toxicity observed for other taxa (see acute effects section of BO). It seems highly unlikely that the amphibians species included in these data sets were among the most sensitive amphibians. Nevertheless, for two of the seven pollutants the single amphibian species in the data set ranked among the most sensitive species/genera in the multi-taxa data sets used to develop criteria. For the remaining five pollutants the GMAVs for amphibians ranged from the 26th percentile to the 100th percentile. Considering the low number of species used to represent amphibians in the analysis and the fact that amphibians were among the most sensitive species/genera for 28% of the pollutants examined we believe that there is a more than a discountable chance that some amphibian species may be highly sensitive to cyanide. Therefore, we do not believe these results alone support EPAs determination that the listed amphibian species are not likely to be adversely affected by cyanide at criteria concentrations.

To better understand how to interpret the results from EPAs analysis we extended our evaluation to include rainbow trout; a species frequently included in criteria development and often among the more sensitive species tested (Table 20). Using data for the same seven pollutants we found that the over all pattern of rankings for rainbow trout were much like those for amphibians, i.e. most near or above the median and two or three falling among the most sensitive species. However we know that in terms of cyanide, rainbow trout is the most sensitive freshwater species that has been tested, more sensitive than the 5th percentile estimated species (EPA 1985). (That is, rainbow trout fell in the "sensitive tail" of the species sensitivity distribution.) So, there is at least one example where the "ranking profile" (for these 7 pollutants) shared by amphibians and rainbow trout was associated with a species that was highly sensitive to cyanide. In addition, we found that for these seven pollutants amphibian species were more sensitive than rainbow trout 43% of the time (3 of 7). To further investigate the relative sensitivity of amphibians to other taxa we reviewed other references on amphibian toxicology.

Table 20. Rank and corresponding percentile of GMAVs (genus mean acute values) for amphibians and rainbow trout versus all aquatic taxa and chordates (fishes) only. Data for amphibians are from Appendix D of EPAs Cyanide Biological Evaluation (EPA 2007). Data for rainbow trout are from criteria documents (see footnotes).

Chemical	Amphibian Species	Amphibian GMAV Rank Vs. Other Taxa	Rainbow (GMAV) Rank vs. Other Taxa	Percentile (Amphibians)	Percentile (Rainbow trout)	Amphibians more (+) or less (-) sensitive than Rainbow trout
Atrazine	Bufo americanus	11 of 19	4 of 19[1]	0.58	0.21	-
Atrazine	Rana sp.	14 of 19		0.74		
Cadmium	Ambystoma gracile	29 of 57	4 of 57[2]	0.51	0.07	-
Cadmium	Xenopus laevis	33 of 57		0.58		
Diazinon	Rana clamitans	8 of 21	12 of 21[3]	0.26	0.57	+
Lindane	Pseudacris triseriata	22 of 23	10 of 23[4]	0.96	0.43	-
Lindane	Bufo woodhousei	23 of 23		1.00		
Nonylphenol	Bufo boreas	2 of 15	8 of 15[5]	0.13	0.53	+
Parathion	Pseudacris triseriata	23 of 31	25 of 31[5]	0.74	0.81	+
Pentachlorophenol	Rana satesbeiana	4[6] of 32	3 of 32[5]	0.13	0.09	-

[1] Draft aquatic life ambient water quality criteria for atrazine (EPA 2003)
[2] 2001 update of the aquatic life ambient water quality criteria for cadmium (EPA 2001)
[3] Aquatic life ambient water quality criteria for diazinon (EPA 2005)
[4] 1995 updates: water quality criteria documents for the protection of aquatic life in ambient water (EPA 1996)
[5] Aquatic life ambient water quality criteria for nonylphenol (EPA 2005)
[6] Rank was changed from 5 to 4 based on GMAV ranks for pentachlorophenol (EPA 1996)

Birge at al. (2003) performed a comparative toxicity analysis for 29 amphibian species in contrast to various species of fish. Amphibian testing included seven salamander species (family Ambystomidae) and 22 frog species (families Microhylidae, Hylidae, Ranidae, and Bufonidae). Though no toxicity testing was performed for cyanide, sufficient data was produced to generate comparisons between amphibians and fish for 34 inorganic compounds and 27 organic compounds. Comparisons include all amphibian test species for 50 of these 61 compounds. Although exposure times varied among species due to differences in hatching times, comparable stages of development (eggs, embryos, and early larvae) were included in all tests. Fish species included in this study for which sensitivity to cyanide is known are the rainbow trout (LC_{50} = 59.22ug/g), largemouth bass (101.7 ug/g) and fathead minnow (138.4 ug/g).

When compared to rainbow trout, LC_{50} values for amphibians were more sensitive 52% of the time for metals (N=203), 36% for organics (N=44), and 49% for all compounds combined (N=247). For largemouth bass, amphibians were more sensitive 83% of the time (N=182), 60% for organics (N=15), and 81% for all compounds (N=197). For fathead minnow, amphibians were more sensitive 89% of the time for metals (N=18), 63% for organics (N=24), and 74% for all compounds (N=42). The generally more sensitive species of Microhylidae and Hylidae were not available for toxicity testing for several organic compounds. For the 15 most sensitive amphibian species, LC_{50} values were below fish values (including species used above, plus channel catfish and goldfish) 74% of the time.

Bridges et al (2002), performed toxicity testing for five compounds on southern leopard frog (*Rana sphenocephala*) tadpoles and compared results with published values for the boreal toad (*Bufo boreas*), rainbow trout, fathead minnow, and bluegill. The two amphibian species showed the highest correlation of LC_{50} values for the rainbow trout. Correlations for the fathead minnow and bluegill were much weaker. The authors suggest that rainbow trout thus may be the most appropriate species for assessing toxicity to anuran tadpoles. However, the authors also argue that since amphibians are very tolerant to some chemicals, and very sensitive to others, individual toxicity testing is suggested rather than relying on surrogate species.

The comparative toxicity data sets from Birge et al. (2003) provided an opportunity to construct ICE-like regression models that could be used to estimate cyanide LC_{50}s for amphibians (Table 21; Figures 5 and 6). Following EPA guidelines (EPA 2003b), regression models were developed to estimate the sensitivity of two amphibian genera (Rana and Ambystoma) using rainbow trout as the surrogate species.

Table 21. Estimated cyanide LC_{50}s for two amphibian genera (*Rana* and *Ambystoma*) using rainbow trout as a surrogate species

Predicted Taxon	Surrogate Species	LCI LC_{50} (ug/L)	MLE LC_{50} (ug/L)	UCI LC_{50} (ug/L)	Corr. Coeff. (r)	MSE	log-log a	log-log b	p	n	Chem.
Rana (genus)	Rainbow Trout	30.82	54.25	95.51	0.789	0.648	0.259	0.832	<0.001	84	32
Ambystoma (genus)	Rainbow Trout	60.56	120.61	639.63	0.638	0.415	0.966	0.629	<0.002	23	4

Figure 6. Rainbow Trout Interspecies Correlation Plot for the Genus Ambystoma

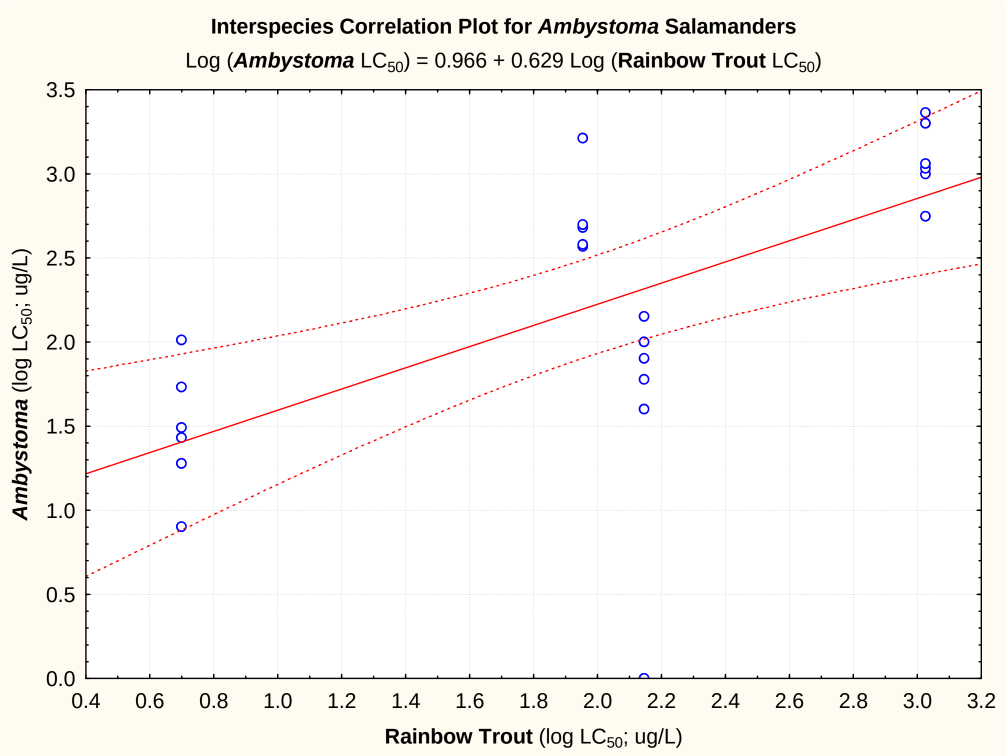
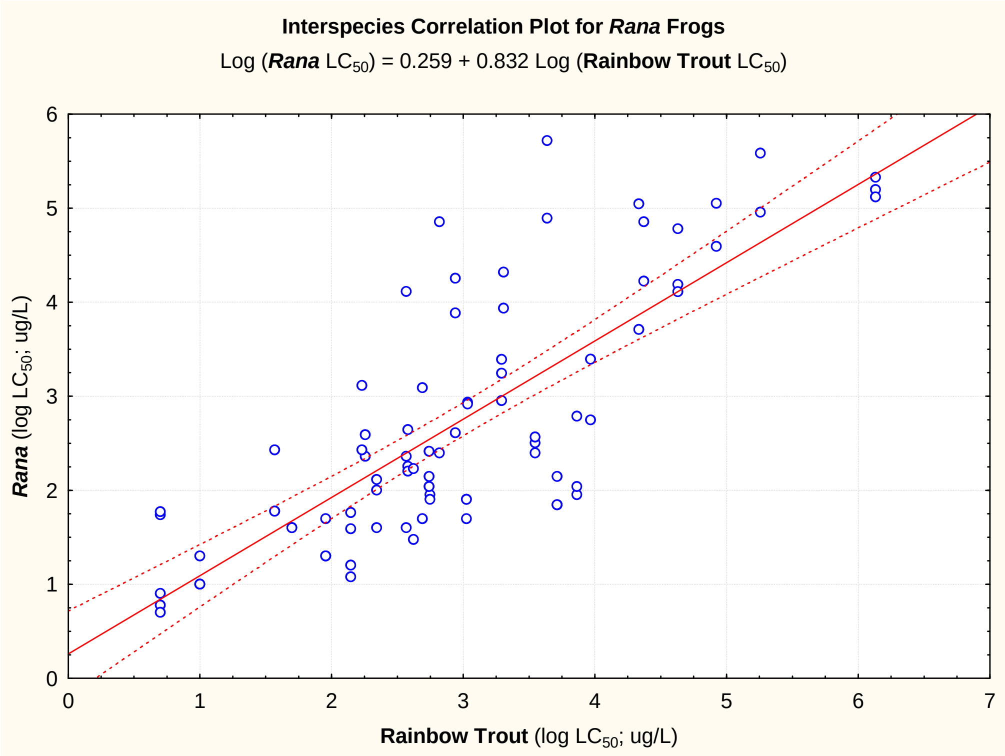


Figure 7. Rainbow Trout Interspecies Correlation Plot for the Genus Rana



Formal Draft Biological Opinion.

Estimated LC_{50}s for the two amphibian genera, Ambystoma (LC_{50} 60.56 ug CN/L) and Rana (LC_{50} 30.82 ug CN/L), are approximately equal to or less than the LC_{50} for rainbow trout (59 ug CN/L).

As previously mentioned, rainbow trout had the lowest measured cyanide LC_{50} of all fish species considered in the cyanide criteria document as well as the cyanide BE. Based on the method described in the *Fish* section of Appendix B, the chronic EC_A for rainbow trout would be 2.54 ug CN/L (i.e. 59 ug CN/L / 23.22) and the acute EC_A would be 51.75 ug CN/L (i.e. 59 ug CN/L / 1.14). Because the chronic EC_A is below 5.2 ug CN/L rainbow trout would likely be adversely affected by exposure to cyanide at the CCC. Thus, amphibian species are estimated to be as or more sensitive to cyanide than rainbow trout and thus likely to be adversely affected by exposure to cyanide at the chronic criterion.

Individual Species and Critical Habitat Accounts

Ambystomatidae

RETICULATED FLATWOODS SALAMANDER
Ambystoma bishop

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that reticulated flatwoods salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, reticulated flatwoods salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The life history of the reticulated flatwoods salamander can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Reticulated flatwoods salamanders breed in relatively small, isolated ephemeral ponds where the larvae develop until metamorphosis. Post-metamorphic salamanders migrate out of the ponds and into the uplands where they live until they move back to ponds to breed as adults. The flatwoods salamander reproduces at 1 year of age for males and two years of age for females. Males and females court before the breeding sites flood. Females then lay their eggs, either singly or in clumps, beneath leaf litter, under logs, sphagnum moss mats, small trees, bushes or clumps of grass at dry locations in seasonal wetlands. If rainfall is insufficient to result in adequate pond flooding, breeding may not occur or, if larvae do develop, they may die before metamorphosis. Egg development from deposition to hatching occurs in approximately 2 weeks, but eggs do not hatch until they are inundated. Depending on when they are inundated, the larvae metamorphose 11 to 18 weeks after hatching. Exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, and in the case of the reticulated flatwoods salamander such reduced growth rates could preclude emergence prior to pond drying.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the reticulated flatwoods salamander’s fertility rates substantially. The salamander’s fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). Taylor et al. (2006) constructed a model, based on extensive population data available for the marbled salamander (*Ambystoma opacum*), to look at how many years of reproductive failure would be required to result in local extinction of pond-breeding salamanders (with varying lifespans) and found that even without total reproductive failure, populations required moderate to high upland post-metamorphic survival to persist. Catastrophic reproductive failure in this study created fluctuations in the population, raised the threshold of survival required to achieve persistence, and imposed the possibility of extinction even under otherwise favorable environmental conditions. Even in populations with multiple breeding ponds, amphibian populations may be unable to recolonize areas after local extirpations due to their physiological constraints, relatively low mobility, and site fidelity. In the case of the reticulated flatwoods salamander, only 20 populations are known and 14 (70 percent) of these populations are supported by only one breeding site. For those populations with only one breeding pond, habitat destruction associated with cyanide at CCC levels may adversely effect flatwoods salamander reproduction and survival resulting in extirpation of the population supported by that breeding pond. For populations with more than one breeding pond, habitat destruction associated with cyanide at CCC levels may result in a reduction of available breeding sites leading to a reduction in population size and range and an increased vulnerability to catastrophic events that may adversely affect breeding and survival.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the reticulated flatwoods salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. The reticulated flatwoods salamander has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected flatwoods salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. The majority of extant populations are supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, reticulated flatwoods salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the reticulated salamander.

Critical Habitat: Critical habitat for the reticulated flatwoods salamander has been designated in: Calhoun, Holmes, Jackson, Santa Rosa, Walton, and Washington Counties Florida; Baker and Miller Counties Georgia. The physical and biological features of critical habitat essential to the conservation of the reticulated flatwoods salamander includes breeding habitats consisting of small, acidic depressional standing bodies of water that are seasonally flooded by rainfall in late fall or early winter and dry in late spring or early summer; and are geographically isolated from other water bodies. In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of reticulated flatwoods salamanders and cause salamanders to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of reticulated flatwoods salamanders and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

FROSTED FLATWOODS SALAMANDER
Ambystoma cingulatum

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that frosted flatwoods salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, frosted flatwoods salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The life history of the frosted flatwoods salamander can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Frosted flatwoods salamanders breed in relatively small, isolated ephemeral ponds where the larvae develop until metamorphosis. Post-metamorphic salamanders migrate out of the ponds and into the uplands where they live until they move back to ponds to breed as adults. The flatwoods salamander reproduces at 1 year of age for males and two years of age for females. Males and females court before the breeding sites flood. Females then lay their eggs, either singly or in clumps, beneath leaf litter, under logs, sphagnum moss mats, small trees, bushes or clumps of grass at dry locations in seasonal wetlands. If rainfall is insufficient to result in adequate pond flooding, breeding may not occur or, if larvae do develop, they may die before metamorphosis. Egg development from deposition to hatching occurs in approximately 2 weeks, but eggs do not hatch until they are inundated. Depending on when they are inundated, the larvae metamorphose 11 to 18 weeks after hatching. Exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, and in the case of the frosted flatwoods salamander such reduced growth rates could preclude emergence prior to pond drying.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the frosted flatwoods salamander's fertility rates substantially. The salamander's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). Taylor et al. (2006) constructed a model, based on extensive population data available for the marbled salamander (*Ambystoma opacum*), to look at how many years of reproductive failure would be required to result in local extinction of pond-breeding salamanders (with varying lifespans) and found that even without total reproductive failure, populations required moderate to high upland post-metamorphic survival to persist. Catastrophic reproductive failure in this study created fluctuations in the population, raised the threshold of survival required to achieve persistence, and imposed the possibility of extinction even under otherwise favorable environmental conditions. Even in populations with multiple breeding ponds, amphibian populations may be unable to recolonize areas after local extirpations due to their physiological constraints, relatively low mobility, and site fidelity. Surveys indicate there are 25 populations of the frosted flatwoods salamander, some of which have been inferred from the capture of a single individual. Twenty-two (88 percent) of the known frosted flatwoods salamander populations occur primarily on public land. Sixteen of the populations (64 percent of total populations of the species) on public land represent metapopulations supported by more than one breeding site. For populations with only one breeding pond, if the habitat at that site is destroyed, recolonization would be impossible and the population supported by that breeding pond would be extirpated. For those populations with only one breeding pond, habitat destruction associated with cyanide at CCC levels may adversely effect flatwoods salamander reproduction and survival resulting in extirpation of the population supported by that breeding pond. For populations with more than one breeding pond, habitat destruction associated with cyanide at CCC levels may result in a reduction of available breeding sites leading to a reduction in population size and range and an increased vulnerability to catastrophic events that may adversely affect breeding and survival.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the frosted flatwoods salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. The frosted flatwoods salamander has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected flatwoods salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. The majority of extant populations are supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, frosted flatwoods salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the frosted flatwoods salamander.

Critical Habitat: Critical habitat has been designated for the frosted flatwoods salamander in: Baker, Franklin, Jefferson, Liberty, and Wakulla Counties, Florida; and in Berkeley, Charleston, and Jasper Counties, South Carolina. The physical and biological features of critical habitat essential to the conservation of the frosted flatwoods salamander includes breeding habitats consisting of small, acidic depressional standing bodies of water that are seasonally flooded by rainfall in late fall or early winter and dry in late spring or early summer; and are geographically isolated from other water bodies. In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of frosted flatwoods salamanders and cause salamanders to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of frosted flatwoods salamanders and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

CALIFORNIA TIGER SALAMANDER

Ambystoma californiese

Central California population

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that California tiger salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, California tiger salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The life history of the California tiger salamander can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Adult California tiger salamanders mate in vernal pools and similar water bodies, and the females lay their eggs in the water. Females attach their eggs singly or, in rare circumstances, in groups of two to four, to twigs, grass stems, vegetation, or debris. In ponds with little or no vegetation, females may attach eggs to objects, such as rocks and boards on the bottom. After breeding, adults leave the pool and return to small mammal burrows in surrounding uplands, although they may continue to come out nightly for approximately the next two weeks to feed. In drought years, the seasonal pools may not form and the adults may not breed. The eggs hatch in 10 to 14 days with newly hatched salamanders (larvae) ranging in size from 11.5 to 14.2 mm (0.5 to 0.6 in) in total length. The larval stage of the California tiger salamander usually lasts three to six months, because most seasonal ponds and pools dry up during the summer, although some larvae in Contra Costa and Alameda Counties may remain in their breeding sites over the summer. Amphibian larvae must grow to a critical minimum body size before they can metamorphose (change into a different physical form) to the terrestrial stage. One study found larvae metamorphosed and left the breeding pools 60 to 94 days after the eggs had been laid, with larvae developing faster in smaller, more rapidly drying pools. The longer the inundation period, the larger the larvae and metamorphosed juveniles are able to grow, and the more likely they are to survive and reproduce. The larvae perish if a site dries before they complete metamorphosis. There was a strong positive correlation between inundation period and total number of metamorphosing juvenile amphibians, including tiger salamanders. Size at metamorphosis is positively correlated with stored body fat and survival of juvenile amphibians, and negatively correlated with age at first reproduction

Exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the California tiger salamander could preclude emergence prior to pond drying, resulting in larval mortalities.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the California tiger salamander’s fertility rates substantially. The salamander’s fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section of Population Level Effects, we noted that reduction in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to reproduce, like California tiger salamanders. Lifetime reproductive success for California and other tiger salamanders is low. One study found the average female bred 1.4 times and produced 8.5 young that survived to metamorphosis per reproductive effort. This resulted in roughly 11 metamorphic offspring over the lifetime of a female. Most California tiger salamanders in this study did not reach sexual maturity until four or five years old. While individuals may survive for more than 10 years, many breed only once, and one study estimated that less than five percent of metamorphic juveniles survive to become breeding adults. The mechanisms for recruitment are clearly dependent on a number of factors such as migration, terrestrial survival, and population turnover, whose interaction is not well understood.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the California tiger salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. The California tiger salamander has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected California tiger salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, California tiger salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the California Tiger salamander.

Critical Habitat: The exact locations of the critical habitat are depicted on maps in the federal register. The physical and biological features of critical habitat essential to the conservation of the California tiger salamander includes standing bodies of fresh water (including natural and manmade (e.g., stock)) ponds, vernal pools, and other ephemeral or permanent water bodies which typically support inundation during winter rains and hold water for a minimum of 12 weeks in a year of average rainfall. In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of California tiger salamanders and cause salamanders to experience adverse effects to growth, locomotion, condition, and development. Based on data for the genus *Oncorhyncus*, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of California tiger salamanders and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

CALIFORNIA TIGER SALAMANDER
Ambystoma californiese
Santa Barbara County population

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that California tiger salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, California tiger salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects *Overview*. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

This population is restricted to Santa Barbara County, California. The life history of the California tiger salamander can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Adult California tiger salamanders mate in vernal pools and similar water bodies, and the females lay their eggs in the water. Females attach their eggs singly or, in rare circumstances, in groups of two to four, to twigs, grass stems, vegetation, or debris. In ponds with little or no vegetation, females may attach eggs to objects, such as rocks and boards on the bottom. After breeding, adults leave the pool and return to small mammal burrows in surrounding uplands, although they may continue to come out nightly for approximately the next two weeks to feed. In drought years, the seasonal pools may not form and the adults may not breed. The eggs hatch in 10 to 14 days with newly hatched salamanders (larvae) ranging in size from 11.5 to 14.2 mm (0.5 to 0.6 in) in total length. The larval stage of the California tiger salamander usually lasts three to six months, because most seasonal ponds and pools dry up during the summer, although some larvae in Contra Costa and Alameda Counties may remain in their breeding sites over the summer. Amphibian larvae must grow to a critical minimum body size before they can metamorphose (change into a different physical form) to the terrestrial stage. One study found larvae metamorphosed and left the breeding pools 60 to 94 days after the eggs had been laid, with larvae developing faster in smaller, more rapidly drying pools. The longer the inundation period, the larger the larvae and metamorphosed juveniles are able to grow, and the more likely they are to survive and reproduce. The larvae perish if a site dries before they complete metamorphosis. There was a strong positive correlation between inundation period and total number of metamorphosing juvenile amphibians, including tiger salamanders. Size at metamorphosis is positively correlated with stored body fat and survival of juvenile amphibians, and negatively correlated with age at first reproduction

Exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the California tiger salamander could preclude emergence prior to pond drying, resulting in larval mortalities.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the California tiger salamander’s fertility rates substantially. The salamander’s fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section of Population Level Effects, we noted that reduction in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to reproduce, like California tiger salamanders. Lifetime reproductive success for California and other tiger salamanders is low. One study found the average female bred 1.4 times and produced 8.5 young that survived to metamorphosis per reproductive effort. This resulted in roughly 11 metamorphic offspring over the lifetime of a female. Most California tiger salamanders in this study did not reach sexual maturity until four or five years old. While individuals may survive for more than 10 years, many breed only once, and one study estimated that less than five percent of metamorphic juveniles survive to become breeding adults. The mechanisms for recruitment are clearly dependent on a number of factors such as migration, terrestrial survival, and population turnover, whose interaction is not well understood.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the California tiger salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. The California tiger salamander has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected California tiger salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, California tiger salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the California Tiger salamander.

Critical Habitat: Critical habitat has been established in Santa Barbara County for this population segment of the California tiger salamander in six separate locations east of Vandenberg Air Force Base. The physical and biological features of critical habitat essential to the conservation of the California tiger salamander includes standing bodies of fresh water (ponds, vernal pools, dune ponds, or other ephemeral or permanent water bodies) sufficient for the aquatic portion of the salamander's life cycle. In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of California tiger salamanders and cause salamanders to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of California tiger salamanders and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

CALIFORNIA TIGER SALAMANDER

Ambystoma californiese

Sonoma County population

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that California tiger salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, California tiger salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The Sonoma population appears to have been geographically isolated from the remainder of the California tiger salamander population by distance, mountains and major waterway barriers for more than 700,000 years. It occurs only in association with vernal pool ecosystems and stock ponds remaining on the Santa Rosa Plain of Sonoma County, California. There are 8 known breeding sites within the Santa Rosa Plain. The life history of the California tiger salamander can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Adult California tiger salamanders mate in vernal pools and similar water bodies, and the females lay their eggs in the water. Females attach their eggs singly or, in rare circumstances, in groups of two to four, to twigs, grass stems, vegetation, or debris. In ponds with little or no vegetation, females may attach eggs to objects, such as rocks and boards on the bottom. After breeding, adults leave the pool and return to small mammal burrows in surrounding uplands, although they may continue to come out nightly for approximately the next two weeks to feed. In drought years, the seasonal pools may not form and the adults may not breed. The eggs hatch in 10 to 14 days with newly hatched salamanders (larvae) ranging in size from 11.5 to 14.2 mm (0.5 to 0.6 in) in total length. The larval stage of the California tiger salamander usually lasts three to six months, because most seasonal ponds and pools dry up during the summer, although some larvae in Contra Costa and Alameda Counties may remain in their breeding sites over the summer. Amphibian larvae must grow to a critical minimum body size before they can metamorphose (change into a different physical form) to the terrestrial stage. One study found larvae metamorphosed and left the breeding pools 60 to 94 days after the eggs had been laid, with larvae developing faster in smaller, more rapidly drying pools. The longer the inundation period, the larger the larvae and metamorphosed juveniles are able to grow, and the more likely they are to survive and reproduce. The larvae perish if a site dries before they complete metamorphosis. There was a strong positive correlation between inundation period and total number of metamorphosing juvenile amphibians, including tiger salamanders. Size at metamorphosis is positively correlated with stored body fat and survival of juvenile amphibians, and negatively correlated with age at first reproduction

Exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the California tiger salamander could preclude emergence prior to pond drying, resulting in larval mortalities.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the California tiger salamander's fertility rates substantially. The salamander's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Formal Draft Biological Opinion.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). In the section of Population Level Effects, we noted that reduction in fecundity and juvenile survival would have a greater population-level effect on species that have fewer opportunities to reproduce, like California tiger salamanders. Lifetime reproductive success for California and other tiger salamanders is low. One study found the average female bred 1.4 times and produced 8.5 young that survived to metamorphosis per reproductive effort. This resulted in roughly 11 metamorphic offspring over the lifetime of a female. Most California tiger salamanders in this study did not reach sexual maturity until four or five years old. While individuals may survive for more than 10 years, many breed only once, and one study estimated that less than five percent of metamorphic juveniles survive to become breeding adults. The mechanisms for recruitment are clearly dependent on a number of factors such as migration, terrestrial survival, and population turnover, whose interaction is not well understood.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the California tiger salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. The California tiger salamander has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected California tiger salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, California tiger salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the California Tiger salamander.

SONORA TIGER SALAMANDER

Ambystoma tigrinum stebbinsi

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that Sonora tiger salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, Sonora tiger salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

This species probably once inhabited springs, streams, backwaters, and cienegas that held permanent or nearly permanent water sources in the San Rafael Valley, Arizona, and Sonora, Mexico. Cattle ponds or tanks are now the primary habitat for Sonora tiger salamanders. Terrestrial salamanders likely spend much of the year in rodent burrows, rotted logs, and other moist cover sites. The life history of the Sonora tiger salamander can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that may be fully aquatic or primarily terrestrial. Sonora tiger salamanders begin their life as jelly-coated eggs laid in water. They hatch and grow as aquatic larvae with gills, and then either mature as gilled aquatic adults called branchiate adults, neotenes, or paedomorphs, or metamorphose into terrestrial salamanders without gills. Sonora tiger salamanders begin breeding as early as January, and eggs can be found in ponds as late as early May. Breeding after monsoon rains in July and August is rare. Courtship takes place under water, and after fertilization, female tiger salamanders lay 200 to 2000 eggs attaching them to aquatic vegetation, sticks, rocks, or substrate individually or in clumps of up to 50. Eggs take from 2-4 weeks to hatch; the colder the water, the longer the eggs take to develop. Following hatching, Sonora tiger salamander larvae can develop to the minimum size necessary to metamorphose in as little as two months. However, because many sites with Sonora salamanders hold water all year, larvae often remain in the water longer before metamorphosing, or develop into branchiate adults instead of metamorphosing. The proportion of larvae that metamorphose depends heavily on pond permanence. In ponds that dry, all larvae that are large enough metamorphose. In ponds that do not dry, approximately 17 percent of larvae that are large enough metamorphose (Collins *et al.* 1988).

Exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the Sonora tiger salamander could preclude emergence prior to pond drying, resulting in larval mortalities.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the Sonora salamander's fertility rates substantially. The salamander's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

In permanent water bodies, approximately 83% of the Sonora salamander larvae develop into branchiate adults instead of metamorphosing. In these cases, individuals could experience increased exposure to cyanide at the CCC due to their continued use of aquatic habitats throughout all stages of their life cycle. Whereas metamorphosed terrestrial adults may experience reduced adult growth, survival and fitness due to the long-term effects of cyanide exposure during egg and larval development, fully aquatic adults will be at greater risk of such adverse and compounded affects due to continued exposure to cyanide at CCC as adults.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). Metamorphs are the only life history stage that can disperse from pond to pond and establish new populations. Data suggest that only a small proportion of salamanders in a pond are likely to have dispersed from another pond, so salamanders in each pond are referred to as a population. For those populations consisting primarily of branchiate adults, habitat destruction associated with cyanide at CCC levels may adversely effect Sonora tiger salamander reproduction and survival to the extent that the population supported by that breeding pond becomes extirpated. Due to the low occurrence or dispersal amongst terrestrial adults, habitat destruction associated with cyanide at CCC levels may also result in population extirpation.

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Sonora tiger salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. The Sonora tiger salamander has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are either wholly aquatic or primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e.

breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Sonora tiger salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, Sonora tiger salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Sonora tiger salamander.

SANTA CRUZ LONG-TOED SALAMANDER

Ambystoma macrodactylum croceum

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that Santa Cruz long-toed salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, Santa Cruz long-toed salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The life history of the Santa Cruz long-toed salamander can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that may be fully aquatic or primarily terrestrial. Santa Cruz long-toed salamanders spend most of their lives underground in small mammal burrows and along the root systems of plants in upland chaparral and woodland areas of coast live oak (*Quercus agrifolia*) or Monterey pine (*Pinus radiata*) as well as riparian strips of arroyo willows (*Salix lasiolepis*) and other species. Ideal breeding locations appear to be shallow, temporary, freshwater ponds that lack fishes and hold water at least through the spring months. Eggs are laid singly on submerged stalks of spike rush (*Eleocharis* spp.) or other vegetation about two to three centimeters apart. Free floating, unattached, and clustered eggs have also been observed. Each female lays about 300 (range 215 to 411) eggs per year. Eggs usually hatch in 15 to 30 days. The larvae remain in the pond environment for 90 to 145 days. Larvae metamorphose when they reach a minimum size of about 32 mm snout to vent length. Metamorphosis can be accelerated by adverse pond conditions, such as reduction in food resources, water pollution, increased temperatures, and drying of the pond environment. Metamorphosed salamanders leave the pond. These salamanders become sexually mature in 3-4 years and do not return to the pond except to breed.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the Santa Cruz long-toed salamander could preclude emergence prior to pond drying, resulting in larval mortalities.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the Santa Cruz long-toed salamander's fertility rates substantially. The salamander's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). Santa Cruz long-toed salamanders are known from 3 metapopulations, each comprised of one or more subpopulations, located in Santa Cruz and Monterey Counties. Habitat destruction associated with cyanide at CCC levels may result in a reduction of available breeding sites and the loss of subpopulations that could lead to a reduction in population size and range and an increased vulnerability to catastrophic events that may adversely affect breeding and survival. Pollution, siltation, and the degradation of water quality in breeding ponds resulting from nearby development and agriculture was cited as one of the primary threats to the Santa Cruz long-toed salamander at the time of listing and is continues to be a threat to species survival

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Santa Cruz long-toed salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. The Santa Cruz long-toed salamander has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Santa Cruz long-toed salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, Santa Cruz long-toed salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Santa Cruz long-toed salamander.

Plethodontidae

SAN MARCOS SALAMANDER
Eurycea nana

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that San Marcos salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, San Marcos salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The San Marcos salamander is aquatic throughout its life cycle. Information on the reproduction of these salamanders is limited. It is found in rocky spring openings and rocky areas downstream from the dams at Spring Lake in San Marcos, TX, as well as in shallow spring areas on the northernmost portion of Spring Lake on a limestone shelf. Six essential elements are required for the San Marcos salamander: thermally constant water, flowing water, clean and clear water, sand, gravel, and rock substrates with little mud or detritus, vegetation for cover, and finally an adequate food supply. Males and females are sexually mature at 19 to 23.5 and 21 mm, respectively. No eggs have been found in nature, but the presence of gravid females and small larvae throughout the year suggests year round breeding. Artificial habitat studies have indicated that the average clutch size is 20, and that eggs are laid in standing pools with thick vegetation. Larvae emerge from the jelly covered eggs after 24 days.

The recovery plan lists a number of major threats to the San Marcos salamander, including decreases in water quantity and quality (including dissolved ions, trace elements, pH, nutrients, dissolved oxygen, and organic contaminants). As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and larval survival. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because the San Marcos salamander is aquatic throughout its entire life cycle, adults could also be at risk of increased adverse and compounded affects due to continued exposure to cyanide at CCC.

Little is known about the salamander's reproduction or longevity in the wild. We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the San Marcos Salamander's fertility rates substantially. The salamander's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

The San Marcos Salamander consists of a single population that is found only in Spring Lake and downstream in the San Marcos River below Spring Lake for 150 m. The San Marcos salamander population is estimated to exceed 53,000 individuals. Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the San Marcos salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected San Marcos salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, San Marcos salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the San Marcos salamander.

Critical Habitat: Critical habitat has been established for the San Marcos salamander in Hays County, Texas. It includes Spring Lake and its outflow and the San Marcos River downstream for 50 meters from the Spring Lake Dam. Primary constituent elements were not identified in the final rule designating critical habitat, but in order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of San Marcos salamanders and cause salamanders to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of San Marcos salamanders and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

BARTON SPRINGS SALAMANDER
Eurycea sosorum

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that Barton Springs salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, Barton Springs salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The Barton Springs salamander has been found only at the four spring outlets that make up Barton Springs within the City of Austin's Zilker Park in Travis County, Texas. The salamander requires stable aquatic environmental conditions, including perennially flowing spring water which is clear, clean, near neutral, varies very little in temperature (annual average must be 21 to 22 degrees Celsius) and proper flowing conditions to maintain dissolved oxygen content. The salamander also prefers clean, loose gravel substrates. The Barton Springs salamanders retain their larval gills throughout their lives, becoming mature and reproducing underwater. Known longevity for Barton Springs salamanders in captivity is at least 10 Years. Gravid females, eggs, and larvae are typically found throughout the year in the Barton Springs, which indicates that the salamander can reproduce year-round. Captive salamanders indicate that females are sexually mature at 11 to 17 months. During courtship the male deposits a spermatophore, which becomes attached to a plant, rock, or other substrate. Females can store the spermatophore in a specialized portion of the cloaca, known as the spermatheca for a month or longer. Females of some salamander species may store spermatophores for up to 2.5 years before ovulation and fertilization occur. In most salamanders, fertilization is internal and occurs during egg-laying whereby sperm are released onto eggs as they pass through the female's cloaca. Clutch sizes range from 5 to 39 eggs with an average of 22 eggs. Hatching of eggs in captivity occurred within 16 to 39 days after the eggs were laid, and the first three months following hatching were a critical period for juvenile survival.

Both the listing notice and the recovery plan for the Barton Springs salamander cite diminished water quality as a critical threat to the species. Analysis has shown that the water quality at Barton Springs has decreased. Dissolved oxygen has decreased (16 percent over 25 years) and conductivity (which has shown levels nearing the rate at which 100% mortality can be expected in 24 hours), sulfates, turbidity, nitrate-nitrogen, and total organic carbon have all increased. While data is limited concerning the Barton Springs salamander's vulnerability to contaminants, its semipermeable skin and reproductive processes suggest that it may be similar to other amphibians. As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and larval survival. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because the Barton Springs salamander is aquatic throughout its entire life cycle, adults could also be at risk of increased adverse and compounded affects due to continued exposure to cyanide at CCC.

Little is known about the salamander's reproduction or longevity in the wild. We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the Barton Springs Salamander's fertility rates substantially. The salamander's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

The Barton Springs Salamander consists of a single population that is found only within Barton Springs in Austin, TX. Total population estimates for the entire species are difficult because of the challenge posed by population surveys. Previous surveys would indicate a number in the hundreds at most, especially as some surveys have found no salamanders even in years with sufficient water flow. Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation). .

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Barton Springs salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Barton Springs salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, Barton Springs salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Barton Springs salamander.

TEXAS BLIND SALAMANDER
Typhlomolge rathbuni

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that Texas blind salamanders exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, Texas blind salamanders exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of larval salamanders.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The Texas blind salamander lives only underground in the Edwards Aquifer in Hays County, Texas. It is neotenic (non-transforming) and aquatic throughout its life. It lives in water filled caverns in the aquifer, and is well adapted to the environment, but is believed to be sensitive to temperature changes, as the aquifer has a near constant temperature of 21 degrees Celsius. It is likely that the Texas blind salamander is sexually active year round, which results from the very little seasonal change in the conditions of the aquifer. Gravid females have been found each month of the year. This species reproduced in captivity at the Cincinnati Zoo. In two months, three spawning events occurred. Clutch sizes ranged from 8 to 21 eggs. The unpigmented eggs were attached in ones, twos, and threes to pieces of gravel. Temperatures of close to 21 degrees Celsius are required for proper egg development.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and larval survival. Larval salamanders that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Reduced growth rates could also delay reproductive maturity and productivity. Because the Texas blind salamander is aquatic throughout its entire life cycle, adults could also be at risk of increased adverse and compounded affects due to continued exposure to cyanide at CCC.

The Texas blind salamander has been found only in Hays County, Texas and the total distribution of the species may be as small as 10 square kilometers. Population estimates have not been established. Little is known about the salamander's reproduction or longevity in the wild. We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the Texas blind salamander's fertility rates substantially. The salamander's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Texas blind salamander's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of larval salamanders. Salamanders may also experience effects on growth, locomotion, condition, and development. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the salamander's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Texas blind salamander population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, Texas blind salamanders are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Texas blind salamander.

Bufonidae

WYOMING TOAD
Bufo baxteri

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that Wyoming toads exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, Wyoming toads exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of tadpoles.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The life history of the Wyoming can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Wyoming toads inhabit floodplains, ponds, and the margins of small seepage lakes in the shortgrass communities of the Laramie Basin. The toad is known to exist in an area between 10 and 20 miles west of Laramie, Wyoming. Sightings since 1987 have come only from a 2 square mile area around Mortenson Lake and its associated meadows. Breeding occurs in shallow water typically less than six inches deep. Vegetated margins and bays of lakes, ponds, and irrigated meadows are preferred breeding areas. Breeding sites are often dry by late summer. Adult toads appear at breeding sites in May after daytime temperatures reach 70 degrees Fahrenheit. Males appear first and attract females with their calls. Breeding congregations are not large, usually consisting of half a dozen males and a few females gathering at a pond or lake margin. Breeding takes place from mid-May to mid-June depending upon weather conditions in any given year. Eggs are deposited in gelatinous strings containing 2,000 to 5,000 eggs and strands are often intertwined among vegetation. Eggs hatched in less than 1 week in water temperatures ranging between 77 degrees Fahrenheit during the day and 50 degrees at night. Tadpoles transformed into toadlets by 4 to 6 weeks following egg deposition.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Tadpoles that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the Wyoming toad could delay reproductive maturity and productivity.

No long-term studies have been performed to determine the maximum longevity of Wyoming toads in the wild. Corn (1993a) observed that few adult toads lived >2 yr at Mortenson Lake, but that was in a population afflicted with chytrid fungus. In captivity, one female toad with an estimated birth date in 1989 lived in captivity from 1994 until its death in 1997 (Callaway, 1998) and produced large numbers of healthy young from 1994 - 96. Little else is known about the toad's reproduction or longevity in the wild. We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the Wyoming toad's fertility rates substantially. The toad's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Wyoming toad's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of tadpoles. Toads may also experience effects on growth, locomotion, condition, and development. The Wyoming toad has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the toad's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Wyoming toad population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, Wyoming toads are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Wyoming toad.

ARROYO TOAD
Bufo californicus

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that arroyo toads exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, arroyo toads exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of tadpoles.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The arroyo toad occurs principally along coastal drainages, but it has been recorded at several locations on the desert slopes of the of the Transverse and Peninsular Mountain ranges south of the Santa Clara River, Los Angeles County, California. The life history of the Wyoming toad can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. The arroyo toad requires shallow, slow-moving stream habitats, and riparian habitats that are disturbed naturally on a regular basis, primarily by flooding. In the northern portion of their range, arroyo toads are found in foothill canyons and intermountain valleys where medium- to large-sized rivers are bordered closely by low hills, riverbed gradients are low, and the surface stream flows frequently pool or are intermittent for at least a few months of the year. In southern California (central portion of the arroyo toad's range), they also occur on the coastal plain and on a few desert slopes. For breeding, adult arroyo toads use open sites such as overflow pools, and old flood channels which are less than 30 cm deep with clear water. Breeding sites usually have flow rates less than 5 cm per second. The breeding period lasts from late January or February to early July, although weather can extend the period. If conditions are unsuitable, females may not obtain sufficient resources for egg production and will forgo breeding during that year. Anywhere from 2000-10,000 eggs are laid in two parallel gelatinous strings on substrates of sand, gravel, cobble, or mud generally located away from vegetation in the shallow margins of the pool. Embryos usually hatch in 4 to 6 days at water temperatures of 12 to 16 degrees Celsius (54 to 59 degrees Fahrenheit). The larval period for arroyo toads lasts about 65 to 85 days, depending on water Temperatures. Newly metamorphosed juveniles remain on sparsely vegetated sand and gravel bars bordering the natal pool for 3–5 wk (Sweet, 1992). Male arroyo toads can reach sexual maturity in 1 year, if conditions are favorable, but females require 2 or 3 years. Mark–recapture studies suggest that few arroyo toads survive into their fifth year, and that these are predominantly females (Sweet, 1993). In the absence of American bullfrogs, adult arroyo toads have a high survivorship during the active season, but suffer 55–80% mortality as they overwinter (Sweet, 1993).

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Tadpoles that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the arroyo toad could delay reproductive maturity and productivity.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the arroyo toad's fertility rates substantially. The toad's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the arroyo toad's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of tadpoles. Toads may also experience effects on growth, locomotion, condition, and development. The arroyo toad has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the toad's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected arroyo toad population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, arroyo toads are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the arroyo toad.

Critical Habitat: Critical habitat consists of 11,695 acres in five counties in California: Riverside, San Bernardino, Los Angeles, Ventura, Santa Barbara. The physical and biological features of critical habitat essential to the conservation of the arroyo toad include rivers or streams with hydrologic regimes that supply water to provide space, food, and cover needed to sustain eggs, tadpoles, metamorphosing juveniles, and adult breeding toads. In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of arroyo toads and cause toads to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of arroyo toads and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

HOUSTON TOAD
Bufo houstonensis

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that Houston toads exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, Houston toads exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of tadpoles.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Houston toads are associated with forest ecosystems and sandy soils. The life history of the Houston toad can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Adult Houston toads respond to cold or summer heat by burrowing into moist sand or hiding under rocks, leaf litter, logs, or in abandoned animal burrows. Breeding occurs in ephemeral, rain-fed pools, flooded fields, and permanent ponds from late January to June with a peak from February to March. Reported egg-laying dates range from February 18 through June 26 and clutch sizes range from 512 to 6,199. Depending on environmental conditions, eggs may hatch within a week and tadpoles develop into toadlets within 40-80 days. Mortality rates are high, and only 1% of eggs laid are believed to survive to adulthood. Captive raised males are sexually mature at 1 year and females at 1 to 2 years.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Tadpoles that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the Houston toad could delay reproductive maturity and productivity. For tadpoles developing in ephemeral ponds, reduced growth rates resulting in a prolonged time to metamorphosis could preclude full development prior to pond drying, resulting in larval mortalities.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the Houston toad's fertility rates substantially. The toad's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Houston toad's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of tadpoles. Toads may also experience effects on growth, locomotion, condition, and development. The Houston toad has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the toad's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Houston toad population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, Houston toads are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Houston toad.

Critical Habitat: Critical habitat has been established in Bastrop, Burleson and Harris County, Texas. Although not described when critical habitat was designated, physical and biological features essential to the conservation of the Houston toad include seasonally-flooded breeding ponds, deep sandy soils, and forest or woodlands. In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of Houston toads and cause toads to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of Houston toads and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

Eleutherodactylidae

GUAJON
Eleutherodactylus cooki

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that guajons exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, guajons exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of tadpoles.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The guajon is associated with the granitic rocks found in the Cuchilla de Panduras mountain range in southeastern Puerto Rico where it inhabits caves formed by large boulders of granite rock known as “guajonales,” but can also be found in associated streams with patches of rock without caves systems. In streams, the guajón has been found only in patches of rock in the streambed. The streams can be perennial, or ephemeral formed during heavy rain and are surrounded by secondary forest. Rocks in the streambed form crevices and grottoes. The guajón deposits eggs on humid boulders within grottoes and on flat surfaces. Eggs are guarded by males. The mean clutch size of the guajón is 17.35 eggs, the developmental time of eggs is 20 to 29 days, and parental care contributes to hatching success. Hatching success of this species is 85 percent, with hatchlings remaining together as a group in the nest for several days before dispersing.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Tadpoles that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the guajon could delay reproductive maturity and productivity. For tadpoles developing in ephemeral ponds, reduced growth rates resulting in a prolonged time to metamorphosis could preclude full development prior to pond drying, resulting in larval mortalities.

Little else is known about the frog's reproduction or longevity in the wild. We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the guajon's fertility rates substantially. The frog's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the guajon's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of tadpoles. Frogs may also experience effects on growth, locomotion, condition, and development. The guajon has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the frog's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected guajon population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, guajons are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the guajon.

Critical Habitat: Critical habitat units were established for Humacao, Las Piedras, Maunabo, Patillas, and Yabucoa, Puerto Rico. The physical and biological features of critical habitat essential to the conservation of the guajon includes plutonic, granitic, or sedimentary rocks/boulders that form caves, crevices, and grottoes (interstitial spaces) in a streambed, and that are in proximity, or connected, to a permanent, ephemeral, or subterranean clear-water stream or water source. The interstitial spaces between or underneath rocks provide microenvironments characterized by generally higher humidity and cooler temperatures than outside the rock formations. In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of guajon and cause these frogs to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of California red-legged frogs and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

Ranidae

CALIFORNIA RED-LEGGED FROG

Rana aurora draytonii

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that California red-legged frogs exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, California red-legged frogs exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of tadpoles.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

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California red-legged frogs have been documented in 46 counties in California, but now remain in only 238 streams or drainages in 31 counties. The life history of the California red-legged frog can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily aquatic but use a variety of aquatic, riparian, and upland habitats. The California red-legged frog's larvae, tadpoles, and metamorphs can be found in streams, deep pools, backwaters, creeks, ponds, marshes, sag ponds, dune ponds, and lagoons. Breeding adults are commonly associated with water deeper than 0.7 m (2 feet) which is slow moving and choked by shrubby riparian or emergent vegetation. California red-legged frogs breed from November to April. Males appear at breeding sites 2-4 weeks before females. Once a pair of frogs has moved into the breeding position, they move to where the eggs are laid and fertilized. The 2,000 to 5,000 eggs float near the surface, attached to emergent vegetation, roots, or twigs. Eggs hatch within 6 to 14 days depending on water temperatures and require approximately 20 days to develop into tadpoles. Tadpoles in turn require anywhere between 11 to 20 weeks to develop into terrestrial frogs. At some locations, larvae may overwinter before metamorphosing. Water bodies suitable for tadpole rearing must remain watered at least until the tadpoles metamorphose into adults, typically between July and September. Adult California red-legged frogs can survive in moist upland areas after breeding habitat has dried, and can live several years to make new breeding attempts. Therefore, aquatic breeding habitat need not be available every year, but it must be available often enough and for appropriate hydroperiods to maintain a California red-legged frog population during most years.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Tadpoles that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the California red-legged frog could delay reproductive maturity and productivity. For tadpoles developing in ephemeral ponds, reduced growth rates resulting in a prolonged time to metamorphosis could preclude full development prior to pond drying, resulting in larval mortalities.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the California red-legged frog's fertility rates substantially. The frog's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the California red-legged frog's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of tadpoles. Frogs may also experience effects on growth, locomotion, condition, and development. The California red-legged frog has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the frog's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected California red-legged frog population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, California red-legged frogs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the California red-legged frog.

Critical Habitat: California red-legged frog critical habitat has been established in 34 locations in Alameda, Butte, Contra Costa, El Dorado, Kern, Los Angeles, Marin, Merced, Monterey, Napa, Nevada, San Benito, San Luis Obispo, San Mateo, Santa Barbara, Santa Clara, Santa Cruz, Solano, Ventura and Yuba Counties, California, and includes aquatic breeding and non-breeding habitats that provide the physical and biological features of critical habitat essential to the conservation of the California red-legged frog. Aquatic breeding habitat is essential for providing space, food, and cover necessary to sustain the early life history stages of larval and juvenile California red-legged frogs. It consists of low-gradient fresh water bodies, including natural and manmade (e.g., stock) ponds, backwaters within streams and creeks, marshes, lagoons, and dune ponds. It does not include deep lacustrine water habitat (e.g., deep lakes and reservoirs 50 ac (20 ha) or larger in size). To be considered essential breeding habitat, the aquatic feature must have the capability to hold water for a minimum of 20 weeks in all but the driest of years.

Nonbreeding aquatic habitat consists of those aquatic elements identified above, and also includes, but is not limited to, other wetland habitats such as intermittent creeks, seeps, and springs. California red-legged frogs can use large cracks in the bottom of dried ponds as refugia to maintain moisture and avoid heat and solar exposure. Without these non-breeding aquatic features, California red-legged frogs would not be able to survive drought periods, or be able to disperse to other breeding habitat.

In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of California red-legged frogs and cause frogs to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of California red-legged frogs and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

CHIRICAHUA LEOPARD FROG
Rana chiricahuensis

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that Chiricahua leopard frogs exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, Chiricahua leopard frogs exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of tadpoles.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

The range of the Chiricahua leopard frog is split into two disjunct parts - northern populations along the Mogollon Rim in Arizona east into the mountains of west-central New Mexico, and southern populations in southeastern Arizona, southwestern New Mexico, and Mexico The life history of the Chiricahua leopard frog can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily aquatic. It is an inhabitant of montane and river valley cienegas, springs, pools, cattle tanks, lakes, reservoirs, streams, and rivers. It is a habitat generalist that historically was found in a variety of aquatic habitat types, but is now limited to the comparatively few aquatic systems that support few or no non-native predators (e.g. American bullfrogs, fishes, and crayfishes). The species also requires permanent or semi-permanent pools for breeding, water characterized by low levels of contaminants and moderate pH, and may be excluded or exhibit periodic die-offs where a pathogenic chytridiomycete fungus is present. Egg masses of Chiricahua leopard frogs have been reported in all months except January, November, and December, but reports of oviposition in June are uncommon. Hatching time of egg masses in the wild has not been studied in detail. Eggs of the Ramsey Canyon leopard frog hatch in approximately 14 days depending on temperature, and hatching time may be as short as eight days in geothermally influenced springs. Tadpoles metamorphose in three to nine months (Jennings 1988, 1990), and may overwinter.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Tadpoles that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the Chiricahua leopard frog could delay reproductive maturity and productivity. For tadpoles developing in ephemeral ponds, reduced growth rates resulting in a prolonged time to metamorphosis could preclude full development prior to pond drying, resulting in larval mortalities.

Formal Draft Biological Opinion.

Little is known about age and size at reproductive maturity or the longevity of the Chiricahua leopard frog. We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the Chiricahua leopard frog's fertility rates substantially. The frog's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the Chiricahua leopard frog's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of tadpoles. Frogs may also experience effects on growth, locomotion, condition, and development. The Chiricahua leopard frog has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily aquatic. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the frog's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected Chiricahua leopard frog population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, Chiricahua leopard frogs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the Chiricahua leopard frog.

MOUNTAIN YELLOW-LEGGED FROG
Rana muscosa
Southern California Population

Relatively few studies were available for estimating the magnitude of effects that could occur following exposure to cyanide at criterion concentrations. Based on multiple lines of evidence, including the relative sensitivity of amphibians to other pollutants, their relative sensitivity to rainbow trout and the sensitivity of rainbow trout to cyanide we conclude that mountain yellow-legged frogs exposed to cyanide at the CCC could experience substantial reductions in recruitment due to reductions in fecundity, hatching success, and larval development and survival. Compared to control populations, we estimate rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in the number of hatched eggs and that reduction could be as much as, but is not likely to be greater than 52% (Appendix E). We estimate that rainbow trout exposed to cyanide at the CCC could experience a substantial reduction in survival of young fish through the first year and that reduction could be as much as, but is not likely to be greater than 61% (Appendix E). Based on the relative sensitivity of amphibians as compared to rainbow trout we estimate the effects of cyanide at the CCC for amphibians could be of similar or greater magnitude than effects estimated for rainbow trout. As such, mountain yellow-legged frogs exposed to cyanide at the CCC could experience a substantial reduced egg hatching and decreased survival to metamorphosis of tadpoles.

Although no comparable data are available for amphibians, cyanide may also adversely effect growth, locomotion, condition, and development as described above for fish in the Effects Overview. The effects of chronic toxicity could also be influenced by other stressors such as dissolved oxygen concentration, temperature, and the presence of other pollutants in the water column.

Currently the mountain yellow-legged frog is known from only seven locations in southern California in portions of the San Gabriel, San Bernardino, and San Jacinto Mountains. The life history of the mountain yellow-legged frog can be characterized as a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily aquatic. Water depth, persistence, and configuration (*i.e.* gently sloping shorelines and margins) appear to be important for mountain yellow-legged frogs, allowing for shelter from predators along shores or in deeper waters, and habitat for breeding, foraging, egg-laying, thermoregulation (to regulate the body temperature through behavior), and overwintering. Breeding activity typically begins in April at lower elevations, to June or July at upper elevations and continues for approximately a month. Egg masses vary in size from as few as 15 eggs to 350 eggs per mass, which is considered low, relative to a range of several hundred to several thousand for other true frogs. Egg masses are normally deposited in shallow waters where they may be attached to rocks, gravel, vegetation, or similar substrates. As larvae develop, they tend to gravitate towards warmer waters to elevate body temperatures which may facilitate larval and metamorphic development by allowing for a higher metabolic rate. Even with this behavior, larvae apparently must overwinter at least two times for 6 to 9 month intervals before attaining metamorphosis because the active season is short and the aquatic habitat maintains warm temperatures for only brief intervals. Time to develop from fertilization to metamorphosis appears to be variable, ranging up to 3.5 years, with reproductive maturity reached from 3 to 4 years following metamorphosis. Little is known about adult longevity, but the species is presumed to be long-lived due to adult survivorship.

As noted above, exposure to cyanide at the CCC could result in a substantial reduction in egg hatching and survival to metamorphosis. Tadpoles that do survive could experience reduced growth rates and reduced swimming abilities which would increase their vulnerability to a host of potential stressors including, temperature, flow, predation, and inter- and intraspecific competition for food and cover. Because amphibian larvae must grow to a critical minimum body size before they can metamorphose to the terrestrial stage, reduced growth rates of the mountain yellow-legged frog could delay reproductive maturity and productivity.

We anticipate that the estimated reductions in hatched eggs and larval survival would reduce the mountain yellow-legged frog's fertility rates substantially. The frog's fertility rates would be reduced based upon (a) reductions in numbers of eggs hatched, (b) reductions in larvae surviving through the first year and (c) the additive effect of reduced fecundity and survival. The combined effect could be less than additive if the eggs that hatch in the presence of cyanide represent individuals that are less sensitive. These reductions in reproduction could be exacerbated further if reduced growth rated delay maturity or lengthen the time between mating.

Reductions in reproductive performance and survival represent reductions in the fitness of the individuals exposed to cyanide at the CCC. Changes in the fitness of the individuals in a population will affect the population as a whole. Effects to populations can be measured in terms of changes in population growth rates and changes in risk of extinction (or extirpation).

In summary, exposure to cyanide concentrations at the chronic criterion could substantially reduce the mountain yellow-legged frog's reproduction by reducing the number of eggs laid by females, the hatchability of laid eggs, and a reduction of survivorship of tadpoles. Frogs may also experience effects on growth, locomotion, condition, and development. The mountain yellow-legged frog has a complex life cycle, consisting of eggs and larvae that are entirely aquatic and adults that are primarily terrestrial. Although exposure to cyanide concentrations at the chronic criterion may be isolated to the aquatic phases of the frog's life cycle (i.e. breeding activities and egg and larval development), the long-term effects of exposure could result in reduced adult growth, survival and fitness. Because of the high magnitude of effects, we would expect density-dependent compensatory mechanisms, if they exist, to be overwhelmed. An effected mountain yellow-legged frog population's decline could stabilize at a reduced absolute population number or could continue to decline until it's extirpated. For extant populations supported by a single breeding pond, reproductive failure at that site could result in the extirpation of the population and even those populations with multiple breeding sites may have difficulty recolonizing after local extirpations due to physiological, morphological, and behavioral constraints. Based upon the magnitude of effect we anticipate could occur, we conclude that ultimately, mountain yellow-legged frogs are likely to become extirpated from waters where they are exposed to cyanide toxicity at the chronic criterion concentration. Continued approval of the chronic criterion could reduce the reproduction, numbers, and distribution of the mountain yellow-legged frog.

Critical Habitat: The physical and biological features of critical habitat essential to the conservation of the mountain yellow-legged frog include (1) Water source(s) found between 1,214 to 7,546 feet (370 to 2,300 meter) in elevation that are permanent. Water sources include, but are not limited to, streams, rivers, perennial creeks (or permanent plunge pools within intermittent creeks), pools (i.e., a body of impounded water that is contained above a natural dam) and other forms of aquatic habitat. Aquatic habitats that are used by mountain yellow-legged frog for breeding purposes must maintain water during the entire tadpole growth phase, which can last for up to 2 years. During periods of drought, or less than average rainfall, these breeding sites may not hold water long enough for individuals to complete metamorphosis, but they would still be considered essential breeding habitat in wetter years. Further, the aquatic includes: a.) Bank and pool substrates consisting of varying percentages of soil or silt, sand, gravel cobble, rock, and boulders; b.) Open gravel banks and rocks projecting above or just beneath the surface of the water for sunning posts; c.) Aquatic refugia, including pools with bank overhangs, downfall logs or branches, and/or rocks to provide cover from predators; and d.) Streams or stream reaches between known occupied sites that can function as corridors for movement between aquatic habitats used as breeding and/or foraging

In order for these aquatic areas to provide the necessary conditions for successful breeding and survival they must include water of sufficient quality to allow the species to carry out normal feeding, movement, sheltering, and reproductive behaviors and to experience individual and population growth. Approval of the CCC in State and Tribal water quality standards would authorize States and Tribes to continue to manage cyanide in waters to the criteria concentrations. This approval could adversely affect the quality of water to the degree that it would impair individual reproduction and survival of California red-legged frogs and cause frogs to experience adverse effects to growth, locomotion, condition, and development. Based on data for the rainbow trout, our surrogate species for assessing impacts to amphibians, we estimate the reduction in number of hatched eggs could be as much as, but is not likely to be greater than 52% and the reduction in larval survival to metamorphosis could be as much as, but is not likely to be greater than 61%. These effects are estimated to of a magnitude great enough to reduce numbers of California red-legged frogs and result in the extirpation of local populations. Approval of the CCC would adversely affect the quality of water to the degree that normal population growth is likely to be impacted and could result in extirpation from critical habitat containing cyanide at the CCC. Impacts to water quality resulting from management of cyanide to the CCC would diminish the ability of critical habitat to provide for conservation of the species.

8.0 CUMULATIVE EFFECTS

Cumulative effects include the effects of future State, tribal, local or private actions that are reasonably certain to occur in the action area considered in this biological opinion. Future Federal actions that are unrelated to the proposed action are not considered in this section because they require separate consultation pursuant to section 7 of the ESA.

As noted in Section 5.0 above, the action area for this consultation consists of all waters of the United States, including territorial seas, which extend seaward a distance of three miles from the coast (CWA section 502), where federally listed endangered, threatened, and proposed species reside. The action area includes such waters within and surrounding Indian country, the 50 States, and all U.S. territories. Given the size of the action area, it is not practical to specifically evaluate cumulative effects in this biological opinion.

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In general, the threatened and endangered aquatic species and designated critical habitats considered in this biological opinion are likely to be adversely affected by non-federal activities that affect the quantity, quality, and hydrographic patterns of water, waterways, and habitats important to these species and critical habitats. These activities could include changes in land and water use and management patterns in ways that increase erosion and sedimentation, increase introduction of pollutants into waterways, and result in introductions and spread of non-native invasive species that directly or indirectly affect listed species and critical habitats. These species and their critical habitats could also be affected by illegal harvest. States or private entities may also engage in activities to restore, enhance, and improve water quality and quantity and restore more natural hydrographic patterns that benefit listed species and their habitats. All of the species and critical habitats considered in this document are likely to be subject to these types of activities in the future to varying extents. The final listing and designation rules, recovery plans, and 5-year reviews for these species and critical habitats are good sources of information, in part, on the threats and benefits associated with these types of activities. These documents are cited in Appendix A.

9.0 CONCLUSION

After reviewing the current status of the following listed species, the environmental baseline for the action area, the effects of EPA's continuing approval of state water quality standards that rely on their nationally recommended criteria for cyanide, and cumulative effects, it is the Service's biological opinion that the action, as proposed, is likely to jeopardize the continued existence of the following species:

Gulf sturgeon, Kootenai River white sturgeon, Pallid sturgeon, Alabama sturgeon, Ozark cavefish, Alabama cavefish, Waccamaw silverside, Modoc sucker, Santa Ana sucker, Warner sucker, Shortnose sucker, Cui-ui, June sucker, Lost River sucker, Razorback sucker, Pygmy sculpin, Blue shiner, Beautiful shiner, Devils River minnow, Spotfin chub, Slender chub, Mojave tui chub, Owens tui chub, Borax Lake chub, Humpback chub, Sonora chub, Bonytail chub, Gila chub, Yaqui chub, Pahranagat roundtail chub, Virgin River Chub, Rio Grande silvery minnow, Big Spring spinedace, Little Colorado spinedace, Spikedace, Moapa dace, Palezone shiner, Cahaba shiner, Arkansas River shiner, Cape Fear shiner, Pecos bluntnose shiner, Topeka shiner, Oregon chub, Blackside dace, Woundfin, Colorado pikeminnow (=squawfish), Ash Meadows speckled dace, Kendall Warm Springs dace, Loach minnow, Unarmored threespine stickleback, Tidewater goby, White River springfish, Hiko White River springfish, Railroad Valley springfish, Delta smelt, Slackwater darter, Vermilion darter, Relict darter, Etowah darter, Fountain darter, Niangua darter**,** Watercress darter, Okaloosa darter, Duskytail darter, Bayou darter, Cherokee darter, Maryland darter, Bluemask darter, Boulder darter, Amber darter, Goldline darter, Conasauga logperch, Leopard darter, Roanoke logperch, Snail darter, Big Bend gambusia, San Marcos gambusia, Clear Creek gambusia, Pecos gambusia, Gila topminnow, Bull trout, Little Kern Golden trout, Apache trout, Lahontan Cutthroat trout, Paiute Cutthroat trout, Greenback Cutthroat Mountain trout, Gila trout, Atlantic salmon, Illinois cave amphipod,

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Noel's amphipod, Cumberland elktoe, Dwarf wedgemussel, Appalachian elktoe, Fat three-ridge, Ouachita rock pocketbook, Birdwing pearlymussel, Fanshell, Dromedary pearlymussel, Chipola slabshell, Tar River spinymussel, Purple bankclimber, Cumberlandian combshell, Oyster mussel, Curtis pearlymussel, Yellow blossom, Tan riffleshell, Upland combshell, Catspaw, White catspaw, Southern acornshell, Southern combshell, Green blossom, Northern riffleshell, Tubercled blossom, Turgid blossom, Shiny pigtoe, Finerayed pigtoe, Cracking pearlymussel, Pink mucket, Fine-lined pocketbook, Higgins eye, Orangenacre mucket, Arkansas fatmucket, Speckled pocketbook, Shinyrayed pocketbook, Alabama lampmussel, Carolina heelsplitter, Scaleshell mussel, Louisiana pearlshell, Alabama moccasinshell, Coosa moccasinshell, Gulf moccasinshell, Ochlockonee moccasinshell, Ring pink, Littlewing pearlymussel, White wartyback pearlymussel, Orangefoot pimpleback, Clubshell, James spinymussel, Black clubshell, Southern clubshell, Dark pigtoe, Southern pigtoe, Cumberland pigtoe, Flat pigtoe, Ovate clubshell, Rough pigtoe, Oval pigtoe, Heavy pigtoe, Fat pocketbook, Alabama heelsplitter, Triangular kidneyshell, Rough rabbitsfoot, Winged mapleleaf, Cumberland monkeyface, Appalachian monkeyface, Stirrupshell, Pale Lilliput, Purple bean, Cumberland bean, reticulated flatwoods salamander, frosted flatwoods salamander California tiger salamander (central California DPS), California tiger salamander (Santa Barbara County DPS), California tiger salamander (Sonoma DPS), Santa Cruz long-toed salamander, Sonora Tiger salamander, San Marcos salamander, Barton Springs salamander, Texas blind salamander, Wyoming toad, arroyo toad, Houston toad, guajon, California red-legged frog, Chiricahua leopard frog, mountain yellow-legged frog

Exposure of the above listed fish species to cyanide at the proposed chronic criterion concentration is likely to substantially reduce their reproduction by reducing the number of eggs spawned by females, reducing the hatchability of spawned eggs, and by reducing the survivorship of young fish in their first year. These fish may also experience effects on growth, swimming performance, condition, and development. In addition, Fountain darters, Bull trout, Apache trout, and Lahontan cutthroat trout exposed to cyanide at the acute criterion are likely to experience substantial reductions in survival. Based upon the magnitude of adverse effects caused by the exposure of these listed species to cyanide at the proposed acute and/or chronic criteria concentrations, these fish species are likely to become extirpated from waters where they are exposed to cyanide toxicity at the CMC and/or CCC. Continued approval of the acute and/or chronic criteria at the rangewide scale of these listed species is likely to reduce their reproduction, numbers, and distribution.

Exposure of the Illinois cave amphipod and Noel's amphipod to cyanide at the chronic criterion concentration is likely to result in the loss of individuals, especially in situations when these amphipods are subject to interspecific resource competition or predation. Because both amphipod species exist in populations that are geographically isolated from one another, the ability of amphipods to recolonize perturbed habitats is limited. Thus, cyanide exposure may result in the elimination of a population unit that cannot rebound. The loss of a population unit for either amphipod species would substantially reduce the reproduction, numbers, or distribution of these species.

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For the above mentioned mussel species, exposure of their host fish to cyanide at criterion concentrations is likely to reduce the abundance of fish hosts for glochidia, thereby decreasing the likelihood that glochidia will survive because they will be unable to attach to suitable host. Since attachment of glochidia to a suitable host is a rare and necessary event in the mussel reproductive cycle, reductions in host fish abundance are likely to negatively impact mussel reproductive output and ultimately population numbers. Thus, host fish abundance for these species is anticipated to substantially decline to levels that will reduce reproduction, numbers, or distribution of these mussel species.

After reviewing the current status of critical habitat, the environmental baseline for the action area, the effects of EPA's continuing approval of state water quality standards that rely on their nationally recommended criteria for cyanide, and cumulative effects, it is the Service's biological opinion that the action, as proposed, is likely to result in the destruction or adverse modification of critical habitat that has been designated for the following species:

Gulf sturgeon, Kootenai River white sturgeon, Alabama sturgeon, Alabama cavefish, Waccamaw silverside, Modoc sucker, Santa Ana sucker, Warner sucker, June sucker, Razorback sucker, Beautiful shiner, Devils River minnow, Spotfin chub, Slender chub, Owens tui chub, Borax Lake chub, Humpback chub, Sonora chub, Bonytail chub, Gila chub, Yaqui chub, Virgin River Chub, Rio Grande silvery minnow, Big Spring spinedace, Little Colorado spinedace, Spikedace, Arkansas River shiner, Cape Fear shiner, Pecos bluntnose shiner, Topeka shiner, Woundfin, Colorado pikeminnow (=squawfish), Loach minnow, Tidewater goby, White River springfish, Hiko White River springfish, Railroad Valley springfish, Delta smelt, Slackwater darter, Fountain darter, Niangua darter**,** Maryland darter, Amber darter, Conasauga logperch, Leopard darter, Snail darter, San Marcos gambusia, Bull trout, Little Kern Golden trout, Cumberland elktoe, Appalachian elktoe, Fat three-ridge, Chipola slabshell, Purple bankclimber, Cumberlandian combshell, Oyster mussel, Upland combshell, Southern acornshell, Fine-lined pocketbook, Orangenacre mucket, Shinyrayed pocketbook, Carolina heelsplitter, Alabama moccasinshell, Coosa moccasinshell, Gulf moccasinshell, Ochlockonee moccasinshell, Southern clubshell, Dark pigtoe, Southern pigtoe, Ovate clubshell, Oval pigtoe, Triangular kidneyshell, Rough rabbitsfoot, Purple bean, reticulated flatwoods salamander, frosted flatwoods salamander, California tiger salamander (central California DPS), California tiger salamander (Santa Barbara County DPS), San Marcos salamander, arroyo toad, Houston toad, guajon, California red-legged frog, mountain yellow-legged frog

The physical and biological features of critical habitat essential to the conservation of these listed species include water of sufficient quality for species to carry out normal feeding, movement, sheltering, and reproductive behaviors, and to experience individual and population growth. Approval of the CCC in state water quality standards would authorize states to manage cyanide in waters to these levels. This approval and cyanide in waters to these levels is likely to adversely affect the quality of water to the degree that it would impair individual reproduction and survival of these listed fish species as well as fish species that are hosts for the above mentioned listed mussels. Approval of the CCC and cyanide in waters to these levels is likely to adversely affect the quality of water to the degree that it would impair normal population growth and likely cause the extirpation of these listed fish from their critical habitat containing cyanide at the CCC. In addition, the majority of fish hosts identified for the above mentioned mussel species are likely to exhibit population declines at cyanide criteria concentrations. For these reasons, impacts to water quality resulting from cyanide in waters to the level of the CCC would diminish the intended conservation function of critical habitat for these listed fishes and mussels.

10.0 REASONABLE AND PRUDENT ALTERNATIVES

The regulations (50 CFR 402.02) implementing section 7 of the ESA define reasonable and prudent alternatives (RPAs) as alternative actions, identified during formal consultation, that: (1) can be implemented in a manner consistent with the intended purpose of the action; (2) can be implemented consistent with the scope of the action agency's legal authority and jurisdiction; (3) are economically and technologically feasible; and (4) would, the Service believes, avoid the likelihood of jeopardizing the continued existence of listed species or the destruction or adverse modification of critical habitat.

The Service has developed the following RPAs to the EPA's proposed action:

1. By December 1, 2012, EPA shall, subject to Service approval, review the geographic ranges of the listed species and designated critical habitats addressed in this biological opinion and insure that the water bodies or water body segments within those ranges include: a) a designated use for which aquatic life criteria apply; b) aquatic life cyanide criteria at least as stringent as described below; and, c) appropriate language within any EPA-approved general policies requiring State coordination with local Service field offices on implementation of general policies. The Service recommends the EPA consider the following criteria for water bodies and water body segments:

   Fish: The acute and chronic cyanide criteria for protection of listed fish species are shown in Table 21. These values are based on the acute and chronic Assessment Effects Concentrations (EC_A) which represent the *the highest* concentration of cyanide where the effects on listed species are expected to be insignificant (see Appendix B for details).

Table 21. Freshwater acute and chronic cyanide criteria for the protection of listed fish species (NC, no change).

Listed Species		Order/Family	Surrogate Taxa	Recommended Acute Criteria [Acute EC_A (ug CN/L)]	Recommended Chronic Criteria [Chronic EC_A (ug CN/L)]
Gulf sturgeon	*Acipenser oxyrinchus desotoi*	Acipenseriformes Acipenseridae (sturgeon)	Actinopterygii (class)	NC	2.86
Kootenai River white sturgeon	*Acipenser transmontanus*				
Pallid sturgeon	*Scaphirhynchus albus*				

Alabama sturgeon	*Scaphirhynchus suttkusi*				
Waccamaw silverside	*Menidia extensa*	Atheriniformes Atherinopsidae			
Modoc sucker	*Catostomus micorps*	Cypriniformes Catosdomidae (suckers)	Cypriniformes (order)	NC	3.64
Santa Anna sucker	*Catostomus santaanae*				
Warner sucker	*Catostomus warnerensis*				
Shortnose sucker	*Chasmistes brevirostris*				
Cui ui	*Chasmistes cujus*				
June sucker	*Chasmistes liorus*				
Lost River sucker	*Deltistes luxatus*				
Razorback sucker	*Xyrauchen texanus*		*Xyrauchen texanus*	NC	3.61
Spotfin chub	*Cyprinella monacha*	Cypriniformes Cyprinidae	*Cyprinella monacha*	NC	1.58
Blue shiner	*Cyprinella caerulea*		Cyprinidae (family)	NC	4.38
Beautiful shiner	*Cyprinella formosa*				
Devils River minnow	*Dionda diaboli*				
Slender chub	*Erimystax cahni*				
Mohave tui chub	*Gila bicolor mohavensis*				
Owens tui chub	*Gila bicolor snyderi*				
Hutton tui chub	*Gila bicolor* ssp.				
Borax Lake chub	*Gila boraxobius*				
Humpback chub	*Gila cypha*				
Sonora chub	*Gila ditaenia*				
Gila chub	*Gila intermedia*				
Yaqui chub	*Gila purpurea*				
Pahranagat roundtail chub	*Gila robusta jordani*				
Virgin River chub	*Gila robusta seminuda*				
Rio Grand silvery minnow	*Hybognathus amarus*				
Big Spring spinedace	*Lepidomeda mollispinis pratensis*				
Little Colorado spinedace	*Lepidomeda vittata*				
Spikedace	*Meda fulgida*				
Moapa dace	*Moapa coriacea*				
Palezone shiner	*Notropis albizonatus*				
Cahaba shiner	*Notropis cahabae*				
Arkansas River shiner	*Notropis girardi*				
Pecos bluntnose shiner	*Notropis simus pecosensis*				
Topeka shiner	*Notropis Topeka*				
Oregon chub	*Oregonichthys crameri*				
Blackside dace	*Phoxinus cumberlandensis*				
Woundfn	*Plagopterus agrentissimus*				
Ash Meadows speckled dace	*Rhinichthys osculus nevadensis*				
Kendall Warm Springs dace	*Rhinichthys osculus thermalis*				
Foskett speckled dace	*Rhinichthys osculus* ssp.				
Loach minnow	*Tiaroga cobitis*				
Bonytail chub	*Gila elegans*		*Gila elegans*	NC	2.19

Cape Fear shiner	*Notropis mekistocholas*		*Notropis mekistocholas*	NC	2.09
Colorado pikeminnow	*Ptychocheilus lucis*		*Ptychocheilus lucis*	NC	1.87
White River springfish	*Crenichthys baileyi baileyi*	Cyprinodontiformes Goodeidae	Actinopterygii (class**)**	NC	2.86
Hiko White River springfish	*Crenichthys baileyi grandis*				
Railroad Valley springfish	*Crenichthys nevadae*				
Big Bend gambusia	*Gambusia gaigei*	Cyprinodontiformes Poeciliidae			
San Marcos gambusia	*Gambusia georgei*				
Clear Creek gambusia	*Gambusia heterochir*				
Pecos gambusia	*Gambusia nobilis*				
Gila topminnow	*Poeciliopsis occidentalis occidentalis*				
Yaqui topminnow	*Poeciliopsis occidentalis sonoriensis*				
Unarmoned threespine stickleback	*Gasterosteus aculeatus williamsoni*	Gasterosteiformes Gasterosteidae			
Delta smelt	*Hypomesus transpacificus*	Osmeriformes Osmeridae			
Tidewater goby	*Eucyclogobius newberryi*	Perciformes Gobiidae	Perciformes (order)	NC	3.91
Slackwater darter	*Etheostoma boschungi*	Perciformes Percidae	*Etheostoma* (genus)	NC	1.72
Vermilion darter	*Etheostoma chermocki*				
Relict darter	*Etheostoma chienense*				
Etowah darter	*Etheostoma etowahae*				
Niangua darter	*Etheostoma nianguae*				
Watercress darter	*Etheostoma nuchale*				
Okaloosa darter	*Etheostoma okaloosae*				
Duskytail darter	*Etheostoma percnurum*				
Bayou darter	*Etheostoma rubrum*				
Cherokee darter	*Etheostoma scotti*				
Maryland darter	*Etheostoma sellare*				
Bluemask darter	*Etheostoma sp.*				
Boulder darter	*Etheostoma wapiti*				
Fountain darter	*Etheostoma fonticola*		*Etheostoma fonticola* (species)	**17.2**	0.93
Amber darter	*Percina antesella*		Percidae (family)	NC	1.82
Goldline darter	*Percina aurolineata*				
Conasauga logperch	*Percina jenkinsi*				
Leopard darter	*Percina pantherina*				
Roanoke logperch	*Percina rex*				
Snail darter	*Percina tanasi*				
Ozark cavefish	*Amblyopsis rosae*	Percopsiformes Amblyopsidae	Actinopterygii (class**)**	NC	2.86
Alabama cavefish	*Spleoplatyrhinus poulsoni*				
Little Kern golden trout	*Oncorhynchus aguabonita whitei*	Salmoniformes Salmonidae	*Oncorhynchus* (genus)	NC	2.02
Paiute cutthroat trout	*Oncorhynchus clarki seleniris*				

Greenback cutthroat trout	*Oncorhynchus clarki stomias*				
Gila trout	*Oncorhynchus gilae*				
Apache trout	*Oncorhynchus apache*		*Oncorhynchus apache* (species)	**14.47**	0.71
Lahontan cutthroat trout	*Oncorhynchus clarki henshawi*		*Oncorhynchus clarki henshawi* (species)	**20.00**	0.98
Atlantic salmon	*Salmo salar*		*Salmo salar* (species)	NC	3.87
Bull trout	*Salvelinus confluentus*		*Salvelinus* (genus)	**13.77**	0.68
Pygmy sculpin	*Cottus paulus*	Scorpaeniformes Cottidae	Actinopterygii (class)	NC	2.86

Freshwater mussels: The acute and chronic criteria for listed mussels are based on the protection of their host fish. For mussels with obligate host fish, the RPA is based on the acute and chronic Assessment Effects Concentrations (EC_A) which represent *the highest* concentration of cyanide where the effects on host fish species are expected to be insignificant (Table 22, see Appendix B for details).

Table 22. Freshwater acute and chronic cyanide criteria for the protection of listed mussels with known obligate host fish species (NC, no change).

Listed Species	**Host Fish**	**Surrogate Taxa**	**Recommended Acute Criteria [Acute EC_A (ug CN/L)]**	**Recommended Chronic Criteria [Chronic EC_A (ug CN/L)]**
Fat Pocketbook *Potamilus capax*	Freshwater drum	Perciformes (order)	NC	2.86
Scaleshell Mussel *Leptodea leptodon*	Freshwater drum	Perciformes (order)	NC	2.86

For mussels with multiple host fish (non-obligates), or for which host fish are unknown, the recommended acute and chronic criteria for listed mussels are based on the protection of fish from the genus *Etheostoma*, family Pericade (Table 23). Percids make up approximately one-third of all known host fish species for listed mussels. For non-obligate listed mussels for which percids have not been identified as hosts, there is a reasonable possibility that these species serve as hosts where they occur. Protection of fish from the genus *Etheostoma* is expected to protect for the majority of species in this family.

For listed mussels either occurring in areas that do not support percids, or are known not to transform on these species, we recommend acute and chronic criteria for listed mussels based on the protection of fish from the class Actinopterygii (Table 23).

Table 23. Freshwater acute and chronic cyanide criteria for the protection of listed mussels with multiple host fish or for which host fish are unknown (NC, no change).

Listed Species	Host Fish	Surrogate Taxa	Recommended Acute Criteria [Acute EC_A (ug CN/L)]	Recommended Chronic Criteria [Chronic EC_A (ug CN/L)]
Mussels no known obligate host fish	Multiple or unknown	*Etheostoma* (genus)	NC	1.72
Mussels with non-percid hosts[1]	Non-percid hosts	Actinopterygii (class)	NC	2.86

[1] Habitat is known to not support percid species or mussels species are known to not transform on percids.

Amphipods: The acute and chronic cyanide criteria for protection of listed amphipod species are shown in Table 24. These values are based on the acute and chronic Assessment Effects Concentrations (EC_A) which represent the *the highest* concentration of cyanide where the effects on listed species are expected to be insignificant (see Appendix B for details).

Table 24. Freshwater acute and chronic cyanide criteria for the protection of listed amphipod species (NC, no change).

Listed Species		Order/Family	Surrogate Taxa	Recommended Acute Criteria [Acute EC_A (ug CN/L)]	Recommended Chronic Criteria [Chronic EC_A (ug CN/L)]
Illinois cave amphipod	*Gammarus acherondytes*	Amphipoda Cambaridae	*Gammarus* (genus)	NC	3.33
Noel's Amphipod	*Gammarus desperatus*			NC	3.33

Amphibians: EPA shall implement RPA Alternative #2 for Amphibians.

and/or,

2. In place of RPA 1(b), the EPA shall, subject to the Service's approval, develop and implement the research necessary to replace modeled estimates of species sensitivities to cyanide with direct evidence, using listed species or more closely related surrogates, as the basis for defining cyanide criteria to insure an appropriate level of protection is afforded to listed species and critical habitats addressed by this RPA. This RPA shall be implemented for all amphibians addressed in this biological opinion, and is optional for all other taxa. This task shall be completed by December 1, 2012.

Because this biological opinion has found jeopardy and adverse modification, the EPA is required to notify the Service of its final decision on implementation of the reasonable and prudent alternatives.

11.0 INCIDENTAL TAKE STATEMENT

The Service has developed the following Incidental Take Statement based on the premise that the RPA will be implemented.

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Section 9 of the ESA and Federal regulations pursuant to section 4(d) of the ESA prohibit the take of endangered and threatened species, respectively, without special exemption. Take is defined as harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Harass is defined by the Service as an intentional or negligent act or omission which creates the likelihood of injury to a listed species by annoying it to such an extent as to significantly disrupt normal behavioral patterns which include, but are not limited to, breeding, feeding or sheltering. Harm is defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by impairing behavioral patterns including breeding, feeding, or sheltering. Incidental take is defined as take that is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity. Under the terms of section 7(b)(4) and section 7(o)(2), taking that is incidental to and not intended as part of the agency action is not considered to be a prohibited taking under the ESA, provided that such taking is in compliance with the terms and conditions of this Incidental Take Statement.

The Service anticipates that with implementation of the RPA, incidental take of the listed fish, mussel, amphipod, and amphibian species considered in this biological opinion is not likely to occur from exposure to cyanide at revised criteria concentrations. However, other elements of water quality standards could allow for exceedance of criteria concentrations and may contribute to incidental take. The other elements of water quality standards will be the focus of subsequent tiered consultations on individual State and Tribal water quality standards. Therefore, no incidental take exemptions are provided in this biological opinion.

12.0 CONSERVATION RECOMMENDATIONS

Section 7(a)(1) of the ESA directs Federal agencies to utilize their authorities to further the purposes of the Act by carrying out conservation programs for the benefit of endangered and threatened species. Conservation recommendations are discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, to help implement recovery plans, or to develop information. We recommend that EPA implement the following actions:

1. In consultation with the Service, develop a conservation program for threatened and endangered species and, in collaboration with States and Tribes, develop conservation plans that specifically addresses threats to listed species and how implementation of Clean Water Act programs can ameliorate those threats;
2. Work with the Service and the National Marine Fisheries Service to reinvigorate implementation of the 2001 MOA on ESA and the CWA, especially to address local and regional water quality concerns, research needs, and revisions to the criteria derivation process.

In order for the Service to be kept informed of actions minimizing or avoiding adverse effects or benefitting listed species or their habitats, the Service requests notification of the implementation of any conservation recommendations.

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13.0 REINITIATION STATEMENT

This concludes formal consultation on EPA's continuing programmatic approval of cyanide criteria in state and tribal water quality standards. As provided in 50 CFR 402.16, reinitiation of formal consultation is required where discretionary Federal agency involvement or control over the action has been retained (or is authorized by law) and if: (1) the amount or extent of incidental take is exceeded; (2) new information reveals effects of the action that may affect listed species or critical habitat in a manner or to an extent not considered in this opinion; (3) the agency action is subsequently modified in a manner that causes an effect to the listed species or critical habitat not considered in this opinion; or (4) a new species is listed or critical habitat designated that may be affected by the action.

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Fish and Wildlife Service

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

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I. Introduction

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

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

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

II. Methodology

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

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

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II.A. Selection of Species of Concern

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

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

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

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

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

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

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

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

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

II.B. Average Concentration Trophic Level Approach

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

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$$\mathbf{WV} = \frac{\mathbf{RfD \times BW}}{\mathbf{FIR}} \qquad \textbf{(1)}$$

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

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

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

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

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

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BW = Body Weight (kg) for species of concern
FIR = Total Food Ingestion Rate (kg of food/day) for species of concern

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

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

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

In contrast to wildlife species with uniform diets, many predators that feed from aquatic ecosystems are more opportunistic and will consume prey from more than one trophic level. Because of methylmercury biomagnification, aquatic food chains do not attain a steady-state condition whereby aquatic biota from all trophic positions exhibit the same tissue concentrations. Instead, organisms higher on the aquatic food chain contain greater concentrations than those lower on the food chain. Although Equation 1 can be used to calculate a protective WV for wildlife species with multi-trophic level diets, based solely on a total daily food ingestion rate, this value is actually dependent on the amount of prey consumed from each trophic level *and* the methylmercury concentrations in each of the trophic levels from which they feed. In this situation, the trophic level composition of the diet becomes the driving factor influencing the amount of methylmercury ingested on a daily basis. Without an understanding of the dietary composition for these wildlife species, it is impossible to determine limiting concentrations for each trophic level necessary to maintain a protective WV.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

FCM4 = 680,000/160,000 = 4.25

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

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

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

FCM4 = 0.15/0.05 = 3

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

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

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

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

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

III. Calculating Wildlife Targets with Average Concentration TL Approach

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

III.A. Reference Doses

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

III.B. Adult Female Body Weights

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

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

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

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

III.C. Food Ingestion Rates

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

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

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

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

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

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

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

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

III.D. Calculation of Wildlife Values

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

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

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

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

III.E. Trophic Level Dietary Composition and Prey Size

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

WV = (%TL3 × FDTL3) + (%TL4 × FDTL4)

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

WV = (%TL3 × FDTL3) + (%TL4 × FDTL3 × TLR)

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

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

Solving this equation for FDTL3:

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

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

FDTL4 = FDTL3 × TLR
FDTL4 = 0.1018 mg/kg × 2
FDTL4 = 0.2036 mg/kg

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

V. Summary of the Guadalupe River Watershed Wildlife Targets

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

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

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

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

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

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

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

VI. Target Location Based on Habitat Type

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

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

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

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

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

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

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

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