APPENDIX N: PISCICIDE TREATMENT PROTOCOLS

This appendix describes piscicide treatment protocols, data collection, and pre- and post-treatment monitoring methods that would occur only if either alternative B or D in the Sequoia and Kings Canyon National Parks (SEKI) Restoration of Native Species in High Elevation Aquatic Ecosystems Final Environmental Impact Statement (Restoration Plan/FEIS) is selected in the final plan/record of decision.

These protocols are taken directly (with some site-specific modification, especially to species and water quality monitoring plans) from the North Cascades National Park Service Complex *Project Plan: Skymo Lakes Restoration* (NPS 2015C), which uses methods nearly identical to the techniques proposed in this Restoration Plan/FEIS.

All piscicide treatments would strictly adhere to the label requirements for CFT Legumine™ and the detailed protocols found in the Rotenone SOP Manual (Finlayson et al. 2010A). This appendix includes a detailed summary of methodologies planned for (1) pre-treatment, (2) during applications, and (3) post-treatment monitoring. However, this appendix is not intended to be all-inclusive. Further details regarding rotenone and piscicide treatment protocols (including the Rotenone SOP Manual; Finlayson et al. 2010A) are available to the public on the American Fisheries Society Fish Management Chemicals Subcommittee's Rotenone Stewardship Program webpage: http://rotenone.fisheries.org/.

Prior to each piscicide treatment, various surveys and bioassays would be conducted to establish baselines for various physical, chemical, and biological conditions of the treatment area. These surveys would include various water quality and hydrologic assessments and amphibian visual encounter surveys at all treatment sites, and benthic macroinvertebrate and zooplankton surveys at the first two to three treatment sites (and potentially additional treatment sites). Surveys would also be completed after treatments are conducted to assess status of fish removal, water quality, and response of aquatic biota following treatment. In addition, this appendix describes the methodology for safe on-site transport, handling, application, storage, spill response, and deactivation of rotenone (CFT Legumine™), record-keeping for the treatment process, and site close-out operations.

Pre-Treatment Surveys, Bioassay, and Other Tasks

Additional physical, chemical, and biological data would be collected preceding each piscicide treatment to characterize any changes in the physical and chemical conditions of the treatment area that may influence CFT Legumine™ toxicity, and CFT Legumine™ and potassium permanganate ($KMnO_4$) application rates; to provide logistical information to facilitate the project's operations; and to provide baseline data on the status of amphibians prior to treatment. Additionally, for the first two to three piscicide treatments, biological data would be collected to determine the baseline pre-treatment benthic macroinvertebrate and zooplankton assemblages.

Physical, Chemical, Biological Data Collection

The following pre-treatment measurements and surveys would be completed within five days of CFT Legumine™ application to ensure the treatment area conditions are accurately characterized and represented. Further detail on measurements taken can be found in the Rotenone SOP Manual (Finlayson et al. 2010A) and label requirements for CFT Legumine™ (Zoëcon 2015). Additionally, if piscicide use proposed in this Restoration Plan/FEIS is approved, then a full implementation plan would be in place prior to any piscicide treatments.

In brief, data acquisition for these surveys would consist of determining and/or measuring:

- The quantity of rotenone needed for each given lentic habitat by collecting bathymetry data, which would involve:
  - Setup of transects across the waterbody using buoys set at regular intervals,
  - Taking depth measurements at each buoy along each transect,
  - Calculating the average depth based on all depth measurements,
  - Multiplying the average depth by the waterbody surface area to determine treatment volume;
- The quantity of rotenone needed for each given stream reach to be treated, which would involve:
  - Computing stream flow rate (preferably using an electric flow meter).
  - Select exposure time and dilution (generally, flows >25 ft^3/s should use undiluted product and flows <25 ft^3/s should use diluted product),
  - Estimating the amount of rotenone needed and application rate;
- Secchi depth transparency;
- Water temperature, dissolved oxygen, and pH depth profiles;
- Nutrient and chlorophyll-a samples;
- Amphibian visual encounter surveys;
- Water quality samples;
- Littoral benthic macroinvertebrate samples from lakes.

Additionally, benthic macroinvertebrate from lakes and streams, and zooplankton samples from lakes, would be collected in proposed treatment areas and nearby control areas outside of proposed treatment areas. These monitoring efforts would initially occur in the first two to three piscicide treatments. Monitoring would occur one summer before treatment, immediately before and after treatment, and for at least two summers after treatment to more comprehensively characterize the pre-treatment condition, disturbance to, and recovery of these assemblages. For additional information, see the ***Benthic Macroinvertebrates*** section of Post-Treatment Biomonitoring and Evaluation below, and the *Invertebrate Recovery* section at the end of this appendix.

Typically, very few MYLF of any life stage are present in habitats containing fish. Low densities of post-metamorphic frogs are occasionally observed in shallow areas connected to the main waterbody, but inaccessible to fish due to natural barriers (e.g., rock piles or thick emergent vegetation adjacent to the water). Tadpoles are rarely present in fish-containing areas. When present, there are usually only a small number of tadpoles confined to peripheral areas inaccessible to fish (e.g., within boulders at the shoreline). However, immediately prior to any piscicide treatments, visual encounter surveys would be conducted and MYLFs of all life stages would be collected using dip nets and/or seines. Captured MYLFs would be removed from the treatment area and placed in a fishless waterbody in the same basin with the same amphibian chytrid status (i.e., infected or uninfected). (Generally, the presence or absence of amphibian chytrid fungus is the same throughout a basin.) The recipient waterbodies would be disconnected from the treatment area so tadpoles could not swim back into the treatment area, and at least several hundred meters away so frogs would not return to the treatment area within the treatment period.

Benthic macroinvertebrate collections would also occur along with amphibian collection efforts just prior to treatment. Invertebrates would be collected from randomly selected areas within the treatment area using D-ring dip nets. All collected invertebrates would be held at the stream edge in oxygenated aquariums (e.g., plastic bins or plexiglass aquaria) along with substrate collected from their point of capture. Battery-powered aerators would be used to maintain adequate oxygen levels in the aquaria. Once rotenone levels have dissipated following rotenone treatment, invertebrates would be released back to their original point of capture. Although collections like these would not guarantee representative species composition or rare species within the treatment area, this kind of collection could help facilitate benthic macroinvertebrate recolonization of the treatment area.

Project Setup Tasks

Numerous tasks would be completed before the application of CFT Legumine™, including: transportation of personnel and equipment to the project area; verification of conditions in the treatment area (and, if necessary, potential modifications to the treatment plan); set-up and testing of boat application equipment; set-up and calibration of drip stations; set-up and calibration of the rotenone deactivation station; set-up and distribution of live cars (e.g., mesh bags with metal hoops on each end, floats on the top hoop, and a drawstring top to prevent any fish from escaping; Finlayson 2010A) containing sentinel fish (collected on site using a variety of methods, including electrofishers, traps, or seines) that are used to help determine treatment efficacy; locating and setting up sleeping, cooking, and staging areas in a way that minimizes impacts on vegetation and wildlife; posting signs to warn the public around the treatment area; and conducting a pretreatment meeting.

The certified applicator(s) and any individual in a supervisory role during treatments would have attended a piscicide treatment class, typically offered annually. All personnel involved in applying piscicides would receive training related to piscicide treatment prior to being involved in any rotenone treatments (see Safety and Exposure Mitigation section below).

The pretreatment meeting is a critical component of this project and would include: reviewing the treatment plan; reviewing the procedures for the safe handling and application of CFT Legumine™ and potassium permanganate; distributing personal protective equipment and providing instructions on its use; assigning roles to all project personnel and outlining the chain of command; briefing all personnel in the operation of treatment equipment; and completing a Green, Amber, Red (GAR) safety assessment.

Pre-Treatment Bioassay

An on-site bioassay would be conducted to ensure that the CFT Legumine™ is effective and applied in amounts lethal to the target trout species, while minimizing adverse effects on non-target organisms and the environment. The CFT Legumine™ label (Zoëcon 2015) identifies lethal concentrations required to kill many fish species; however, lethal concentrations are affected by environmental and water quality conditions (e.g., pH, alkalinity, water temperature, sunlight exposure) and may require adjustments in the concentration used, given the specific conditions of the treatment area, based on results of the bioassay.

The bioassay would be conducted on-site one day before the treatment. Methods for conducting the bioassay would generally follow those given in Finlayson et al. (2010A; SOP 5 and 14). The bioassay would be conducted using replicate sets of five 95 L plastic totes, filled with 70 L of lake water, and situated in the lake near the shoreline. A stock solution of CFT Legumine™ would be mixed to achieve a concentration of 50 ppb rotenone (the active ingredient)/1 ppm of CFT Legumine™ by adding 1 ml of the CFT Legumine™ concentrate to 1 L of water. The active concentrations of CFT Legumine™ tested and amounts of stock solution that are added to achieve these concentrations in 70 liters of water can be found in Table N-1.

Table N-1. Concentrations of CFT Legumine™ achieved by adding the listed amount of stock solution to 70 L of water.

Concentration of CFT Legumine™	0 ppm	0.25 ppm	0.5 ppm	1.0 ppm	2.0 ppm
Amount of Stock Solution	0 ml	17.5 ml	35 ml	70 ml	140 ml

After the CFT Legumine™ has been allowed to mix in each container, five small trout (<150 mm) of the targeted species would be captured on site (using electrofishers, seines, or traps) placed in each tote and monitored every 30 minutes for eight hours of exposure time. Changes in coloration, response to stimuli, respiration stress (measured by gill movement), the number of live fish, fish on their sides, and dead fish would be recorded at each monitoring interval. Dissolved oxygen and water temperature would also be measured at the same time. Each tote would be aerated with aeration stones only if dissolved oxygen levels drop below 8 mg/L.

The Minimum Effective Dose (MED), the lowest concentration of CFT Legumine™ that produces 100% mortality of the test fish after 8 hours of exposure, would then be doubled to come up with a treatment concentration in order to ensure all target organisms are killed (Finlayson et al. 2010A).

Material Handling: CFT Legumine™ and Potassium Permanganate

All project staff would be briefed and clearly understand the details on storage, transportation, decontamination, and spill containment prior to treatment following guidance outlined in the Material Data Safety Sheet (MSDS) for CFT Legumine™ and potassium permanganate labels.

Safety and Exposure Mitigation

A successful CFT Legumine™ application results in no exposure to personnel handling the chemical (i.e. mixers, loaders, and applicators) or the public. To ensure adequate protection, all personnel handling CFT Legumine™ would wear personal protective equipment (PPE) as required by the EPA and listed on the product label (Zoëcon 2015), including, at a minimum, long-sleeved shirt, long pants, chemical resistant gloves, goggles, and approved respirators. Additionally, given the remote environment and limited ability to wash field clothing, Tyvek™ suits with hoods will be provided as PPE to minimize the risk of exposure.

To ensure adequate protection of the public, the following protective measures would be put into place:

- The CFT Legumine™ and potassium permanganate product labels and MSDS would be followed;
- The treatment area would be clearly marked and identified with posted signs;
- Public access to the project area would be prohibited during treatment and for up to two weeks following treatment;
- Dead fish would not be consumed;
- Treated water would not be ingested;
- Out-flow from the treatment area would be deactivated with potassium permanganate.

At least two pre-project safety trainings would be held prior to the start of each piscicide treatment and an Operation Leadership risk assessment would be completed with the participation of all personnel. The first training would be held at SEKI headquarters at the start of each project's mobilization. This training would be attended by all personnel who would be involved with handling or transporting the CFT Legumine™ from the storage facility to the field during mobilizations. The second training would be held on site at the project area one day prior to the application of the CFT Legumine™. Topics covered at these meetings would include:

- A review of the CFT Legumine™ product label and MSDS;
- A review of the potassium permanganate product label and MSDS;
- The proper use of the required PPE, exposure control, the safe mixing and application of CFT Legumine™, health effects of the CFT Legumine™ formulation, and first aid measures;
- Environmental considerations including: driving conditions, helicopter safety, water safety, dehydration, sun exposure, safe use of application equipment, radio communication, and situational awareness.

Storage

CFT Legumine™ would be stored in the original five gallon metal containers until it is applied to the treatment area. Prior to transportation to the project area, the CFT Legumine™ would be stored according to the label, with guidance provided in Finlayson et al. (2010A, SOP 4: CFT Legumine™ Storage, Transportation, and Spill Containment Plan) and the MSDS. During transport to the project area the CFT Legumine™ would be stored in the original five gallon containers and inside of a large steel box that is lined with plastic and locked. Absorbent padding would be placed around the containers inside each box. The five gallon containers would be removed from the box the day of treatment and placed in plastic-lined berms located near the shoreline of each lake or stream within the treatment area.

Transportation

A helicopter or packstock would be used to transport the CFT Legumine™, potassium permanganate, and other equipment and materials to each project area. Transport operations would be staged at the packstock corral or helispot (i.e., the landing zone, which will vary depending on the site and act as the staging area) that is closest to each treatment area. The CFT Legumine™ would be transported from the storage area at SEKI's frontcountry headquarters in Ash Mountain to each staging area, packaged in the previously described steel box. The CFT Legumine™ would also be transported from each staging area to each treatment area while packaged CFT Legumine™ in the same steel box configuration.

On-site Transfer and Mixing of CFT Legumine™

All measurement and mixing of the CFT Legumine™ would be done on the day of treatment and over the waters to be treated. For boat application, the CFT Legumine™ would be hand pumped into a graduated bucket and transferred to a 20 gallon tank that is located on each boat. Each tank would then be filled with water so that each tank is mixed with 1 gallon of CFT Legumine™ in 10 gallon of water.

Measurement and mixing of CFT Legumine™ for backpack sprayers and drip stations would also be conducted near the shoreline and/or in a shallow area of the lake. Using a graduated cylinder, 150 ml of CFT Legumine™ would be measured and placed in labeled, opaque, wide-mouth Nalgene bottles. These bottles would then be placed in sealed plastic bags that are packed with absorbent material. On the day of the treatment each packaged bag of CFT Legumine™ would be hand carried to the treatment area that would either be sprayed in the treatment area are or used to charge a drip station.

Spill Response and Containment

In the event of a CFT Legumine™ spill on land or in water, the spill would be controlled and contained as described below. The project manager would document any spill, regardless of the amount spilled. Documentation would include: the amount CFT Legumine™ spilled; specific location of the spill; and the description, date, and time of the spill, and control/containment/cleanup activities. The SEKI Dispatch radio communication center would be immediately notified for spills occurring outside of the treatment area (e.g., enroute from the helicopter staging area to the treatment area). Following the initial contact, SEKI dispatch personnel would make contact with other park staff as described in the SEKI Spill Prevention, Control, and Countermeasure Plan (NPS 2013C). In addition, dispatch personnel would notify the Washington Emergency Management Division as soon as possible after the spill.

If the <u>spill is located in an area accessible to the public</u> then immediate action would be taken to isolate the hazardous area by keeping everyone a safe distance and upwind from the site. After donning the appropriate personal protective equipment, the first step of the spill response team would be to control the flow of CFT Legumine™ by taking all possible steps to stop or contain the leak or spill.

To contain <u>spills on land</u>, staff would stop the spill at its source; dike the liquid in pools with booms; absorb with clay, soil, or other noncombustible, absorbent material. Following containment, the CFT Legumine™ would be deactivated with an oxidizing agent such as potassium permanganate.

To contain spills in water, a determination would be made as to whether or not the location of the spill is designated for immediate treatment. For an accidental spill of CFT Legumine™ into waters not designated for immediate treatment, the project manager would initiate neutralization procedures using potassium permanganate to minimize further contamination (as described in Finlayson et al. (2010A, SOP 7: Determining the Need and Methods for Chemically induced Deactivation). For an accidental spill of CFT Legumine™ into waters designated for immediate treatment, the project manager would note the time of the spill, the amount spilled, and actions taken. Additionally, the project manager and project personnel would keep detailed notes on the effects on sentinel fish in cages and conduct on-site monitoring to determine if material from the spill travels downstream from the treatment area, and thus the immediate need for operating the potassium permanganate deactivation station.

Public Communication

SEKI would follow public notification standards and treatment area restrictions as outlined in the American Fisheries Society (AFS) Rotenone SOP Manual, Supplement 1 (Finlayson et al. 2010A) and EPA label requirements for CFT Legumine™ (Zoëcon 2015). Some of the specific plans for public communication are listed below.

Park personnel would provide multiple news releases regarding individual piscicide treatments to local media outlets and on the SEKI website. At the minimum, a short-term release notice would be provided one to three weeks ahead of the application date (Finlayson et al. 2010A). An additional medium- to long-term release notification may also be provided three to 12 months prior to the treatment date. Media outlets may include local newspapers, radio, and television stations (e.g., those located in Fresno, Tulare, and/or Inyo counties). News releases would include: a description of the project area, the general reason for treatment, name and concentration of rotenone formulation used and the deactivation agent, public and/or water use restrictions, posting procedures, the anticipated amount of time that the area would be affected, and the names and contact information of designated applicator(s) and/or agency contact person(s). Press releases would be provided to media outlets for either voluntary publication or to broadcast to the public.

Notices would be provided to sport shops in park gateway communities via mail, email, or directly-given handbills. Piscicide application would only be conducted within SEKI park boundaries, therefore, no private property owners or water rights holders would need to be contacted separately, since rotenone deactivation and natural degradation would occur long before treated water flows anywhere near private land. Wilderness permit desks and stations would be provided information to inform visitors regarding which areas would be receiving upcoming treatments, the duration of the treatment, and any which areas would be closed to the public during the treatment period.

SEKI and affiliated personnel would also place signs in and around the treatment area to notify the public. Signs would be placed in various locations, including public access points to the treatment area (e.g., trailheads and trail junctions) and/or at least every 250 ft (76 m) along the treatment area. Signs would be placed at least 7-14 day prior to treatment at public access points and on the day of application at the site itself. During treatment, the application area would be off-limits to the public and non-project staff.

According to CFT Legumine ™ label requirements (Zoëcon 2015), application notification signs can be removed and the area can technically be re-opened to the public and non-project staff once application is complete because <90 ppb of active rotenone would be applied. However, SEKI management and affiliated personnel would use their discretion to decide when to remove placards. The site closures would likely average around 7 days after neutralization, but the exact length of closures would vary depending on site-specific characteristics. Generally, there would be a minimum closure time of 72 hrs and a maximum closure time of 14 days.

The notification signs would be made of heavy waterproof stock or laminated paper signs to withstand weather, would be the minimum size necessary to reduce impacts on wilderness, and would include the following information:

- "DANGER/PELIGRO"
- "DO NOT ENTER WATER/NO ENTRE AGUA: Pesticide Application"
- CFT Legumine Fish Toxicant
- The purpose of the application
- The start date and time of application
- The end date and time of application
- "Recreational access (e.g., wading, swimming, boating, fishing, etc.) within the treatment area is prohibited while rotenone is being applied."
- "Do not swim or wade in treated water while placard is displayed."
- "Do not consume dead fish from treated water."
- The name, address, and telephone number of the responsible agency or entity performing the application.
- "DO NOT DRINK WATER/NO BEBA AGUA" signs would be installed during the treatment period (Although rotenone and additives found in CFT Legumine™ are applied at levels well below EPA levels of concern, the parks' decision to advise visitors not to drink water in or near treatments is precautionary and only for a limited amount of time post-treatment.).

CFT Legumine™ Application

Rotenone would be applied as the liquid CFT Legumine™ formulation below water using: (1) a small boat with oars and/or an electric motor for lakes and ponds; (2) direct metering into flowing streams; and (3) hand-held equipment, such as backpack sprayers, in difficult to reach aquatic areas (Finlayson et al. 2000, EPA 2007A). Sentinel fish would be collected prior to the treatment process from the project area using electrofishing units, seines, or fish traps and placed in live car net baskets just upstream of the drip stations to monitor the effectiveness of the treatments. Rotenone would be applied according to label instructions for normal species (trout in this plan), which allow for treatment concentrations up to 1.0 parts per million (ppm) of CFT Legumine™ product [50 parts per billion (ppb) active ingredient rotenone] in flowing streams and standing water (lakes/ponds/marshes; Zoëcon 2015). Piscicide treatments would occur in mid-late summer, with the specific timing determined by site-specific characteristics, including streamflow (targeting lower flows), water temperature (targeting warmer conditions; i.e., late summer conditions, when water temperatures are typically >10°C, NPS unpublished data), and site accessibility.

Liquid rotenone would be applied to lakes and ponds using an inflatable boat. GPS units would be used to place buoy transects that divide lentic waterbodies into manageable sections to more easily keep track of where rotenone would be applied. Rotenone formulations would be pumped (using small electric pumps) below the surface of the lake. Application to streams would also apply a diluted liquid form of rotenone for 4 to 8 hours through a series of metered dispensing stations (gravity-fed) placed at specified intervals along the stream's course. Stations would be placed in secure and stable locations, either on the stream bank or on a stand in the stream channel, and actively monitored by project staff for the duration of the treatment. The drip nozzles of the stations would be placed very close to the water's surface to reduce the potential for piscicide drift to terrestrial environments. Because rotenone breaks down and loses its toxicity within hours in flowing water (Finlayson et al. 2000), drip stations would be placed at intervals between about 0.5 to 2 miles apart (anywhere between 1 and 2 hours of stream travel time) along the stream, depending on conditions, to maintain concentrations lethal to fish. The placement and number of drip stations would be determined at the time of treatment to address current conditions. A non-toxic dye would be used to determine chemical travel time (Finlayson et al. 2010A, SOP 11). Backpack sprayers with hand-held wands would be used to apply highly diluted liquid rotenone in backwater areas along streams and the littoral zones of lakes where mixing may be incomplete due to minimal water movement. This would help to ensure that untreated refuges do not occur and would minimize the likelihood of project failure from incomplete eradication. When applied from backpack sprayers, small amounts of piscicide are applied directly to the water surface in a small, steady stream (as opposed to an actual spray), which minimizes drift onto terrestrial environments.

The total combined concentration of CFT Legumine™ from boat applications, backpack dispensers, and drip stations would be up to 1.0 ppm in streams and lakes, as required by the product label for normal use given the water characteristics such as pH, temperature, depth, and sensitivity of the target species. The final concentration would be calculated as described in the "Pre-treatment Bioassay" section of this chapter. Based on water quality conditions measured during pre-treatment surveys, the estimated amount of CFT Legumine™ needed to treat each treatment area would be a concentration of up to 1.0 ppm in streams and lakes.

Each treatment area would be divided into application units consisting of lakes and streams. The piscicide would be apportioned to each of these units based on the volume of water contained in the lake treatment habitat, or a combination of discharge and travel time for the stream treatment habitat.

Each application of CFT Legumine™ would begin at approximately 08:00 and would take up to eight hours. For treatment areas with no barriers to fish passage within the treatment area, all application units would be treated at the same time to ensure the fish are continuously exposed to a lethal dose of rotenone downstream to a barrier. For treatment areas with barriers to fish passage within the treatment area, application units can be treated at different times. Deeper lakes may be treated in two stages, with the first stage treating depths below the thermocline and the second stage treating depths above the thermocline. The deep-water treatment is expected to discourage fish from leaving shallower waters and provide better distribution of CFT Legumine™ through the water column. Two boats may be used for this stage, with each boat pumping a diluted CFT Legumine™ solution from tanks via weighted hoses. Boats would follow the buoyed grid system during the application process. Following deep-water treatment, shallow water would be treated with the remaining CFT Legumine™. Shallow lakes would not require a separate treatment below the thermocline.

The lake inlet (up to a barrier) and outlet stream(s) of each treatment area would be treated for 4 to 8 hours and would start at the same time as lake treatments to ensure that fish cannot find a refuge from the piscicide. The outlet stream would be treated with a drip station placed at the start of each outlet and additional stations placed along the stream at appropriately intervals, based on stream travel time to the downstream barrier. Drip stations would consist of a 10 gallon bucket attached to a float controlled dish that has been calibrated to dispense at a consistent flow rate of 80 ml per minute. The amount of CFT Legumine™ added to 10 gallons of water would vary depending on the rate of stream flow (Table N-2). The initial drip station flow rate, amount of CFT Legumine™, and water added to the bucket would be recorded. The flow rate would be monitored, adjusted, and recorded at 1 hour intervals during the 8 hour treatment period.

Table N-2. The total amount of CFT Legumine™ that should be added to 10 gallons of water to treat a stream at 1 ppm for 8 hours at a rate of 80 ml/hour given a range of flows between 0.1 to 1.0 cubic feet per second (cfs).

Stream Discharge (cfs)	CFT Legumine™[1] (ml)
0.1	82
0.2	163
0.3	245
0.4	326
0.5	408
0.6	490
0.7	571
0.8	653
0.9	734
1.0	816

[1]Amount of CFT Legumine™ is calculated as: X = Y(102 F)H where: X = CFT Legumine™ diluted into 10 gallons of water (ml), Y = desired treatment concentration of CFT Legumine™ (ppm), Q = stream discharge (cfs), and H = the duration of the treatment (hours).

Several backpack sprayers would treat shallow (boat inaccessible) areas around each lake shoreline, seeps, and sections of the inlet and outlet stream channel that did not show good dispersal based on pre-treatment surveys. Six backpack spraying applications would be completed at 1.5 hour intervals, beginning at approximately 09:00. Backpack sprayers would be filled with a 2% solution of CFT Legumine™ (mixed as 150 ml CFT Legumine™ to 2 gallons of water).

Monitoring the effects of each treatment would begin shortly after the completion of the lake application and would continue for three days. Sentinel fish kept in live cars (5 fish/cage) would be used to help monitor treatment effectiveness. Live cars would be set the day before treatment to acclimate trout and minimize the potential confounding effects of post-handling stress. Sentinel fish would be checked again immediately before treatment to ensure no trout died overnight. In lakes, live cars would be located near the surface, at mid-depth, and at the lake bottom. To ensure sentinel fish exposure accurately reflects conditions experienced during treatment, live cars would not be checked for at least 24 hours post treatment (i.e., if live cars are pulled up to the surface earlier, trout may be moved through higher piscicide concentrations in shallow areas that may not be present at lower depths, which could confound results). In streams, live cars would be placed in semi backwater areas (if available) just upstream of drip stations. The final live car within the treatment area would be located just upstream of the deactivation site. Additionally, a drift net would be placed across the stream channel at the 30-minute stream travel time distance to prevent fish from leaving the treatment area. The drift net would be checked periodically to remove dead trout and debris.

All dead fish collected would have their swim bladders punctured and would be dropped into the deepest part of each treatment lake and/or deeper adjacent lakes. Data forms for recording rotenone use and monitoring fish sentinel cages would be used. In lakes, numerous gill nets (dependent on treatment lake size) would be continuously fished (checked and reset every 24 hrs) for three days following the treatment day. In streams, electrofishers and visual searches would be used to determine initial treatment effectiveness. Short-term determination of treatment effectiveness would be made if there is 100% mortality of all sentinel fish and no live fish are observed or caught using any survey method (electrofishers or gill nets). Final determination of treatment success would be made during site revisits in the following two summers.

Rotenone Deactivation

Environmental factors such as pH, sunlight, stream discharge, water temperature, and stream gradient affect the toxicity and breakdown of rotenone. As rotenone travels downstream, light exposure, aeration, and agitation due to the physical movement of the water contribute to the degradation of rotenone and decreases its toxicity. Steep stream gradients would help to deactivate rotenone. In addition, tributaries contributing flow below each treatment area would reduce the toxicity of rotenone through dilution. As an additional precaution, rotenone would be deactivated using liquid potassium permanganate dispensed into the outlet stream below each treatment area.

For a typical piscicide application, 1 ppm of potassium permanganate for every 1 ppm of rotenone formulation used would be applied at the most downstream point of the stream section from which fish removal is desired. In addition to the 1 ppm potassium permanganate used to neutralize the rotenone, another 1 ppm is applied to satisfy the background oxidation demand, and another 1 ppm is applied as residual or buffer. Background oxidation demand is determined using an oxygen meter. Clear and low-productivity waters typical of SEKI's high elevations are expected to have relatively low background oxidation demand, and thus to be satisfied by 1 ppm potassium permanganate. However, in cases where the background oxidation demand is more than 1 ppm potassium permanganate, more neutralizing agent must be used. The typical target concentration for neutralizing a piscicide treatment in a stream is therefore 3 ppm, but in cases where background demand is high it could range up to 4 to 5 ppm. Potassium permanganate would be applied to each stream using a 2.5% solution for deactivation (Table N-3).

The rotenone deactivation station would typically be located at the top of the downstream fish barrier in each treatment area. The station would be operated continually, starting at a certain point after the beginning of each treatment, until rotenone has fully dissipated.

Neutralization occurs within 30 minutes of contact between the treated water and the potassium permanganate, so fish and other aquatic organisms may still be affected by piscicide the distance water moves downstream in 30 minutes. This water would be considered part of the project area. A 1 ppm potassium permanganate residual would be maintained at the 30-minute travel time downstream location by increasing or decreasing the amount of potassium permanganate to ensure complete neutralization of rotenone leaving the project area. A chlorimeter to test for total chlorine can be used for this measurement by multiplying the total chlorine value by 0.89 (Finlayson et al. 2010A, SOP 7, pg. 71: "A less accurate estimate of $KMnO_4$, but an easier approach, is the DPD [N, N-diethyl-p-phenylenediamine sulfate] method for measuring total chlorine. Through the introduction of a powder containing DPD, potassium iodine, and a buffer, the oxidizing potential of the solution is measured spectrophotometrically or using a color wheel. The oxidizing potential of permanganate is about 89% of total chlorine, so results from this method can be multiplied by 0.89 to get an approximate measure of KMnO4 concentrations in water.") One live car of five sentinel fish would be placed just upstream of the neutralization station to confirm rotenone efficacy through the treatment area and to signal when neutralization should commence. Sentinel fish would be replaced at least once per day (Finlayson et al. 2010A, SOP 5) from an oxygenated tank with healthy fish, which will be collected on site (using methods mentioned above) just before treatment begins. Neutralization would be stopped when fish in this live car remain alive and appear unstressed for at least four hours. Additionally, one live car with sentinel fish would be placed at each of 15-minute, 30-minute, and approximately 1-hour flow travel time below the deactivation station as a backup, to ensure fish are not being killed beyond the 30-minute travel time below the deactivation station. Behavior of these fish would alert workers to the efficacy of rotenone deactivation in the stream section below the neutralization station.

Reservoirs with a metering device that can be manually controlled would be used to dispense potassium permanganate. The reservoirs would be initially calibrated prior to operation and adjusted at regular intervals as needed during operation. To calibrate metering devices, the valve on one tank is opened and a large graduated cylinder is used to measure the amount diluted potassium permanganate exiting the drip box over a 15 second period. Immediately before and during operation, the contents of the reservoir would be stirred (with a boat paddle) to ensure that all of the potassium permanganate is in solution. If the reservoirs or metering system begin to precipitate potassium permanganate, the reservoir and lines would be flushed with clean water prior to refilling for the next round.

Stream discharge at the site would be recorded daily before and during operation and adjustments in delivery rates made according to Table N-3. For any given discharge, the weight of potassium permanganate added to each barrel would be calculated and recorded.

Table N-3. Dispensing rates for a 2.5% solution of potassium permanganate calculated for various stream discharge rates to achieve an application rate of 3 ppm.

Stream Discharge (cfs)	Potassium permanganate (g/hr)	Potassium permanganate (g/24 hr)	Dispensing Rate for 2.5% Potassium permanganate Solution[1]		
			ml/min	L/hr	L/24 hrs
0.1	30.5	732	21	1.26	30.24
0.2	61.0	1464	42	2.52	60.48
0.3	91.5	2195	63	3.78	90.72
0.4	122.0	2927	84	5.04	120.96
0.5	152.5	3659	105	6.30	151.20
0.6	183.0	4390	126	7.56	181.44
0.7	213.0	5112	147	8.82	211.68
0.8	244.0	5856	168	10.08	241.92
0.9	274.5	6588	189	11.34	272.16
1.0	305.0	7320	210	12.60	302.40

[1] A 2.5% solution of potassium permanganate is achieved by dissolving 1 pound of KMn04 crystals into 5 gallons of water (25g/L).

[2] The dispensing rate is calculated as: LF = Y * 70 * Q where LF = dispensing rate of 2.5% solution of KMn04 (ml/min), Y = desired concentration of KMn04, and Q = stream discharge.

Rotenone treatments in standing waters (lakes, ponds, and marshes) would not be neutralized within the treated standing water habitats. If the outlet stream(s) of treated standing waters are flowing at the time of treatment, then they would be neutralized with potassium permanganate beginning at approximately 100 ft (30 m) in elevation below the elevation of the lake. This method would allow for natural degradation and breakdown of a substantial amount of rotenone-containing water, and thus less potassium permanganate would be needed to achieve neutralization. Any rotenone that remains in the treated standing water habitats would detoxify through natural degradation and breakdown.

Water Quality Monitoring

During and after rotenone treatments, water quality would be monitored to assess the effects of treatment on surface waters. The monitoring would determine if: (1) effective piscicide concentrations of rotenone have been applied; (2) sufficient degradation of rotenone has occurred prior to the resumption of public contact; and (3) rotenone toxicity has occurred outside the project area. An analytical laboratory would analyze water samples for rotenone and rotenolone (a common breakdown product of rotenone) (Ling 2003) concentrations as well as for volatile organic compound and semi-volatile organic compound concentrations.

During the treatment and neutralization period, water quality samples would be collected from various locations within the treatment area, and within and below the neutralization area. Sampling locations and collection frequency are specified in the permit. In streams, samples are expected to be collected from at least three locations within the stream treatment reach (top, middle, and bottom), plus three monitoring locations in the neutralization area (two minutes, 30 minutes, and 120 minutes flow time below the neutralization station). In lakes, samples are expected to be collected from the surface and at various depths at several locations throughout each treatment lake. The sample collection frequency is expected to be several times per day, including just prior to treatment, during treatment, and until rotenone degrades to <40 ppb. Following stream neutralization, water samples are expected to be collected once every seven days, until rotenone is no longer detected in samples. For several recent CFT Legumine™ treatments in Silver King Creek, California, water quality samples were non-detect for rotenone within seven days following neutralization (Mussulman S., pers. comm., 2016). It is possible it could take longer in some areas.

Holding times and temperature requirements of water samples would be adhered to. Water quality monitoring would follow the methods of SOP 16 in Finlayson et al. (2010A). These requirements would present a challenge given the remote locations of most of the proposed treatment areas, and the likelihood that storage and processing would take place at a non-local laboratory; however, these challenges have previously been overcome in SEKI wilderness (Clow et al. 2013).

There are several factors that can influence water quality related to the degradation process of the rotenone formula. Those include, but are not limited to: lake vegetation, groundwater influence, ultraviolet radiation, suspended solids, pH, temperature, rotenone travel time, substrate, oxidation-reduction potential, stream gradient, and lake depth and mixing regime (Britton et al. 2011; Brown and Zale 2012; NPS 2013B; Finlayson et al. 2014; Chapter 4, Impacts of Alternative B; Appendix G, Ecological Risk Assessment, Water). Detailed, on-the-ground habitat assessments would be conducted prior to piscicide treatments to ensure applications are at an effective concentration unique to the habitat and its biological and physical properties, and to investigate how these features would influence rotenone's persistence and impact on water quality.

Water quality monitoring would adhere to the state and federal criteria established by the required permit(s) from the California Regional Water Quality Control Board (CRWQCB) and/or EPA in order to conduct piscicide treatments in California. A unique permit would likely be required for each piscicide treatment area. The registration of CFT Legumine™ through the EPA and CDPR results in a product label that stipulates requirements for treatment concentration, duration, neutralization, and placarding (Zoëcon 2015). Permits obtained from the CRWQCB and/or EPA include stipulations for water quality monitoring.

During treatments, access areas to the treatment area would be placarded, and the project area would be closed to the public and all non-project staff. Detailed instructions for placarding are described in the *Communication Plan* section above. Placards would be removed and closures would be lifted once sampling has shown rotenone to be <40 ppb in water samples and/or sentinel trout can survive for 24 hours, which is expected to be a maximum of 7 to 14 days following neutralization (Finlayson et al. 2010A, Zoëcon 2015).

Post-Treatment Biomonitoring and Evaluation

To ensure that all fish have been removed from each treatment area, fish surveys using gill nets, backpack electrofishers, and visual searches would be conducted for two summers following treatment.

To monitor recovery of amphibians resulting from the absence of nonnative fish and possible treatment impacts, amphibian visual encounter surveys would be conducted for two or three summers following treatment.

Benthic Macroinvertebrates: Information to address concerns regarding benthic macroinvertebrates can be obtained using a replicated Before-After-Control-Impact experimental time series using multiple reaches (Green 1979, Underwood 1994, 1997). The following approach would be used in at least the initial two or three basins selected to receive any approved piscicide treatments. If information from the initial benthic macroinvertebrate studies suggests additional monitoring is needed (e.g., there are significant differences among basin benthic macroinvertebrate species assemblages, especially in regard to species more sensitive to piscicides), benthic macroinvertebrate monitoring would continue in subsequent basins proposed for piscicide treatment.

It is important to sample multiple sites within multiple Control and Experimental reaches in each watershed, if possible, to provide replication at the reach scale (Vinson et al. 2010), and such nested replication has been used in the ongoing work at Sixty Lake Basin (Holmquist and Schmidt-Gengenbach 2014). In some small and/or unbranched SEKI streams, it may be difficult to designate multiple experimental reaches, and in these cases replication would need to be at the scale of groups of individual samples within the same reach. Fifteen to twenty sites in each of the two reach categories would generally provide good power (which should be estimated a priori rather than retrospectively; Hoenig and Heisy 2001, Nakagawa and Foster 2004). Control reaches would be upstream of Experimental reaches. Efforts would be made to locate Control and Experimental reaches that are similar in terms of stream order, gradient, substrate, depth, and flow. A stratified random process would be used to select sites from within areas of comparable habitat. If post-sampling analyses indicate differences between Control and Experimental reaches, comparisons can still be made, because pre-application data would be available for both categories of reaches.

Sampling would be done one year before rotenone application (pre-treatment), shortly before and after piscicide application (to evaluate immediate effects), and at least two additional summers following application (to evaluate medium-term effects). In each study year, sampling would be done during the month anticipated for piscicide application (typically August) and during the following month (September). Additional monitoring may occur if time, personnel, and funding allow. Control and Experimental stream reaches would thus typically be sampled in August and September in the year preceding application, immediately before and after August rotenone treatment during the application year, during September of the application year, and in August and September of the year following application.

Control and Experimental stream reaches would thus be sampled multiple times and at multiple places in the years preceding application, immediately before and after rotenone application, later during the application year, and at least two years following application. Use of the Control reaches and multiple, nested, sampling locations, sampling months, and years would allow accounting for natural spatial and intra- and inter-annual variability. Interactions would be of particular interest: for instance, no Control-Experimental differences prior to application, divergence of the Experimental reaches from Before, and Control reaches after, application, followed by a possible reduction in differences with increased time after application.

Data collection in streams would be density-based (Vinson et al. 2010), which would enable a variety of response variables to be assessed, including abundance, richness, dominance, evenness, diversity, rank-abundance, functional feeding groups, responses of individual taxa, algal depth and cover, and siltation, using both uni- and multi-variate approaches. Faunal collections would be made with a standard Surber sampler (Surber 1937, Hauer and Resh 2007, Southwood and Henderson 2000; see also Waddle and Holmquist 2013, Holmquist and Waddle 2013). The Surber sampler is a 0.3 m x 0.3 m quadrat with a connected 0.3 m x 0.3 m framed net that is aligned perpendicular to the substrate. Mesh size is 0.5 x 0.5 mm. The quadrat is pressed against, and demarcates, a portion of the stream bed. The associated substrate is disturbed by hand, and organisms are swept downstream by the current into the net. Samples would be sorted completely in the lab, rather than subsampled, because complete sorting reduces the variance of metrics, increases taxon richness, and improves proportion-based metrics (Courtemanch 1996, Doberstein et al. 2000).

There may be cascading effects of piscicide treatment on algal growth in streams; a proliferation of algae following removal of invertebrate herbivores may occur. A transect 0.5 m upstream of each subsample location would be used for measurement of algal cover and layer using the rapid-assessment methodology of Fetscher et al. (2009). Each transect associated with each Surber sample would include algal thickness measurements and cover estimation at five equidistant points across sampled habitat (Holmquist et al. 2015).

A variety of physical data would be collected at each subsampling site after biological sampling is completed. Depth would be recorded as a mean of four equidistant measurements within the Surber quadrat. Substrate characteristics within each quadrat can be categorized via the Wentworth substrate scale (Allan and Castillo 2007) and embeddedness would also be estimated. A flow meter would be used to measure velocity at 0.6 depth and 0.25 m upstream of each sampler location. Water temperature would also be recorded. Percent tree canopy cover would be determined with a convex spherical densiometer and slope can be estimated with a Suunto clinometer or equivalent. In addition to providing overall habitat information, these physical measurements would be used to test/confirm assumptions regarding habitat similarity between Control and Experimental reaches.

Benthic macroinvertebrate sampling in lakes would follow standard methods, such as those used by Deiner et al. (2013). Samples would be collected in the littoral zone of lakes and taken via 15 sweeps with a D-net outfitted with a 0.5 mm mesh bag. Sweeps will be taken from all available habitat types, and the number of sweeps in a particular habitat type would reflect the proportional availability of that habitat type. For example, if 20% of the littoral zone is made up of silt, three sweeps would be taken from silt habitats.

One standard sweep is about 1 m in one direction, followed immediately by a 1 m sweep in the opposite direction through the same area. Sweeps would be made after vigorously stirring up the substrate. After sweeping, net contents would be transferred into a sorting pan containing lake water. All benthic macroinvertebrates would be removed and placed in a labelled sample bottle.

Samples would for both stream and lake monitoring would be preserved in 95% ethanol and shipped to a reputable laboratory for identification to the lowest possible taxonomic level possible for each taxon collected.

Zooplankton: Zooplankton sample collection and laboratory-based taxon identification would be conducted using the following methods from Knapp et al. (2001). Collections would occur during the day from the deepest portion of the lake using float tubes (small, portable, inflatable rafts).

Samples would be collected by taking vertical tows from the bottom to the surface with a conical plankton net (29.5 cm diameter, 64-mm mesh) in the deepest part of each lake. One to five replicate tows would be made until substantial numbers of zooplankton are present in samples (Stoddard 1987, Bradford et al. 1998). Samples would be preserved in ethanol and shipped to a reputable laboratory for identification to the lowest possible taxonomic level possible for each taxon collected.

Record Keeping and Reporting

Daily records of rotenone and potassium permanganate application would be maintained by staff at each treatment and detoxification station. These records include the following information; date, operator name, station #, flow (cfs), concentration of CFT Legumine™, duration, grams of potassium permanganate/hr, starting time and ending time. Water temperature, rotenone, and comments or observations would be recorded hourly, including any deviations from the treatment plan. All records would be given to the project manager at the completion of each day's work. Information from the records would then be summarized by the project manager.

A final report would be completed after each treatment. The report would include background information on the project, a treatment overview, and steps taken to meet compliance with FIFRA and CWA (NPS 2008D; AOP 10).

Project Close-out Operations

At the conclusion of the final potassium permanganate application and prior to exiting each project area, the field team would gather and remove all items brought into each project area. The project manager would perform a final walk-through of each project area to ensure no chemicals or equipment are left behind. The project manager would have any postings or notices removed no more than two weeks following each treatment.

Piscicide Use Adaptive Management Framework

If alternative B or D is selected, no piscicide treatments would occur until 2017, at the earliest. The estimated implementation timeline of proposed piscicide treatments is shown in chapter 2 (alternative B, Development of the Site-Specific Treatment Plans). An adaptive management approach would allow SEKI to incorporate the results of piscicide treatments in order to adjust future treatments. Results of the monitoring of the initial piscicide treatments would be used to better understand the likely impacts of subsequent treatments, and if necessary, to redesign subsequent treatments to further minimize anticipated impacts. The following describes the approach for implementing a piscicide use adaptive management framework.

Number of Treatments: The general protocol would be to treat habitat once in mid- to late summer, monitor in September, and monitor again in the following early to mid-summer. If live fish are found during the monitoring period, a second treatment would be conducted. If no live fish are found during the monitoring period, then a second treatment would not be necessary. Some of the proposed treatment areas in SEKI are small (e.g., one outlet stream of Upper Bubbs) and one treatment would likely be effective for eradicating all fish. Other proposed treatment areas are larger (e.g., lower portion of Sixty Lake Basin), and while one treatment may eradicate all fish in these areas, additional treatment(s) may be necessary. Whether reapplications may be necessary in a treatment area is based on several site-specific factors, such as fish species, habitat size and complexity, streamflow and gradient, water temperature, water quality, and others. If fish eradication is unsuccessful after three treatment years (initial year plus two reapplication years) at a particular site, then piscicide use would be abandoned at that site.

Treatment Concentrations: One goal is to use the lowest piscicide concentration in each treatment area that still allows eradication of all fish after one treatment. The CFT Legumine™ label stipulates application concentrations for trout (normal species) to be from 0.5 to 1 ppm of product (25 to 50 ppb active ingredient rotenone). The initial two to three treatments would likely be applied with a target concentration of 1 ppm. If it appears that eradication could have been achieved using less than 1 ppm of product in the initial treatments, then a portion of the next treatment may be treated with a target concentration of 0.5 to 0.75 ppm of product. This would allow testing to determine whether fish eradication could be achieved in certain sites in the lower range of prescribed concentrations, and thus minimize potential environmental effects. Habitat complexity is one factor that may affect whether lower concentrations are feasible.

Treatment Design: Results of initial treatments in SEKI as well as other recent high elevation treatments in the U.S. would be evaluated to inform the implementation design of subsequent treatments. Each new treatment would attempt to use the most up-to-date practices that should maximize the potential for successful fish eradication using the minimum number of treatments and/or the lowest piscicide concentration.

Invertebrate Recovery: To evaluate recovery of benthic macroinvertebrate and zooplankton assemblages resulting from (1) the absence of nonnative fish predation and (2) possible treatment impacts, benthic macroinvertebrate and zooplankton assemblages in streams and lakes in the first two or three treatments would be sampled for two or three summers following each treatment. If results at these study sites show recovery has occurred by the second or third summer following treatment, then macroinvertebrate and zooplankton studies would not be conducted for the remaining piscicide treatments.

Benthic macroinvertebrate and zooplankton "recovery" is defined as the following. *Assemblages in treated habitats largely resemble assemblages in untreated fishless habitats, having a similar number of taxa and abundance. This would suggest that ecological function typically provided by benthic macroinvertebrate and zooplankton assemblages in fishless habitat has returned to the treated habitat; and the treated habitat would thus be in a restored ecological state compared to when it was impacted by nonnative fish.* If results of the initial studies are highly variable, in which some treatments show sufficient recovery of benthic macroinvertebrates and zooplankton but some treatments show poor recovery by the second or third summer following treatment, then invertebrate studies would continue following subsequent treatments until a consistent pattern of sufficient recovery is shown. If the first two or three treatments all show poor recovery of benthic macroinvertebrates and zooplankton by the second or third summer following treatment, then piscicide use in this plan would be re-evaluated.