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Natural Resource Program Center

A Field Manual for the Use of Antimycin A for Restoration of Native Fish Populations

Natural Resource Report NPS/NRPC/NRR—2008/033




ON THE COVER

Clockwise from top left: Pictures of a small stream in Great Smoky Mountains NP (NPS photo), a high elevation lake in Great Sand Dune NP (NPS photo), an antimycin detoxification site in Great Smoky Mountains NP (NPS photo), and boat application of antimycin A via a Venturi device on a high elevation lake in Rocky Mountain National Park (USFWS photo).

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Executive Summary

"The national park system was created to conserve unimpaired many of the world's most magnificent landscapes, places that enshrine our nation's enduring principles, and places that remind us of the tremendous sacrifices Americans have made on behalf of those principles" (NPS, 2006). To accomplish this, an important component of the current management strategies calls for the National Park Service to maintain as parts of the natural ecosystems of parks all plants and animals native to park ecosystems and to restore, where appropriate, native plant and animal species (NPS, 2006), While management actions today make every attempt to meet this mandate, during the formative years of the National Park Service, managers sometimes promoted parks by managing scenery and enhancing tourism activities such as recreational fishing, sometimes to the detriment of natural systems. Oftentimes, these recreational activities were readily endorsed by the public and they clamored for more opportunities including the stocking of non-native fishes. In its early years, the new agency did not have knowledgeable staff to manage the fishery; this function was often turned over to state agencies. The states followed accepted recreational fishery management actions of the day and stocked non-native fish in National Park units across the nation. Only in more recent years have biologists in state agencies and national parks began to realize the irreparable damage that had occurred due to the loss of genetic integrity and the displacement or elimination of native species by non-native species.

National Park Service fishery managers and fishery managers across the nation are now faced with the problem of protecting and preserving native fish and, if possible, restoring them to segments of their historic range. Currently, only two techniques (backpack electrofishing and piscicides) are available to successfully remove non-native fishes in order to restore native fish to segments of their historic range. Projects in Great Smoky Mountains National Park (GRSM) used electrofishing techniques to successfully remove non-native rainbow trout (*Oncorhynchus mykiss*) from segments of small streams with natural barriers to upstream migration of non-native salmonids. Data from these projects demonstrate that they are labor intensive and are subject to failure if all of the right conditions are not met.

In general, the use of piscicides (fish poisons) is the only way to ensure complete eradication of entire non-native fish populations short of dewatering habitats. Historically, Fintrol® (antimycin A) and Rotenone® have been used by fisheries biologists throughout the United States (U.S.) for restoring native fish species in streams and lakes.

The purpose of this manual is to provide fisheries managers' with protocols for conducting a safe, effective, and lawful restoration project with antimycin A. Using the protocols specified in this document, fisheries managers will be able to apply antimycin A consistently to remove unwanted fish species from streams, ponds, or small lakes and impoundments (<50ha) with subsequent re-establishment of natural aquatic communities. This standardized and consistent use of antimycin A will also help reduce potential for human exposure and unintended adverse ecological effects.

Acknowledgements

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Introduction

This report, *A Field Manual for the Use of Antimycin A for Restoration of Native Fish Populations,* provides protocols for the use of antimycin A which have been successfully used in restoration efforts within units of the national park system and in national forest and state projects. This document is based upon experiences of biologists from the National Park Service (NPS), the U.S. Fish and Wildlife Service (USFWS) and New Mexico Department of Game and Fish. The authors' hope that the information included in the manual will be applicable to any application of antimycin for the restoration of native fish populations and that it provides fisheries managers with established protocols for safe, effective, and lawful restoration projects utilizing antimycin A.

Fisheries Management in National Parks

The National Park Service (NPS) was established by Congress in 1916. This action formally recognized the significance of setting aside and preserving areas in the United States because of their important and unique natural and cultural resources. The Organic Act was passed in 1916 and directs the National Park Service to protect and conserve the resources of these areas "unimpaired for both present and future generations." This is a unique mandate among federal land management agencies and it directs park managers to be concerned with the conservation of natural diversity, the functioning of native ecosystems, and the maintenance of natural processes. While today NPS effectively meets these mandates, recognition of the actions required to comply with these mandates developed slowly in the National Park Service due mainly to the incomplete understanding of ecological processes during the formative years (Zuble 1996, Sellers 1997).

By 1916, over 25 National Parks and monuments had been established primarily in remote western areas of the continental United States (Tilmant 2004). Some of these units were relatively pristine initially and others were not. Regardless of the condition, NPS managers were challenged to attract people and build a constituency for the fledgling agency (Tilmant 2004). Management actions during this time included providing easy access to the parks, programs to concentrate wildlife for easy viewing, and promoting recreational fishing, other outdoor sports, and sometimes even cattle grazing within the parks. Early managers appeared to interpret the Organic Act of 1916 to mean managing scenery and wildlife, accommodating tourists, and promoting parks (Tilmant 2004). One product of this early management philosophy was introduction of non-native fish species to support recreational angling and has subsequently led to ongoing restoration efforts to correct these actions.

Introduction of Non-Native Species

The management of natural resources during the formative years of the NPS was often delegated to state agencies and their management actions followed the then current fishery management practices of the day, despite potential inconsistencies with NPS management policies. All of these actions reflected the anthropocentric view of the period that resources were to be managed for the benefit of people. This, in turn, led to the widespread and purposeful introduction of non-native fishes within National Parks. This "Johnny Apple-fish" mentality was so prevalent that in some cases non-native fish rearing facilities were established within National Parks (Tilmant 2004). These efforts aimed primarily to provide popular non-native sport fish and to diversify recreational angling opportunities (Schullery 1979, Rahel 1997). Many of these programs included stocking numerous fishless lakes and streams (Franke 1996) because a “vacant niche” was thought to exist and thus needed to be filled with sport fish. During this time, little if any thought was given to the potential long-term consequences of these actions for indigenous species and their habitat. Even though this description focuses on the NPS, state fishery management agency programs as well as those on U.S. Forest Service, U.S. Bureau of Land Management, state and tribal lands are a mirrored what occurred on National Park lands.

Early managers had few resources to properly understand ecosystem functions and most saw no harm in non-native introductions; rather, they believed their actions to be consistent with Congressional mandates. The impact of non-native sport fish on native fish and aquatic systems became apparent around 1928 when park managers became concerned about the potential adverse effects of stocking fish (Tilmant 1999). Still, the majority of resource managers continued to believe that the public benefits of stocking outweighed the “disadvantages which may incidentally occur” (Wright and Thompson 1933; Sellars 1997).

Because the effects of “non-native” introductions on aquatic systems were less noticeable than in terrestrial systems, they are arguably the most altered and adversely affected resources in park units today. Alterations include native species displacement, population reduction, disease and parasite introduction, and hybridization. The magnitude and extent of these actions make it difficult for park managers to protect and conserve aquatic resources “unimpaired for the enjoyment of future generations” (NPS Organic Act 16 USC). In many cases the impacts on native fish populations have been so great that many species are now Federally listed as Endangered or Threatened (e.g. Gila trout, Rio Grande Cutthroat trout, bull trout, etc.)

Restoration Efforts

In an effort to restore the ecological integrity of historically altered ecosystems and to comply with the intent of the 1916 Organic Act and NPS policies, biologists initiated efforts to restore native fishes to segments of their historic range and these efforts are continuing. It cannot be overemphasized that successful restoration projects depend on appropriate site selection. A natural or man-made waterfall or other barrier must be located at any outflow sites to prevent non-native species from re-entering the treatment area after restoration is complete.

The first effort to protect native fish occurred in 1936 when NPS Director Cammerer issued written fish management policies with the intent of prohibiting the wider distribution of non-native fish in NPS waters (Cammerer 1936). This Order stated that “exotic species were not to be introduced in waters where only native fish existed” and that where exotic and native fish existed, the native species were to be favored. Despite this early effort, fish stocking continued from the 1930s to the 1970s. A policy to phase out non-native fish stocking in National Parks was adopted in 1968.

Currently, only two techniques (backpack electrofishing and piscicides) are available to successfully remove non-native fishes in order to restore native fish to segments of their historic range. Kulp and Moore (2000) and Moore *et al.* (2005) demonstrated that electrofishing techniques were used successfully to remove non-native rainbow trout (*Oncorhynchus mykiss*) from segments of small streams in Great Smoky Mountains National Park (GRSM). While this technique is effective in segments of small streams with natural barriers, electrofishing is more time consuming and more costly than restoration projects using piscicides (Moore et al 2005).

Restoration projects using piscicides typically rely on one of the four chemicals approved by the U.S. Environmental Protection Agency (EPA) for use in fisheries management to eliminate non-native fish: Fintrol®, Rotenone®, Lampricide® and Bayluscide®. In general, the use of fish poisons (piscicides) is the only way to ensure complete eradication of entire non-native fish populations short of dewatering habitats. Historically, Fintrol® (antimycin A) and Rotenone® have been used by fisheries biologists throughout the United States (U.S.) for restoring native fish species in streams and lakes. Lamprecid® (3-trifluromethyl-4-nitrophenol) and Bayluscide® (niclosamide) can only be used legally in the Great Lakes drainages and their tributaries to control sea lampreys (*Petromyzon marinus*).

The NPS Antimycin A Manual

Purpose

The purpose of this manual is to provide fisheries managers' with protocols for conducting lowest risk, yet safe, effective, and lawful fisheries restoration projects with antimycin A. Using the protocols specified in this document, fisheries managers will be able to apply antimycin A to remove unwanted fish species from streams, impoundments, or small lakes (<50ha) with subsequent re-establishment of natural aquatic communities. The standardized and consistent use of antimycin A will minimize if not eliminate the potential for human exposure and unintended adverse ecological effects on non-target organisms. In addition to the literature cited, a list of other pertinent references is listed for readers seeking additional information on topics discussed in this manual.

Scope and Applicability

This document provides a thorough understanding of antimycin A use with regard to permitting, legalities, history, chemistry, risk analysis, safety precautions, application materials, treatment concentrations, application techniques, deactivation procedures, reporting, and project monitoring protocols. The manual contains the information necessary to plan and implement a project using antimycin A, pending the completion of all pertinent certifications, bioassays, environmental planning, permitting, and compliance requirements. Equipment checklists are also provided to help the project manager/applicator purchase, build, and maintain all equipment needed for antimycin A treatments.

It is a violation of Federal law to apply antimycin A in a manner inconsistent with its labeling.

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Structure

This manual is divided into four sections: the main text (pages 1 - 29), administrative operating procedures (pages 30 – 47), technical operating procedures (pages 48 - 88), and appendices (91 – 124. The main text provides an overview of the complexities involved in preparing for and conducting an antimycin A treatment. The administrative operating procedures (AOP) provide detailed instructions on how to meet the administrative requirements of an antimycin A treatment (*e.g.*, obtaining the necessary permits). The technical operating procedures (TOP) provide detailed instructions on how to satisfy the technical requirements of an antimycin A treatment (*e.g.*, recommendations on how to mix and apply the compound). The appendices provide additional resources for conducting antimycin A treatments.

Updates

Every effort has been made to ensure that the most accurate and up-to-date information is contained in this manual. The National Park Service recognizes that, from time to time, this manual may require revisions based on new information (*e.g.*, improved treatment techniques), changes in state and Federal regulations (*e.g.*, changes to the EPA-approved product label), and/or other factors. As appropriate, NPS will revise this manual in accordance with the appropriate NPS procedures.

Deviations

Although every effort should be made to adhere to the protocols outlined in this document, the NPS recognizes the potential need to deviate from certain protocols under extenuating circumstances. Such circumstances include situations which, if left unchecked, may jeopardize the safety of an individual, result in exposure beyond the treatment area, violate Federal or other regulations, or render the treatment ineffective. In such instances, the project manager will document the deviation and rationale in accordance with AOP 11.

It is a violation of Federal law to apply antimycin A in a manner inconsistent with its labeling. A protocol deviation that violates the antimycin A product label, while properly documented, does not absolve the violator(s) from potential administrative or legal action under FIFRA.

Antimycin A Overview

This section provides an overview of the piscicide antimycin A including its background, approved uses, formulation and application methods, environmental fate, and toxicity. Additional information can be found on the Fintrol® product label and the literature cited in Appendix A.

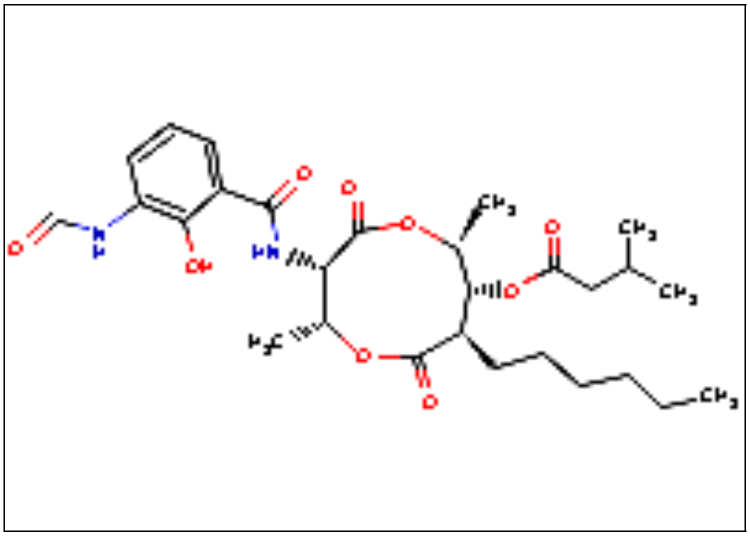
Background

Antimycin A, discovered in 1945, is an antibiotic derived from the soil mold *Streptomyces*. Antimycin A is currently registered with the U.S. Environmental Protection Agency (EPA) as a restricted use pesticide under the trade name Fintrol® (EPA Registration number 39096-2). Restricted use chemicals may be applied only by certified pesticide applicators.

Antimycin A applications are made directly to water. The putative mode of action for antimycin A is that the compound uncouples oxidative phosphorylation by blocking the electron transport pathway to Complex III within the mitochondria (Bettermann *et al.* 1996). Antimycin A (3-methylbutanoic acid 3[[3-(formylamino)-2-hydroxylbenzoyl]amino]-8-hexyl-2,6-dimethyl-4,9-dioxo-1,5-dioxonan-7-yl_ester) consists of four major components which in turn consist of a pair of compounds, for a total of eight significant homologues (Abidi and Adams 1987; Abidi 1988) — four "major" and four "minor"—that are distinguished by their chemical constituents. Figure 1 presents the chemical structure of the A3 component of antimycin A which is believed to be the most toxic isomer.

This section provides an overview of the piscicide antimycin A including its background, approved uses, formulation and application methods, environmental fate, and toxicity. Additional information can be found on the Fintrol® product label and the literature cited in Appendix E.

Figure 1. Chemical structure of antimycin A (A3) molecule.



Source: http://toxnet.nlm.nih.gov/cgi-bin/sis/search

Approved Uses

Antimycin A is a restricted use pesticide and therefore may only be applied by pesticide applicators certified in the aquatic category by the state in which the application will occur. Employees working under the direct supervision of the certified project manager may assist in antimycin A application.

There are two broad uses for antimycin A as a piscicide: complete kill and selective kill. In a complete kill, the water body is treated with antimycin A at water column concentrations ranging from 5 to 25 µg/L antimycin A to eliminate all fish in the treatment area. A common objective of a complete kill is to eliminate invasive or non-native species in an area in order to restore listed or indigenous species (Wormell 2005).

In a selective kill, the water body is treated with antimycin A at 0.5 to 1.0 µg/L of antimycin A to eliminate only small, scaled fishes. A common objective of a selective kill is to eliminate smaller fishes to free up food and other resources for larger fish. Selective kills at higher concentrations are also used in catfish production (aquaculture) to eliminate scaled fish that commonly reduce the catfish yields of commercial catfish farmers. According to the Fintrol® label, scaled fish in aquaculture ponds succumb to treatment with antimycin A at 5 to 10 µg/L of antimycin A, whereas catfish generally tolerate antimycin A at concentrations up to 20µg/L (Wormell 2005). Based on information provided by the registrant (Personal communication, Mary Romeo, president of AquaBiotics), the majority of Fintrol® use in [catfish] aquaculture is for food-fish production and not fingerling-production. According to aquaculture use information collected by the Southern Regional Aquaculture Center (Personal communication, Dr. Craig Tucker, U. S. Department of Agriculture), Fintrol® use is roughly equally divided between food-fish and fingerling-production ponds to eliminate scaled fish at various points within the production cycle.

Formulation

Antimycin A is produced as a fermentation product from Streptomyces mold and is sold as a "unit" of biological activity (480 ml). A "unit" of antimycin A consists of one bottle of active ingredient (240 ml) and one bottle of diluent (240 ml) that contains a surfactant. According to label instructions, the active ingredient and diluent must be mixed together prior to use of the product. Once the product is mixed, the label states it must be used within seven days (Ayerst, 1970). The surfactant is intended to enhance the solubility of antimycin in water and to increase the transfer efficiency of antimycin across cell membranes (*e.g.* gill uptake).

Environmental Fate

The primary route of antimycin A degradation is alkaline hydrolysis and the chemical becomes inactive approximately eight hours after being mixed with water (USEPA 2006**)**. Antimycin breaks down more rapidly in warmer (*i.e.* >12C) and more alkaline (*i.e.* >pH 8.0) water. Field studies of downstream movement also show that the compound can extend and remain active for linear distances of 1.75 km beyond desired treatment areas when no effort is made to deactivate the compound (Tiffan and Bergersen 1996). However, field observations from projects in New Mexico, Tennessee, Nevada and South Carolina demonstrate that antimycin A is naturally neutralized in much shorter distances, often 500 m or less. There are no direct measurements of antimycin adsorption; however, field observations provide evidence that antimycin adsorbs significantly to soil, sediments and organic material in the stream thereby reducing potential exposure outside of the immediate treatment area (USEPA 2006). Field observations from NPS supervised projects in South Carolina provide evidence that adsorption to organic material also limits the linear and vertical distance the antimycin is effective (Moore and Kulp, personal observations).

Environmental factors such as water body size, discharge, pH, water temperature, and stream gradient may affect the quantity of antimycin A and the number of application stations that must be used to achieve the desired concentration in the treatment area. As antimycin A travels downstream, aeration and agitation due to the physical movement of the water contribute to the degradation of antimycin and decrease the piscicidal activity of antimycin. In Sams Creek (GRSM), a 15-20 m (50-60 ft) drop in elevation effectively deactivated antimycin, whereas in Bear Creek (GRSM) a drop in elevation of 24-30 m (80-100 ft) was required to deactivate antimycin (Moore et al 2005). In some western parks such as Rocky Mountain and Crater Lake NP, effective vertical change in elevation for antimycin deactivation was 40-60 m (120-200 ft) Rosenlund, personal observation). These data provide an indication of the variability between project areas and provide insights into the necessity for on site bioassays described in TOP 9.

Toxicity

When antimycin is applied according to the procedures outlined in this manual for native fish restoration, it is not toxic to terrestrial organisms. Information derived from studies in GRSM also demonstrates that it is not toxic to salamanders or crayfish (Moore *et. al.* 2005). The lethal toxicity of antimycin varies between families and species of fish. Appendix C provides information on the amount of antimycin required for a lethal dose for many fish species. Although fish eggs are susceptible to laboratory exposure of antimycin A (100% mortality using 10 ppb for 1 hour), impacts to eggs in the gravel have not been observed in actual treatments. Instream studies by Berger *et al.* (1975) and Olson and Marking (1965) demonstrated that treated water does not completely mix with the water flowing through gravel where salmonid eggs incubate and therefore does not have an impact on this life phase. Some native greenback cutthroat trout (*O. clarki stomias*) restoration projects in Rocky Mountain National Park failed because treatment occurred while non-native trout eggs were incubating in the gravel (personal communication, B. Rosenlund, U. S. Fish and Wildlife Service 2006).

Regulatory Compliance

Applying antimycin A in a lawful manner may require users to comply with at least four Federal laws: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), the Clean Water Act (CWA), the National Environmental Policy Act (NEPA), and the Endangered Species Act (ESA) (AOP 3). Note that adherence to the provisions of the laws discussed herein does not absolve the applicator from complying with appropriate state, tribal, or territorial regulations. The project manager is responsible for ensuring and, where appropriate, documenting legal compliance with all applicable regulations.

Additionally, projects on federal lands must comply with The Department of Interior, Department Manual 517 Integrated Pest Management (DOI, 2007) requires use of an integrated pest management approach when managing pest species (Title 7 136r-1), (Appendix B.1). At the NPS and FWS bureau levels pesticide use proposals should be submitted through the respective bureau's IPM Program's Pesticide Use Proposal Process where all aspects of the project are reviewed for compliance with the IPM approach, insures the problem not the symptom is addressed, and plans for long term management. Pesticide requests are reviewed, approved, denied, or approved on a conditional basis. Every park project or project on federal lands is required to go through an annual review when requesting approval for the use of a pesticide.

For the context of this document, pests are defined as "living organisms that interfere with the purposes or management objectives of a specific site within a park or that jeopardize human health or safety" (MP 2006 4.4.5.1). The procedures in this manual support the "11 Step the IPM Process" implemented by NPS and USFWS (Appendix B.2). This approach fosters science-based decision-making on a site specific basis as described in this manual.

Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) require all pesticides sold or distributed in the United States (including imported pesticides) to be registered by the EPA. Registration typically requires submission of toxicity, environmental fate, and other data which EPA then evaluates to determine whether or not the pesticide will pose “unreasonable adverse effects” to human health or the environment.

FIFRA also requires that pesticide products be labeled appropriately and that use is consistent with both the affixed product label and any accompanying or referenced materials, together making up the product’s labeling. Each product’s labeling provides users with all the necessary information to conduct a FIFRA-compliant application. This information includes directions for use (including storage and disposal), permitted and prohibited use sites, personal protective equipment that must be worn when handling or applying the product, and precautionary statements based on the product's toxicity.

It is a violation of FIFRA to apply a pesticide in a manner inconsistent with its label. To ensure compliance with FIFRA, any and all antimycin A applications must be made in strict accordance with the EPA-approved product labeling.

Clean Water Act

The Federal Water Pollution Control Act Amendments of 1972, (as amended in 1977 and commonly known as the Clean Water Act or CWA), established the basic structure for regulating discharges of pollutants into the waters of the United States. CWA gives EPA the authority to implement pollution control programs and to set water quality standards for all contaminants in surface waters, and makes it unlawful for any entity to discharge a pollutant from a point source into navigable waters unless a permit is previously obtained under the National Pollutant Discharge Elimination System (NPDES) program (Clean Water Act Section 402).

EPA issued a Final Rule (FRL-8248-1) on November 27, 2006 clarifying two specific circumstances in which a Clean Water Act permit is not required to apply pesticides to or around waters of the United States: (1) the application of pesticides directly to water in order to control pests and (2) the application of pesticides to control pests that are present over (including near) water, where a portion of the pesticides will unavoidably be deposited into the water in order to target the pests effectively.

Antimycin A is an aquatic pesticide applied directly to water to control fish; therefore, provided that antimycin A is registered with EPA, an NPDES permit is not required to make a CWA-compliant treatment. Note that EPA's Final Rule does not absolve the project manager from ensuring appropriate state, tribal, or other compliance with CWA.

National Environmental Policy Act

In 1969, Congress passed the National Environmental Policy Act (NEPA) and this legislation became effective January 1, 1970. NEPA established the environmental policies for the United States with the intent of ensuring that the physical environment is protected for present and future generations. This legislation requires that any action or activity funded, authorized, or accomplished by a federal agency adhere to NEPA. This requires that the action agency inform interested public of the proposed action, document the decision-making process to ensure that the proposed action is evaluated in context of reasonable alternatives, if any, and make public the final decision regarding the proposed action.

Public interest in the management of natural resources in National Parks and other federal lands has increased as has public knowledge of management efforts. With water resources, management actions related to the removal of non-native fish with piscicides is controversial, considered a major Federal action, and is not categorically excluded under NEPA. Because of this, an environmental assessment (EA) or environmental impact statement (EIS) must be prepared for a native fish restoration project using piscicides in National Parks or other federal lands. The EA is a concise public document that explains the need for the proposed action, alternatives to the proposed action, the environmental impact of each alternative and a list of the agencies and people consulted. The EA is intended to provide sufficient evidence to determine if an EIS or finding of no significant impact (FONSI) should be prepared (Director's Order #12, 2001). If the park or federal land owner has multiple projects in the same area, it is recommended that all proposed streams be listed in the EA or EIS. By following this recommendation, NEPA compliance will only need to be done once. Native fish restoration projects on other federal lands require NEPA compliance but the requirements may vary slightly between federal agencies.

Once completed, a public notice of the availability of the document for public review must be issued. As a general rule for the NPS the document must undergo a 30 day public review and comment period. This process is to be guided by agency specific mandates and guidelines. Based on the information gained and developed from the review, the park will decide if an EIS is necessary or to issue a FONSI. If the latter, then the FONSI must specify the reasons why the action will not have a significant impact on the human or natural environment and state why the EIS is not necessary. This document is recommended for approval by the Park Superintendent and forwarded to the regional director for final approval and signature. This process is to be guided by agency specific mandates and guidelines.

Endangered Species Protection Act

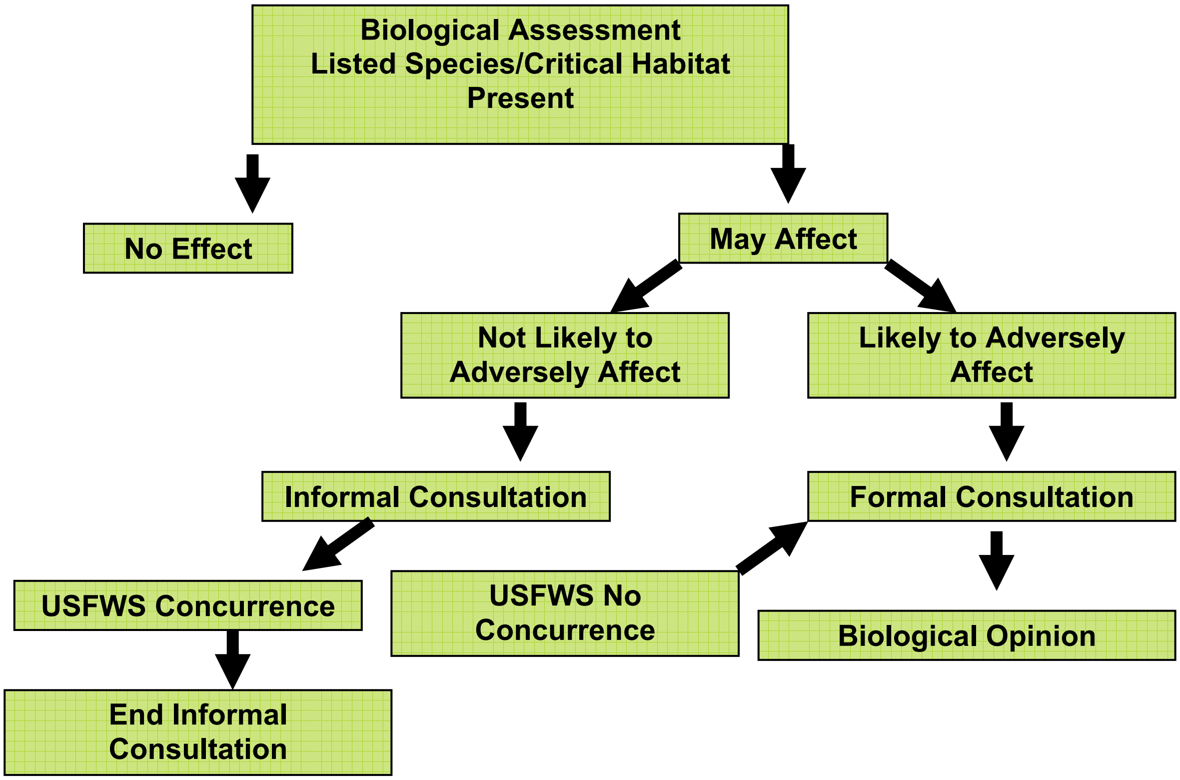
Federal regulations implementing Section 7 of the Endangered Species Act, 16 U.S.C. Section 1536(a) (2), require all Federal agencies to consult with the National Marine Fisheries Service (NMFS) for marine and anadromous listed species, or the United States Fish and Wildlife Services (FWS) for listed wildlife and freshwater organisms (hereafter referred to as the "Services"), if they are proposing an "action" that may affect listed species or their designated habitat. Each Federal agency is required under the Act to ensure that any action they authorize, fund, or carry out is not likely to jeopardize the continued existence of a listed species or result in the destruction or adverse modification of designated critical habitat. EPA, a Federal agency, authorizes through product labeling the use of pesticides such as antimycin A. Pesticide users, in turn, must comply with product labeling that EPA approves under the Federal Insecticide, Fungicide and Rodenticide Act, 7 U.S.C. Section 136-136y. , *et seq*, for the purpose of preventing jeopardy to listed species or adverse modification of their critical habitat. FWS defines adverse modification to designated critical habitat as "a direct or indirect alteration that appreciably diminishes the value of critical habitat for both the survival and recovery of a listed species" (50 CFR 402.02). To jeopardize the continued existence of a listed species means "to engage in an action that reasonably would be expected, directly or indirectly, to reduce appreciably the likelihood of both the survival and recovery of a listed species in the wild by reducing the reproduction, numbers, or distribution of the species" (50 C.F.R. § 402.02).

To facilitate compliance with the requirements of the Endangered Species Act subsection (a)(2), the Environmental Protection Agency (hereafter referred to as the EPA or "the Agency") Office of Pesticide Programs has established methodologies for evaluating whether a proposed registration action may directly or indirectly affect listed species or their designated critical habitat (U.S. EPA 2004). Based on the Agency's screening-level risk assessment, levels of concern (LOCs) are exceeded for direct or indirect effects for listed or candidate aquatic species that co-occur in the area of the proposed antimycin use or areas downstream that could be contaminated if the proper neutralization procedures are not used (U. S. EPA 2006). If determined that listed or candidate species are present in the proposed treatment area, further biological assessment must be undertaken. The extent to which listed species may be at risk from the proposed action determines the need for development of a more comprehensive consultation package as required by the Endangered Species Act (ESA).

The process for determining risk to endangered species is discussed in the document entitled "Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs."[1] If the assumptions associated with the screening-level action area result in risk quotients (RQ or ratio of exposure to toxicity) that are below the Agency's level of concern (LOC) for risk to Federally-listed endangered and/or threatened ("listed") species, a "no effect" determination conclusion is made with respect to listed species in the project area. EPA's 'no effect' determinations also takes into account the potential indirect effects to listed species by also evaluating the RQs for species that listed species may depend upon for survival. However, in situations where the screening assumptions lead to RQs in excess of the listed species LOCs for a given taxonomic group, a potential for a "may affect" conclusion exists and may be associated with direct effects on listed species belonging to that taxonomic group or may extend to indirect effects upon listed species that depend upon that taxonomic group as a resource. In such cases, additional information on the biology of listed species, the locations of these species, and the locations of use sites could be considered to determine the extent to which screening assumptions regarding an action area apply to a particular listed organism. These subsequent refinement steps should consider how this information would impact the action area for a particular listed organism and may potentially include areas of exposure that are downwind and downstream of the pesticide use site.

[1] http://www.epa.gov/oppfead1/endanger/consultation/ecorisk-overview.pdf

Figure 2. A conceptual model outlining the interaction processes of working with the Services under the Endangered Species Act (ESA).



Once the action area is defined, the U.S. Fish and Wildlife Service and/or the National Marine Fishery Service, hereafter referred to as "the Services," will provide action agencies such as the National Park Service and the EPA with a list of designated critical habitat and listed species that occur in the action area and the action agency determines that the action "may affect" listed species. If designated critical habitat or listed species co-occur or overlap with the action area, then the Agencies will engage in consultation with the Services. The consultation package will include: use site information, listed species biological characteristics (direct effects analysis), biological/ecological information for indirect and critical habitat effects. Additional details on information to be provided to the Services as required by the ESA are presented in (AOP 3).

The action agency is responsible for determining whether or not the proposed action "may affect" designated critical habitat or protected species. This determination must be submitted to the appropriate Service for concurrence.

No Effect Determination
If the action agency determines that the proposed action has "no effect" on designated critical habitat or listed species it is not required to consult with the Services on the action.

Not Likely to Adversely Affect Determination
If the action agency determines that the proposed action "may affect" designated critical habitat or protected species, there are two possible determinations. If the determination is that the proposed action is "not likely to adversely affect" and the Services provide written concurrence, the action agency has completed its consultation obligation under the ESA.

Likely to Adversely Affect Determination
If the determination is that the proposed action is "likely to adversely affect," (LAA) or the Services "non-concur" with the action agency's "not likely to adversely affect determination" (NLAA) the action agency must enter "formal consultation" with the appropriate Service.

In response to a LAA determination where the Service provides a nonjeopardy opinion, it may issue reasonable and prudent measures (RPMs) when incidental take is anticipated that are necessary or appropriate to minimize the impacts incidental of any such incidental take.

In response to a LAA determination where the Service provides a jeopardy opinion, the Services will provide (1) reasonable and prudent alternatives (RPAs) to the action agency's proposed action in its Biological Opinion, (2) issue an incidental take statement together with reasonable and prudent measures that are necessary or appropriate to minimize take, or (3) determine that there are no RPA(s) nor can it issue an incidental take statement

Overview of Project Planning and Implementation

This section provides an overview of the qualifications for the project manager(s), required training for project personnel, planning, steps and documentation required to conduct a safe, effective, and lawful antimycin A application. Detailed information and instructions for training and documentation in compliance with federal law and this manual are presented in the referenced Administrative Operating Procedures (AOP) and Technical Operating Procedures (TOP).

Training
Detailed information on training is presented in AOP 5 and 6. Because antimycin A is a restricted use pesticide, it may only be applied by or under the direct supervision of applicators that have passed the required state pesticide applicator certification course. Additionally, a project manager is required to participate in all aspects of a minimum of two antimycin projects prior to supervising an antimycin A application. Formal training from the Rotenone and Antimycin Use in Fishery Management course taught at the National Conservation Training Center (http://training.fws.gov/, can be substituted for participation in one on-site application. Adherence to these actions will insure that professional and public credibility are maintained during native fish restoration projects using antimycin.

Project Manager Qualifications
The project manager must have demonstrated experience in the following areas:

a. Certified Pesticide Applicator: the project manager must be certified by the state in which the restricted use pesticide will be applied
b. Water chemistry: pH, conductivity, dissolved oxygen, and temperature
c. Determination of on-site toxicity
d. Determination of water travel time using a tracer dye
e. Discharge measurements
f. Project Implementation: The project manager must have experience in all aspects of an antimycin project. This includes the use and operation of all equipment used in applications (drip stations, handling antimycin, and washing cans, using all safety equipment and keeping project records.) and the application of potassium permanganate for neutralization of antimycin A. Experience can come from participation in a minimum of two antimycin A projects under the supervision of an experienced project manager.
g. The Project Manager is responsible for keeping all records pertaining to the project as specified in Administrative Operating Procedure (AOP) 8. The Project Manager should also oversee the preparation of the final report.

Project Personnel Qualifications
The project manager(s) must document that all project personnel have attended the Safety Meeting and have received the following training:

a. Safety: each team member must be trained on how to safely handle antimycin A. This training will stress compliance with label directions
b. Personal Protective Equipment: each team member must be trained on how to properly use the personal protective equipment required for handling and/or applying antimycin A.

Reporting
For each antimycin A project, the project manager will prepare a preliminary project plan to document the purpose and rational for the project. Additionally once completed the project manager will complete a final report that will include documentation of project planning, treatment records, protocol deviations and project evaluation. These documents are to be filed in the project manager's office and a copy of the final report should be filed in the agency's central office. The actual amount of antimycin A and potassium permanganate applied should also be recorded on a pesticide use log and submitted annually per each agency's recording requirements. NPS and USFWS require annual reporting of pesticide use through the agency's Pesticide Use Proposal System

Preliminary Project Plan
Detailed information on preparing the Preliminary Project Plan is presented in AOP 1. As part of the Preliminary Planning stage, the project manager will prepare a "Preliminary Project Plan." The primary objective of this document is to communicate the purpose, rationale, and a general overview of the intended application. The document will consist of the following draft sections:
Background: This section will include the objectives, rationale, fish species management strategy, legal authority, and project team.

Treatment Overview: This section will include the application dates, treatment area, project area, and equipment needs.
Regulatory Compliance: This section will include relevant information on NEPA and ESA.

Final Project Plan

Detailed information on preparing the Final Project Plan is presented in AOP 1. After the Preliminary Project Plan has been reviewed by management, other relevant federal and state agencies, and the public, the project manager will revise and finalize the document as the "Final Project Plan." The main objectives of the Final Project Plan are to provide the project manager with detailed instructions for conducting the application and to serve as a record of the project which can be used to gauge the success of the treatment and improve best practices for future projects. The document will consist of the following finalized sections:

Background: This section will include the objectives, rationale, fish species management strategy, legal authority, and project team.

Treatment Overview: This section will include the application dates, treatment area, project area, treatment area characterization, pre-treatment bio-monitoring, and equipment needs.

Regulatory Compliance: This section will include relevant information on NEPA, and ESA.

Appendices: This section will include the NEPA compliance records, final environmental assessment decision, and official ESA correspondence.

Treatment Records

Detailed information on documenting treatment records is presented in AOP 8. Treatment records include relevant water body and application data collected during the treatment. The project manager will maintain original data sheets for the treatment and copies of treatment records (including all data collected prior to, during and after the treatment). For NPS units it is recommended that treatment records will be retained for a minimum of five years and that guidance provided in Directors Order 12 for administrative records be followed. Guidance for other agencies may be different and projects on their lands should follow their policies.

Protocol Deviations

Detailed information on documenting protocol deviations is presented in AOP 11; an example Protocol Deviation Form is presented in Appendix B.10. Throughout the project, the project manager will document any deviation from the standard operating procedures identified in this manual.

Final Report

Detailed information on preparing the Final Report is presented in AOP 10. At the completion of the antimycin A project, the project manager will prepare a "Final Report." The primary objective of the Final Report is to document the intended and actual outcomes of the antimycin A application.

Overview of Antimycin A Application

This section provides an overview of the activities to be completed before, during, and after an antimycin A treatment to ensure a safe, effective, and lawful application. Detailed information and instructions for applying antimycin A in compliance with federal law and this manual are presented in the referenced AOP's and TOP's.

Before Application

Prior to applying antimycin A, the project manager and appropriate team members will conduct and/or prepare for the following activities: planning and public involvement, defining the treatment area and project areas, characterization of the physical treatment area, pre-treatment bio-monitoring, public notification and closures, safety training and use of personal protective equipment, and regulatory compliance.

Planning and Public Involvement

Planning identifies the steps, timing of planning, and final decision making process regarding an antimycin A application. Public (stakeholder) involvement informs parties interested in the proposed treatment of any potential direct impacts [on the public] such as area closures or drinking water restrictions (AOP 1 or 2). Through public involvement, potential issues/concerns are identified and resolved and are later discussed in the Final Project Plan. The general steps for planning and public involvement can be divided into *Preliminary Planning*, *Intermediate Planning*, and *Final Planning* (Figure 3). Ideally, stakeholder involvement occurs concurrently during each stage of the project, with most of the stakeholder involvement occurring during the *Intermediate Planning* stage.

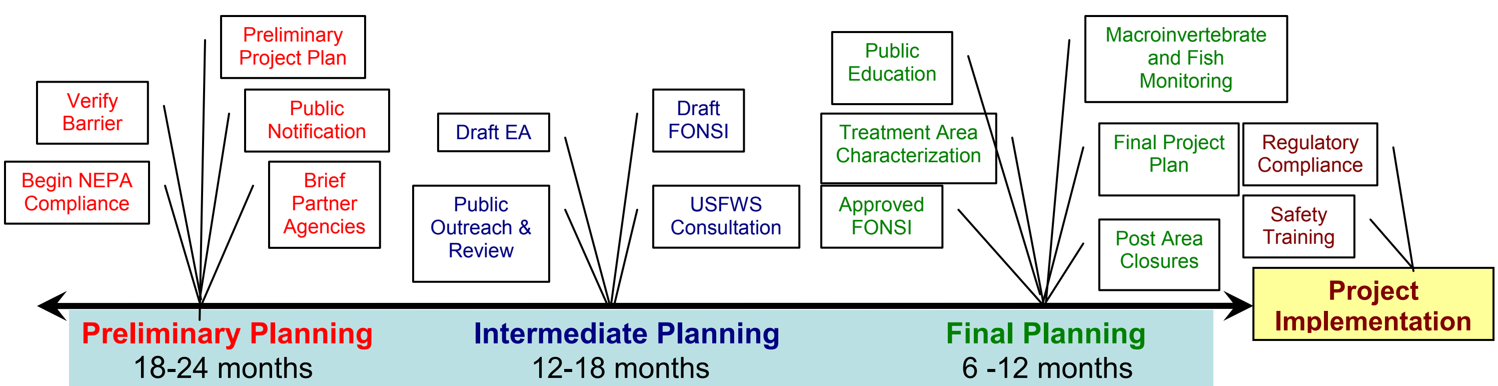


Figure 3. Example of a typical antimycin A project implementation planning and application chronology beginning with project conception through implementation.

Preliminary Planning Stage: *Preliminary Planning* typically begins between one and two years before treatment and initiates the formal documentation process for the project. As part of the *Preliminary Planning* stage, the project manager will prepare a draft Preliminary Project Plan. The draft Preliminary Project Plan will be distributed within the lead agency for appropriate internal review and approval. After comments are incorporated and the document is approved, the revised Preliminary Project Plan is typically distributed to other appropriate federal and state agencies and non-governmental organizations for review. After additional comments are incorporated, the revised draft Preliminary Project Plan is typically distributed to other appropriate federal and state agencies and non-governmental organizations for review. After additional comments are incorporated and the revised document is approved, it is considered the final Preliminary Project Plan.

Although the exact timing is left to the discretion of the project manager, NEPA compliance, and if applicable endangered species consultation with FWS and/or the NMFS, is typically initiated during the Preliminary Planning Stage.

Intermediate Planning Stage: Once the Preliminary Project Plan is completed and approved, the document will be distributed for public review thus beginning the *Intermediate Planning* stage. The *Intermediate Planning* stage allows for appropriate revision of the Preliminary Project Plan to address issues regarding new information on the affected environment, regulatory compliance, and response to agency and public concerns. During this stage, project managers are encouraged to conduct outreach (*e.g.* stakeholder meetings) as appropriate to inform and educate stakeholders about the upcoming application.

Upon completion of the *Preliminary* and *Intermediate Planning* steps, the Preliminary Project Plan will be revised and finalized as the "Final Project Plan." The Final Project Plan will be used to conduct the application and, eventually, will form a large portion of the Final Report. In addition, the Final Project Plan will include documentation demonstrating compliance with NEPA and ESA.

Final Planning Stage: Once the Final Project Plan is approved and all necessary regulatory requirements/permitting are complete, *Final Planning* begins. During the *Final Planning* phase, the project manager will make final preparations to conduct the application such as procuring necessary supplies and equipment and briefing team members on the details of the project.

Defining the Treatment Area and Project Area

As part of the Preliminary Project Plan, the project manager will clearly define the treatment and project areas. Clear definition of these areas facilitates regulatory compliance (*e.g.* NEPA, ESA, etc) and prompts useful stakeholder input. The "treatment area" comprises the water body or bodies, or portions thereof, into which antimycin A is applied. In streams, a typical "treatment area" would begin with the upper treatment stations and end just downstream or beyond the deactivation site. In ponds or lakes, the "treatment area" would consist of the lake or pond, all tributary inlets, and possibly the lake outlet. Clear definition of the treatment area is required to comply with NEPA and ESA, and also provides stakeholders with an understanding of the exact location of areas potentially affected by the treatment. Proper identification will include a written description as well as supporting maps and other appropriate documentation. In streams, proper identification will include the downstream physical barrier (*e.g.* waterfall, cascade or dry channel) that will prevent upstream migration of non-native fish and downstream movement of the piscicide (TOP 1).

The "project area" includes the location(s) where antimycin A will be mixed and measured for use, the treatment area, the areas closed to public access during the project, the location(s) of the deactivation site(s), and the location of the barrier that will prevent invasive species from re-entering the treatment area. Proper identification will include a written description as well as supporting maps and other documentation.

Treatment Area Characterization

Characterizing the treatment area provides the project manager with detailed water body and water quality data needed to complete the Final Project Plan. Characterization includes determining target species distribution, delineating the treatment area, water quality parameters (*e.g*., pH, hardness, temperature, dissolved oxygen), identifying discharge sites (*e.g.* where treated water will or may outflow to or join other water bodies) and, for flowing water bodies, measuring mean stream gradients, approximate flow times and discharge for each treatment section.

Determining Target Species Distribution: The distribution of target fish within the proposed project area will be mapped and the treatment area defined prior to the completion of the Final Project Plan (TOP 1). For stream applications, defining the fish distribution includes the elevation of the upper distribution limit for every tributary (TOP 1). If native fish exist in headwater areas, the distribution work must identify sympatric zones and the treatment area defined based on this information.

Fish distribution information may be collected in a variety of ways, but electrofishing gear is commonly used as this technique efficiently captures fish in small high gradient streams (TOP 1). It is important that only staff experienced in the use of backpack electrofishing gear and fish identification verify all distribution information such that no areas are inadvertently over looked or specimens misidentified. All distribution information will be recorded and important locations noted on either topographical maps or entered into a Geographic Information System (GIS) database so that the project area and treatment area can be clearly defined later in the Preliminary and Final Project Plan.

Delineating the Treatment Area: Proper delineation and marking of key locations throughout the treatment area facilitates site navigation and also provides reference points useful in record keeping and for determining application sites. In some instances, it may be desirable to collect gradient and habitat data for 100m stream sections (TOP 1). If the determination is made to collect these data, it is recommended that sequentially numbered tags (aluminum and/or plastic flagging tape) be placed every 100m (or other appropriate interval) where practical. For flowing water bodies, begin at the lower barrier (typically just upstream of the deactivation site) and continue upstream to the uppermost end of the treatment area. For some applications, determination of elevation of each measured interval is helpful. For example, site markers for the barrier site at 457m elevation and the 100m site at 472m elevation would read “0m, 457m” and “100m, 472m”, respectively. Once completed, the vertical drop between each 100m site, total length of treatment area, and the total vertical elevation drop from the top to the bottom of the treatment area can be determined. A study area map depicting these data is useful for project planning and crew instruction during daily treatment (TOP 1).

For lakes or small impoundments begin site measurements at a marked point and continue around the perimeter of the treatment area to determine the surface area. Currently, a hand held

GPS unit is probably the best way to delineate the size and area of the lake. The location of all inlet streams, springs and wetlands that need to be treated should also be marked on the project map. Additionally, a depth profile needs to be completed for the water body. For preliminary planning purposes, this can be extrapolated from a USGS 1:24000 TOP maps. But this information needs to be verified (ground-truthed) on site prior to the completion of the final project plan.

Discharge Sites: Discharge measurements can be in ft^3/sec or m^3/sec. Based on field surveys of the project area, the project manager or an experienced team member will identify and appropriately mark suitable stream discharge sites or these sites may be arbitrarily selected daily for the collection of these data. Discharge measurements will be taken daily during the treatment to ensure proper daily treatment concentrations (TOP 5).

Areas recommended for the collection of discharge data are:

- just upstream of the confluence of each tributary stream within the treatment area with the main stream;
- just after the confluence of each tributary within the treatment area and the main stream;
- immediately upstream of the detoxification site; and
- immediately downstream of the detoxification site.

Discharge measurements can generally be conducted several months prior to actual treatment if flows are similar to those expected during Antimycin A application and during normal flow conditions (*i.e.* not during or directly after large rain events) for estimating the quantity of antimycin A needed and for project planning purposes only. The day prior to treatment, stream discharge for the main stream and any tributary stream to be treated will be measured at pre-determined measurement locations to determine the amount of antimycin A needed to achieve an effective concentration in the stream. Stream discharge sites and measurements will be noted on the study stream map (TOP 1). All discharge sites will be clearly marked with flagging and denoted on the site map so that discharge measurements can be repeated and compared throughout the treatment period.

Water Travel Times: For flowing water bodies, the determination of approximate water travel times from the upper end of the treatment area to all tributaries and to the downstream end of the project area using fluorescene or rhodamine dye is recommended (TOP 6). Water travel times should also be determined for all tributaries that will be treated. If the project area is sub-divided into sections or reaches, this information should be collected for each reach or section. These data provide project managers with information that aids daily planning and helps determine travel time of antimycin between dispensing stations and for the total project area. This process also aids in the identification of potential problem areas (e.g. backwater eddies, springs, seeps, or other hydrological anomalies where the dye does not mix well). Data may be added to the study area map for easy reference (TOP 1).

Dye studies can generally be completed prior to actual treatment during normal flow conditions and can be completed by one or more teams working simultaneously at different sites. However, travel time measurements also should be made just prior to application at several locations within treatment area to ensure previously collected data still accurately reflect water travel times.

Stream Gradients: For flowing water bodies, mean stream gradient should be calculated for each 100m section within the treatment area. Mean stream gradients help the project manager identify potential trouble spots or areas. Work in Great Smoky Mountains National Park has demonstrated that if the stream gradient exceeds 8%, the length of stream that can be effectively treated by one dispensing unit decreases (TOP 1). This information helps the project manager determine the proper location of dispensing units so an effective concentration of antimycin is maintained throughout the stream segment being treated.

Pre- and Post-Treatment Bio-Monitoring

Pre-treatment fish population biomonitoring is recommended to establish carrying capacity prior to treatment. Recommended sample locations are upstream of the treatment area, within the treatment area and downstream of the treatment area in the deactivation area. Post-treatment biomonitoring is recommended in these same locations to determine if the treatment was successful, to evaluate effects of deactivation on fish populations and to determine when reintroduced native fish species have recovered (TOP 4).

While not required, pre- and post-application bio-monitoring is recommended for aquatic macroinvertebrates upstream of, within and downstream of the treatment area and deactivation area (TOP 4). Aquatic macroinvertebrate monitoring will provide pre-treatment data that can be compared to post-treatment data to assess short and long-term impacts as well as recovery from the antimycin and neutralization with potassium permanganate. However, if data from several projects in different geographical areas provides conclusive evidence that these communities recover from the treatment, then this monitoring should be considered on a project by project basis. If a project is to be conducted in an area for which no information exist, aquatic macroinvertebrate monitoring is highly recommended.

Although there are no specific guidelines for determining the number of locations that aquatic macroinvertebrates should be sampled, at least two locations within the treatment area and one downstream of the neutralization site should be sampled both prior to treatment (optimally within a month) and after treatment (optimally within a month). At each monitoring site, a minimum of three follow-up collections should be made (winter, spring and summer/fall) to evaluate recovery. The area of the country in which the project occurs will dictate the sampling method used (*e.g.* Surber or Hess samplers are commonly used quantitative collection methods). The Rapid Bioassessment method is not as quantitative as the other two methods *(e.g.* number of individuals per unit area) but is a recognized as a semi- quantitative sampling method and is used throughout the U.S.

Pre-treatment fish sampling provides managers with a realistic, measurable population density by which population recovery of native species can be measured. In some projects in the Southeast, four100-m sites for fish sampling (i.e. two in the treatment area and one immediately downstream of the deactivation site, and one control) have been used to collect these data. Three-pass depletion or mark-recapture techniques that are normally used to quantify fish abundance in wadeable streams. Other accepted methods can also be used. Pre-treatment abundance data are the benchmark used to evaluate recovery of the desired native species during post treatment evaluations. Species abundance data from the neutralization site and control/reference site can be compared pre- and post-treatment to evaluate the impacts of potassium permanganate on fishes downstream of the treatment area. Post-treatment collections are used to assess establishment of the desired species and to evaluate the rate of recovery.

Public Notification and Closures

Public access to the treatment area is prohibited while antimycin A is being applied (*i.e.* the dates specified in the Final Project Plan) and during any additional times determined to be necessary by the project manager (AOP 4). The project manager will take the necessary steps to inform the public of the planned closures and to prevent access to the treatment area.

The project manager may inform the public through general press release, public meetings, newspaper, radio, and/or television. At least 3 - 7 days prior to the project start date, notice of closure and dates of closure will be posted on appropriate road crossings or access ways to the treatment area. The information presented to the public and on posted notices will include, at a minimum, the following:

- a description and map of the area closed to public entry
- the time period during which the closure will occur
- a summary of the objectives and rationale of the closure
- a contact name, telephone number, and email address for additional information.

All steps taken to notify the public and prevent access to the treatment area must be documented in the Final Report.

Safety Training and Use of Personal Protective Equipment

Safety training, including the use of personal protective equipment (PPE), is *required* prior to any application of antimycin A (AOP 5). Additional pertinent safety information on required PPE is also presented on the Fintrol® product label. Prior to applying antimycin A, the project manger or their designee will lead a detailed training/briefing on how to safely handle antimycin A and how to properly use the necessary PPE that is required to be worn when handling and applying antimycin A. In addition, the project manager or their designee will brief team members on the legal requirements of the label under FIFRA and any applicable nuances of that particular application related to FIFRA, CWA, NEPA, ESA, and any other applicable regulations.

Participation in this meeting is required for all individuals who will be present in the project area during the application of antimycin A. The project manager will document the presence of all individuals using a sign-in sheet (Appendix B.6) that will be included in the Final Report.

Regulatory Compliance

Prior to and during treatment, the project manager will ensure appropriate compliance with FIFRA, CWA, NEPA, ESA, state, agency specific and other applicable regulations. *All* antimycin A projects on Federal lands must include the proper NEPA compliance documentation prior to treatment (AOP 3).

During the Application Dates

During the application dates, the project team will apply and deactivate antimycin A under the supervision of the project manager (See *Overview of Project Planning and Implementation*). This will include the appropriate storage, transportation, decontamination (*e.g.* proper cleansing of equipment), and spill containment (AOP 7); pre-treatment site preparation and setup (TOP 1-8); pre-treatment bioassay (TOP 9); applying antimycin A (TOP 13, 14); minimize or eliminate personal exposure (AOP 5); and deactivating with potassium permanganate (TOP 16).

In addition, at the beginning and conclusion of each work day, the project manager will assemble all team members. During these meetings, the project manager will provide the team with an update on project status, daily roles (duty roster)/responsibilities emphasize safety and other appropriate matters. As appropriate, team members will inform the project manager and other team members of any pertinent information from his/her duty station.

Storage, Transportation, and Spill Containment of Antimycin A and Equipment

Proper handling of antimycin A is essential to conducting a safe, effective, and lawful application. All project staff should be briefed and clearly understand the details on storage, transportation, decontamination, and spill containment *prior to* treatment (AOP 7).

Storage: A unit of antimycin A is packaged in two glass bottles (antimycin A and dilutent), padded with thick paper sheeting, and housed in a 3.75L metal pail. Prior to use, antimycin A will be stored in the pail provided and according the instructions on the EPA-approved product label.

Transportation: Antimycin A will be transported from the storage area to the project area in appropriate containers that minimize or eliminate breakage. To transport from the project area to the individual application sites, antimycin A and dilutent bottles, with original padding in place, will be removed from the pails, mixed and the appropriate amount for each treatment station to achieve the desired concentration will be measured and placed in drip-proof, glass or plastic (*e.g.* Nalgene®) specimen containers. The date, station number and amount of antimycin in each bottle needs to be recorded on each bottle (duct tape and permanent markers has been used effectively for this purpose). These plastic containers will be placed in sealed plastic bags and placed in padded backpacks or padded, plastic panniers and transported to the individual antimycin station for that day's treatment.

Spill Containment: In the event of an antimycin A spill on land or in water, the spill will be contained as described below. The project manager will document all spills as protocol deviations (AOP 11; TOP 18).

To contain spills on land, stop the spill at its source; dike solution in pools; absorb with clay, soil, or other noncombustible, absorbent material; and deactivate materials resulting from an antimycin spill and containment with an oxidizing agent such as potassium permanganate.

To contain spills in water, first determine whether or not the location of the spill is designated for immediate treatment. An accidental spill of antimycin A into waters not designated for immediate treatment requires the initiation of neutralization procedures using potassium permanganate as described in TOP 16. The project manager will initiate actions to deactivate the chemical to minimize further contamination.

An accidental spill of antimycin A into waters designated for immediate treatment requires the project manager to note the time of the spill and the amount that was spilled on the Antimycin Application SOP Deviation Form (Appendix B.10). Additionally, the project manager and project personnel should keep detailed notes on the effects on fish in live cages (bioassay) and conduct on site monitoring to determine if material from the spill travels further downstream than antimycin applied from a dispensing unit.

Pre-Treatment Bioassay

The Fintrol® label identifies the lethal treatment concentrations required to kill many fish species (Appendix E, Table C.1); however, lethal concentrations for other species and/or varying environmental conditions (*e.g.* pH, alkalinity, temperature, stream gradient) may require adjustments in the concentration to be used. Because of these variations, it is highly recommended that the project manager conduct a bioassay to determine the proper target concentration of antimycin A (TOP 9). These on-site bioassays are conducted to ensure that antimycin A is applied in amounts lethal to target organisms while minimizing adverse effects on non-target organisms and the environment.

The project manager should record this information in the final report. Based upon the results of this bioassay and experience from previous projects, the concentration identified should be applicable to similar streams throughout the region with similar water chemistry.

Bioassays are used to ascertain the concentrations of antimycin and potassium permanganate necessary for a successful treatment and deactivation, including: 1) the treatment concentration in µg/L (parts per billion; ppb) of antimycin and treatment duration (hours) needed to kill 100% of the target species; 2) the concentration of potassium permanganate in mg/L (parts per million; ppm), applied to water to deactivate antimycin and reduce antimycin A non-target mortality to 0%, and 3) the mortality rate to target and non-target species associated with various concentrations of potassium permanganate.

The concept of "*exposure*" is an important principal for project managers to understand and utilize. "Exposure" is defined as the concentration of antimycin A (ppb) multiplied by the amount of time (hours) the organism was exposed to that particular concentration. For example, a typical treatment for rainbow trout may be 8ppb for 8 hours, or 64 exposures. In some cases, the LD_{100} concentration observed during field bioassays for a particular species (*e.g.* 50ppb) over 8 hours (*i.e.* 400 exposures) may be higher than the antimycin A label permits (25ppb) (Appendix E). In order to treat the target species without violating the product label, the project manager could plan a treatment of 25ppb for 8 hours (200 exposures each) for day one and repeat the treatment on day two to achieve project objectives. Using this technique, the project manager can meet restoration goals while limiting impacts on non-target specimens due to higher concentrations.

Application concentration and exposure time varies by species, region, and habitats. In order to determine the proper application concentration to achieve a 100% kill for a given species, the project manager may: 1) use the recommended treatment concentrations from the antimycin A label (Appendix E, 2) conduct laboratory bioassays, *or* 3) on-site bioassays to determine appropriate concentration for specific project objectives (TOP 9). Because of the many chemical and physical differences between different regions of the nation, a laboratory or on-site bioassay is recommended for initial projects to determine appropriate concentrations for the complete removal of non-native fish. If the decision is to conduct a laboratory bioassay, it is recommended that water from the body of water to be treated be brought to the laboratory for the bioassay and that lethality tests are conducted at temperatures that are similar to what may occur under field conditions.

Once the bioassay is completed, it is imperative that the appropriate distance between antimycin stations (dispensing stations) be determined for stream projects (TOP 12). This is best accomplished on day one of the initial treatment, in a region or area.

The person operating the station(s) is also responsible for observing the fish in each live cage (bioassay) located just upstream of each application station. Observations should occur at one to two hour intervals after treatment begins and information on fish appearance and condition must be recorded on the *antimycin dosage and stream temperature* data sheet (Appendix B.7). If fish die during the day, dead fish are to be removed from live cages and the number and time at which the fish were removed recorded. Prior to leaving the station for the day, record the appearance of surviving fish should be recorded on the appropriate data sheet.

In a lake or pond project, live cars with the target species should be randomly distributed throughout the water body as described in TOP 14.

Pre-Treatment Site Preparation and Setup

It is recommended that pre-treatment site preparation and setup proceed in the following order:

1. Set one to two antimycin stations in the upstream reach of the project area.
2. Place holding cages with fish at 100 m intervals for 1 km downstream of the stations.
3. Determining the appropriate spacing for antimycin A dispensing stations based on the distance 100% kill was achieved in 24 hours.
4. Set additional antimycin A stations at the appropriate distance in remainder of treatment reach.
5. Set up and test the potassium permanganate deactivation equipment (neutralization site) at a minimum of two to three days prior to antimycin arrival at the station.

Detoxification Methods:
Potassium Permanganate Station: Based on water travel times, the potassium permanganate station should be setup and calibrated two to three days prior to the anticipated arrival of the antimycin. Additionally, this will ensure that, in the event of an antimycin A spill in the downstream portion of the treatment area that the antimycin can be deactivated prior to movement of the chemical beyond the treatment area (TOP 16).

The detoxification site should be located within 100m, or as close as possible, to the barrier identified in the Final Project Plan. The detoxification site should have a level area of ground large enough to hold the deactivation dispensers. Generally, the dispensing system depends on gravity to deliver the liquified potassium permanganate from the barrels to the dispensing units, therefore, the barrels must have sufficient vertical drop to ensure continuous and constant flow. Experience from previous projects has shown that a drop of approximately eight feet is sufficient to meet this need. Additionally, the site must be accessible for staff and equipment; and have appropriate morphology to safely allow proper installation and hourly calibration of the equipment. Other methods that have been used successfully can also be used.

Application Methods: Antimycin A is applied to water by a drip-feed device (as part of a drip station), backpack sprayer, boat bailer, or sprayer. Drip stations (Figure 4) are typically used in streams and rivers inaccessible to boats. Backpack sprayers may be used to supplement drip stations or other application devices in areas with poor water circulation (*e.g.* stagnant pools that the chemical may not reach through natural stream flow); the Fintrol® label recommends that backpack sprayers be used in areas where water depth is 0.3 meters (1 foot) or less. Boat bailers are used in larger water bodies such as ponds, lakes or small reservoirs. Application to these deeper water bodies may require the use of a pump mechanism to ensure adequate mixing throughout the water column where antimycin A is dispensed through a perforated hose stretching the depth of the water column or is delivered through the propeller wash (Wormell 2005).

Antimycin A Station: Antimycin dispensing stations should be set at pre-determined locations following the results of earlier field trials (TOP 12). Locate a fairly level area next to the water body for the location of the dispensing unit. Dispensing containers should be leveled on the bank at least 15 to 45 mm (6-18 inches) above the water surface (TOP 13). Depending on type of dispensing apparatus, it may be placed directly over the water or on shore. Once the dispensing container is in place, it should be tested to ensure that it is functioning properly. For best results, lift the bucket (filled with a couple gallons of water) for ten seconds to remove air from the hose (Figure 4). Moore et al. (2005) provide additional information on this type of unit. Steffereud and Propst (1996) provide information on an alternative dispensing unit they have used.

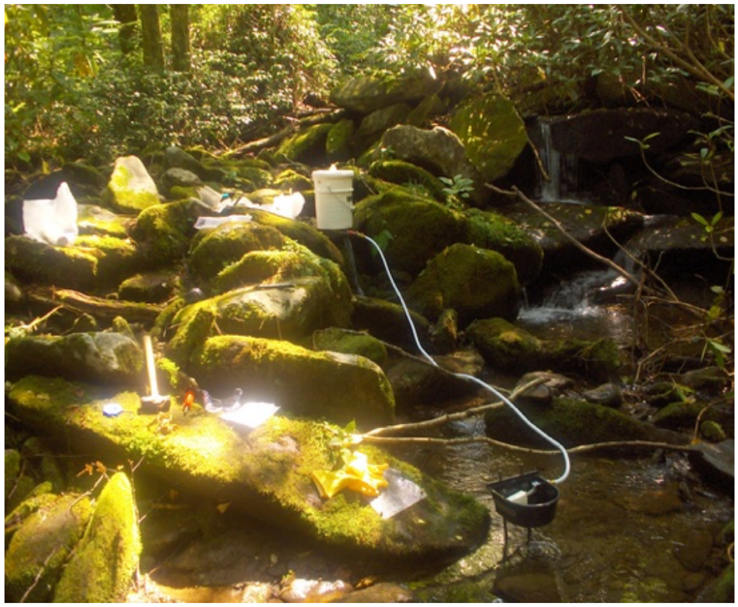


Figure 4. Antimycin A drip station in operation during a small stream treatment. The apparatus includes a dispensing bucket (19L or 5-gal) containing formulated product (water, antimycin A, and the surfactant nonoxyl-9). The dispensing bucket is calibrated to deliver 3.8L or 1-gal per hour.

Applying Antimycin A

Antimycin A must be handled in accordance with EPA-approved product labeling and OSHA standards (AOP 3). Antimycin A may be applied using drip stations (of various designs), boat bailer, and, when necessary, a backpack or hand held sprayer.

Antimycin Station Operation: The project manager will authorize preparation or "loading" of that day's antimycin A treatment stations. For flowing water bodies, begin with the upper treatment stations and work downward. Where possible, the upper treatment stations should be set at least 50m linear distance above the upper distribution of target species to allow sufficient mixing of the antimycin A with water prior to reaching the beginning of the target area. A similar proven approach from another region may also be used. The method should be documented in the agencies procedures antimycin treatments and in the final report.

Once all of the day's treatment stations are loaded (TOP 13), the project manager will authorize treatment initiation. For NPS projects, treatment begins at the upper station(s) and proceeds downstream and/or to lower elevations. When treatment is initiated at the upstream most station, a fluorescene or rhodoamine dye is added to the stream as an aid to tracking the progress of the antimycin flume. The arrival of the dye plume at downstream stations lets these operators know when their antimycin station should be "turned on. A similar proven approach from another region may also be used and should be documented in the final report.

Backpack Sprayer Operation: A backpack sprayer is used to treat backwater areas not readily exposed to antimycin A through natural water circulation (TOP 15). These areas, including pools, springs, and seeps, were initially identified when determining water travel times through the use of a dye. Supplementing drip stations with a backpack sprayer prevents ensures complete elimination of unwanted species. Sprayers capable of holding11 to 15L (3 to 4 gallons) are preferred over larger models (>15 liters) because the weight is difficult to carry safely throughout the day over difficult terrain.

Typically, the sprayer applies two applications. Once the fluorescence dye is applied and treatment begins, the sprayer mixes half the antimycin assigned to them along with enough dye (fluorescene or rhodoamine) to keep track of where they have sprayed. The sprayer slowly follows the dye downstream spraying areas where the dye is not mixing. The sprayer also sprays a short distance up (50 – 200 m) the downstream segment of any small tributaries normally fishless that are within the treatment zone that might provide some refuge for fish. It is important to note that the concentration of antimycin in the sprayer is usually relatively high (>50ppb), therefore areas only need be sprayed for several seconds to be effective. Once all appropriate areas have been sprayed using the backpack sprayer, a second spraying is normally conducted approximately 4 hours later. As a general rule the operator works from the bottom of the treatment area to the top for the second application. Once complete, wash and clean all equipment as discussed in TOP 13 and 15.

Exposure Mitigation

A successful antimycin A application results in no exposure to handlers (*i.e.* antimycin A mixers, loaders, and applicators) or the public. To ensure adequate protection of handlers, all persons handling antimycin A wear personal protective equipment (PPE) as required by the EPA-approved product label including, at a minimum, long-sleeved shirt, long pants, chemical resistant gloves, and goggles (AOP 5).

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

- The antimycin A and potassium permanganate product labels will be followed
- The treatment area will be clearly marked and identified with posted signs
- Public access to the project area will be prohibited during treatment and for at least two days after the last treatment day for streams and two weeks for lakes or small impoundments
- The public will not be permitted to enter (e.g. swim, bathe) or fish in treated waters during treatment
- Dead fish will not be consumed
- Treated water will not be ingested
- Out-flow from the treatment area will be deactivated with potassium permanganate any time treated water will leave the defined project area or if the terminus of the project area is within one kilometer of public access that is not in the defined project area.

Deactivating with Potassium Permanganate

Potassium permanganate is a very strong oxidizing agent capable of inflicting serious burns and must be handled in accordance with product labeling and OSHA/other standards. Care must be taken to minimize exposure to the skin, eyes, and lungs. If skin/eye contact occurs, rinse the affected area with cold fresh water for at least 5 minutes and seek immediate medical attention.

The method described below is one that has proven effective during projects in National park units. A similar proven method from another region may also be used provided the techniques are documented in agency approved plans and in the final report.

The current label indicates that areas downstream of the treatment area may or may not be neutralized with an oxidizing agent such as potassium permanganate to intentionally inactivate antimycin A. The following discussion is intended to provide guidance for the types of situations in which a project manager may decide not to deactivate with potassium permanganate. In remote areas where the risk of human contact is negligible and there is at least a one to one dilution from another tributary just downstream of the treatment area the project manager may decide not to deactivate. In stream reaches downstream of a water fall or steep canyon/gorge that exceed the vertical elevation drop known to naturally deactivate antimycin A, the project manager may decide not to deactivate with potassium permanganate provided access to the area is closed and there is no chance of human exposure . However, to ensure public safety, neutralization is mandatory any time treated water will leave the defined project area. If the terminus of the project is within one kilometer of public access that is not in the defined project area, neutralization is mandatory.

Use the potassium permanganate application chart (TOP 16) to determine how much $KMnO_4$ must be applied per hour, based on stream discharge (ft^3/sec) at the neutralization station. The stream discharge at the neutralization station must be measured daily to determine the correct potassium permanganate application rate for each day of the treatment (TOP 5). Normally, a potassium permanganate concentration of 1 mg/L is necessary to accommodate background potassium demand, 1 mg/L (ppm) to neutralize antimycin A, and 1 mg/L residual to ensure complete neutralization, for a total concentration of 3 mg/L. However, some stream systems may have high organic loads that demand more $KMnO_4$. If a project area is thought to have a high organic demand, pre-treatment bioassays will be necessary to determine the appropriate amount of $KMnO_4$ required to neutralize the antimycin.

Approximately 1- 4 hours prior to the anticipated start time of the deactivation station, measure out the appropriate amount of potassium permanganate and add it to one drum/barrel. Stir the solution thoroughly (avoid splashing) using a boat paddle or other appropriate method and wait for the arrival of the dye to begin the application of potassium permanganate.

Prior to deactivation, calibrate all drip boxes to ensure proper application rates when applied (TOP 16). It is important to re-calibrate the boxes every hour to ensure they are working properly and have not gotten clogged. Although potassium permanganate is relatively soluble in water (6.4 g/mL at 20°C), experience in GRSM projects has shown that adding more than 100g $KMnO_4$ per 3.8 liters of water results in excessive clogging of the drip box drain ports at normal operating temperatures of 7 - 10°C. Therefore, for 114L (30 gallon) drums, you may use 57L (15 gallons) per hour up to 100g and then switch to 114L (30 gal) per hour delivery when application exceeds 100g per hour. If the drip boxes or tanks begin to precipitate potassium permanganate, flush the tanks and lines with clean water prior to refilling the tanks for the next round.

When the dye is observed approximately 100 – 200 m upstream of the deactivation station attendants must carefully stir the potassium permanganate solution again to ensure all potassium permanganate is in solution. Once the dye reaches the detoxification station, begin detoxification by opening the valve of one barrel. Record the date and time deactivation begins on the Detoxification Station Datasheet (Appendix B.8) including any other pertinent information or observations. Use a graduated cylinder to calibrate the discharge of potassium permanganate solution from the dispensing boxes to ensure the proper application rate. If necessary, adjust the valve on the drip box to get the appropriate discharge rate.

Once the station is operating, stir the solution occasionally to prevent the potassium permanganate from settling and check the discharge rate every hour to ensure proper the correct amount of potassium permanganate is being applied. Previously inscribed graduations on the side of the stock containers (barrels) are also a reliable way to ensure the station is running at the desired application rate. Have the secondary barrel filled, mixed, and ready to use to ensure there is no gap in the potassium permanganate application process. During stirring, refilling, and when the barrel is empty, the operator should visually inspect the container for any potassium permanganate precipitate. Report any precipitate to the site supervisor so he/she may determine whether or not it is necessary to flush each off-line system thoroughly before refilling for the next run. These actions are recorded on the detoxification station datasheet and included in the Final Report.

Note that the purpose of the water in the barrels is to act as a delivery mechanism for the $KMnO_4$. Once the barrels are set to deliver a fixed volume of water per hour, you can add the respective amount of $KMnO_4$ for the stream discharge measured. A two barrel (container) system is an efficient means of delivering the $KMnO_4$ because it provides a backup tank in case of part failure, and an uninterrupted flow can be maintained throughout the deactivation process.

The deactivation station attendant will run the station for a previously determined time (typically eight hours) and record the start/stop times of operation. Note any important safety, logistical, or functional problems on the datasheet and report to the project manager or site supervisor. Although not required, most systems perform better when they are flushed with water at the conclusion of each day of use.

At the conclusion of the final potassium permanganate application and prior to exiting the project area, the project team will gather and remove all items brought into the project area. The project manager will perform a final walk-through of the project area to ensure no chemicals or equipment is left behind. At a later date, in accordance with the timing designated in the Final Project Plan, the project manager will have any postings or notices removed.

After Application

Upon completion of the application, and in accordance with the Final Project Plan the project manager will make arrangements to retreat or restock the treatment area and will prepare the Final Report.

Re-Treatment
The goal of every project is to achieve complete removal of the target organisms with one treatment. However, experience has shown this goal may not be achieved for various reasons. When additional antimycin A applications are necessary, all aforementioned procedures should be followed, including the annual application guideline limits detailed in TOP 13.

Restocking
Although the timing of stocking desired fish species is at discretion of project manager, it should not occur until there is a sufficient forage base in stream to support the reintroduced fish.

Post-Treatment Bio-Monitoring
Pre- and post-treatment bio-monitoring of fish species is required to properly identify the treatment area and gauge the application's success (TOP 4). Pre- and post-treatment bio-monitoring of aquatic macroinvertebrates is optional, but *strongly recommended* as these data can be used to evaluate the extent to which non-target species are affected by antimycin A and for determining whether a sufficient forage base exists to support stocking.

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Final Report
At the completion of the antimycin A project, the project manager will prepare a Final Report. In the Final Report, the project manager will document the intended and actual outcomes of the antimycin A application (AOP 10).

Administrative Operating Procedures

AOP 1 Preliminary and Final Project Plan

Applicability
A Preliminary Project Plan and Final Project Plan will be developed for each antimycin A treatment.

Purpose
To provide standardized documents for the Preliminary Project Plan and the Final Project Plan prior to initiation of an antimycin A application.

Procedure
As part of the Preliminary and Intermediate Planning stages, respectively, the project manager will prepare a "Preliminary Project Plan" and "Final Project Plan." These documents communicate the purpose, rationale, and overview of the intended application to promote internal and external feedback. The outlines for completing the required Preliminary and Final Project Plan are presented herein.

Preliminary Project Plan
The Preliminary Project Plan provides an overview of the intended application to promote feedback prior to finalizing the plan. The Preliminary Project Plan will consist of the following sections:

Background:
Objectives: Explain the overall purpose and objectives of the project.
Rationale: Explain the rationale for the project.
Fish Species Management Strategy: explain the overall fish species management strategy and how it relates to the objectives and rationale of the project.
Legal Authority: Explain the legal authority for conducting the application including citations of applicable statutes.
Project Team: Identify the project manager and key team members along with corresponding roles, responsibilities, and a summary relevant experience.

Treatment Overview:
Application Dates: List the projected start and end date of the project.
Treatment Area: Clearly identify the water bodies or portions thereof to which antimycin A will be applied beginning with the highest altitude treatment stations (*i.e.*., the "upper" treatment stations) and ending just beyond the deactivation site.
Project Area: Clearly identify the location(s) where antimycin A will be formulated (*i.e.*, mixed and measured for use), the areas potentially affected by the treatment (*i.e.*, where antimycin A and potassium permanganate will be measurably present in the environment), the location(s) of the deactivation site(s), the location of the barrier that will prevent invasive species from re-entering the treatment area, and potential public access ways.
Equipment Needs: Describe the equipment that will be used to conduct the application.

Final Project Plan: The Final Project Plan provides the project manager with detailed instructions for conducting the application and to serve as a record of the intended project that can be used to gauge the success of the treatment and potentially derive best practices for future projects.

Regulatory Compliance:
FIFRA: Describe the steps that will be taken during the application to comply with the requirements of FIFRA (e.g. Federal and all appropriate state labels are strictly adhered to).
CWA: Describe the steps that will be taken during the application to comply with the requirements of CWA. The project manager must ensure compliance with all federal and state requirements with CWA.
NEPA: Describe the steps that will be taken during the application to comply with the requirements of NEPA
ESA: Describe the steps that will be taken during the application to comply with the requirements of ESA

Appendices:
Final Environmental Assessment (if applicable)
Final Environmental Impact Statement (if applicable)
Official ESA Correspondences

Table 1. Necessary components of the Preliminary and Final Project Plans.

	Preliminary Project Plan	Final Project Plan
Background	X	X
Objectives	X	X
Rationale	X	X
Fish Species Management Strategy	X	X
Legal Authority	X	X
Project Team	X	X
Treatment Overview	X	X
Application Dates	X	X
Treatment Area	X	X
Project Area	X	X
Pre-Treatment Bio-Monitoring		X
Equipment Needs	X	X
Regulatory Compliance		
FIFRA	X	X
CWA	X	X
NEPA	X	X
ESA	X	X
Appendices		X
Final EA or EIS		X
Official ESA Correspondence		X
FONSI		X

AOP 2 Planning and Public Involvement

Applicability
This procedure defines the planning and public involvement steps necessary to develop and implement an antimycin A treatment.

Purpose
The purpose of this procedure is to ensure that an antimycin A treatment is fully planned and public concerns addressed prior to initiation. Actions identified in this procedure are consistent with the requirements of National Environmental Policy Act for federal, state and local agencies and tribes. Adequate planning ensures that environmental, regulatory, and public concerns and information needs are addressed and incorporated into antimycin A treatment design.

Procedure
Adequate planning and public involvement steps are defined below to address the application of antimycin A for control of target fish species in flowing and static water. **Planning** identifies the steps, timing of planning, and final decision process regarding antimycin A treatment. **Public Involvement** educates interested parties on the proposed treatment and any potential direct impacts such as area closures or drinking water restrictions. Through public involvement, potential issues/concerns are identified and resolved to create a more meaningful and complete Final Project Plan. Ideally, public involvement occurs concurrently during each stage of the project, with most of the public involvement occurring during the Intermediate Planning stage.

Planning
The general steps for planning and public involvement can be divided into Preliminary Planning, Intermediate Planning, and Final Planning.

Preliminary Planning: Preliminary Planning begins the formal documentation process for the project. As part of the Preliminary Planning stage, the project manager will prepare a draft Preliminary Project Plan. The draft Preliminary Project Plan will be distributed to management for appropriate internal agency review and approval. After comments are incorporated and management approves the document, the revised Preliminary Project Plan is typically distributed to other appropriate Federal and state agencies for review and comments. After agency comments are incorporated and management approves the revised document, the document is considered the final Preliminary Project Plan.

Although the exact timing is left to the discretion of the project manager, NEPA documentation, and, if necessary, material for endangered species consultation with FWS are typically initiated and prepared during the Preliminary Planning Stage. The steps involved in this process are:

1. Define the project proposal, including relationship to identified fisheries management strategies and description of the treatment area (later used to define action area).
2. Preparation of the Preliminary Project Plan
3. Identification of all applicable laws and regulations.
4. Receive and incorporate agency review and feedback.
5. Public scoping to identify issues and concerns.

6. Presentation of preliminary application plan, including all pre- and post- application activities.

Intermediate Planning: Intermediate planning serves as a response to the results of Preliminary Planning and finalizes project design for implementation. This planning stage allows for revision, as appropriate, of the original project design to address issues regarding new information on the affected environment, regulatory compliance, and response to agency and public concerns. Components of intermediate planning are:

- Finalization of project design and application plan.
- Completion of environmental analysis (e.g. air and water quality, biological resources, recreational uses, economics, benefits and costs).
- Development of public involvement plan.
- Define pre- and post- application activities, including resource protection and monitoring.

Final Planning: Once the Final Project Plan is approved by management and all necessary regulatory requirements/permitting are complete, Final Planning can begin. During Final Planning, the project manager will make final preparations to conduct the application such as procuring necessary supplies and equipment and briefing team members on the details of the project. The Project Managers Planning and Implementation Check list is found in Appendix B.3.

Public Involvement: Public involvement provides for a process to identify significant public issues/concerns and allow input in final project design and implementation. Included in public involvement is the development of a Public Involvement Plan, with primary components identified below:

- Identification of interest groups.
- Definition of process for public information and education and input into final project design.
- Identification of methods to notify public and obtain public comment.
- Public notification of application.
- Post-application public information.

AOP 3 Environmental Compliance

Applicability
Compliance with all environmental laws and regulations required for safe and legal application of antimycin A.

Purpose
The purpose of this procedure is to ensure that relevant environmental compliance measures are met prior to the initiation of an antimycin A treatment. This includes a variety of laws and regulations administered by federal and state agencies, local government, and municipalities and tribes. Satisfaction of requirements in relevant sections of the laws and regulations are necessary to safely and legally apply antimycin A.

Procedure
During initial planning of an antimycin A treatment project, applicable environmental laws and regulations are identified. Federal laws and regulations are contained within Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Clean Water Act (CWA), National Environmental Policy Act (NEPA), and Endangered Species Act (ESA). Applicable State laws vary from state to state. Laws and regulations by local government entities also vary for location to location. Processes for adherence to laws and regulations for State and local governance will vary on a project by project basis.

Federal Laws and Regulations
FIFRA

- Basis for piscicide-use regulations.
- Requires registration of pesticide with EPA.
- Prohibits use of registered pesticide "in a manner inconsistent with its labeling."
- Provides for certification of pesticide applicators**.**

CWA

- NPDES permit not required for pesticides applied directly to water to control pests in the water.
- Application is consistent with relevant requirements of FIFRA.

NEPA: Any antimycin A project occurring on federal lands or otherwise having a federal nexus requires adherence to NEPA. This includes the preparation of an Environmental Assessment (EA) or Environmental Impact Statement (EIS). For the NPS, detailed information on the preparation of an EA or EIS can be found in Director's Order #12 and Handbook. An equivalent manual will guide the preparation of these documents for other federal agencies.

Basic steps for this process include the following:

- Define project proposal and conduct internal agency scoping.
- Identify potential environmental impacts.
- Consider alternatives (action, no action).
- Determine "significance" of impact(s).

- Provide for public review and comment on the proposal.
- Decision notice is completed and provided to agencies and public.

ESA

- EPA is the federal action agency responsible for labeling and use of pesticides. For this reason, all users of antimycin A must act in compliance with the Endangered Species Act.
- Section 7 consultations are required to ensure actions of federal agencies do not jeopardize existence of listed species or adversely modify critical habitat.
- The Federal action agency develops and provides to FWS an environmental assessment that describes the antimycin A application and assessment of impacts.
- If action is likely to "adversely affect," formal consultation is required.
- Biological Opinion is issued by FWS to action agency and renders a decision of "jeopardy" or "non-jeopardy".

Endangered Species Act: In compliance with Section 7 of the Endangered Species Act[2], each Federal agency shall, in consultation with and with the assistance of the Secretary of the Interior (hereafter referred to as "the Secretary"), ensure that any action authorized, funded, or carried out by such agency (hereinafter in this section referred to as an "agency action") is not likely to jeopardize the continued existence of any endangered species or threatened[3] species or result in the destruction or adverse modification of habitat of such species which is determined by the Secretary, after consultation as appropriate with affected States, to be critical[4], unless such agency has been granted an exemption for such action by the Committee pursuant to Section 7 subsection (h) of the Endangered Species Act. In fulfilling the requirements of this paragraph each agency shall use the best scientific and commercial data available.

Species Location Information: Section 7(a) (4) requires Federal agencies to confer with the U. S. Fish and Wildlife Service and/or the National Marine Fisheries Service (hereafter referred to as the "Services") on any agency action which is likely to jeopardize the continued existence of any species proposed for listing or result in the adverse modification of critical habitat proposed to be designated. A conference may involve informal discussions between the Services, the action agency, and the applicant. Following informal conference, the Services issue a conference report containing recommendations for reducing adverse effects. These recommendations are discretionary, because an agency is not prohibited from jeopardizing the continued existence of a proposed species or from adversely modifying proposed critical habitat. However, as soon as a listing action is finalized, the prohibition against jeopardy or adverse modification applies, regardless of the stage of the action.

Defining the Action Area: Actions necessary to define the project area are described in TOP 1. The documentation used by a Federal action agency to initiate consultation should contain a

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[2] http://www.fws.gov/endangered/esa.html

[3] The term "threatened species" means any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range.

[4] The term "critical habitat" for a threatened or endangered species means- (i) the specific areas within the geographical area occupied by the species, at the time it is listed in accordance with the provisions of section 4 of the Endangered Species Act, on which are found those physical or biological features (I) essential to the conservation of the species and (II) which may require special management considerations or protection; and (ii) specific areas outside the geographical area occupied by the species at the time it is listed in accordance with the provisions of section 4 of this Act, upon a determination by the Secretary that such areas are essential for the conservation of the species.

description of the action area[5] as defined in the Services regulations and explained in the Services' consultation handbook.

Issues Related to Incidental Take: When the Services determine that a proposed action may jeopardize the continued existence of a listed species in the wild or result in adverse modification to designated critical habitat, the Services, with the assistance of the Federal agency and/or applicant, develop Reasonable and Prudent Alternatives (RPA) that may be undertaken to avoid the likelihood of jeopardy or adverse modification. While these RPAs must avoid jeopardy or adverse modification, they may result in adverse effects to or take[6] of listed species. If take will occur from the implementation of an RPA, an incidental take statement must be developed to exempt such take from section 9 prohibitions.[7]

An incidental take statement identifies the level of take that is anticipated from implementation of a project as proposed. However, a statement also contains reasonable and prudent measures and terms and conditions that are nondiscretionary actions designed to minimize the effects of the take, and that must be implemented in order for such take to be exempt from the section 9 prohibitions.

State Laws and Regulations

State Pesticide Applicator Training and Certification: FIFRA provides for state certification of pesticide applicators. State requirements are variable, but include requirements for initial applicator certification and renewal processes.
Other State Requirements: Satisfaction of other State laws and regulations may include additional technical and public review and associated compliance measures, above and beyond federal requirements.

Local Government Laws and Regulations

Local laws and regulations may be administered by a variety of agencies, commissions, and/or boards and in a variety of formats. Preliminary planning steps will include identification of these laws and regulations and requirements for implementation of antimycin A application.

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[5] The action area is defined by regulation as all areas to be affected directly or indirectly by the Federal action and not merely the immediate area involved in the action (50 CFR §402.02). This analysis is not limited to the "footprint" of the action nor is it limited by the Federal agency's authority. Rather, it is a biological determination of the reach of the proposed action on listed species. Subsequent analyses of the environmental baseline, effects of the action, and levels of incidental take are based upon the action area.. Taken from http://www.fws.gov/endangered/consultations/sec7_faq.html#4

[6] The term "take" means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct. Taken from http://www.fws.gov/endangered/consultations/sec7_faq.html#4

[7] For additional information see pages 4-41 through 4-48 of the Section 7 Consultation Handbook.

AOP 4: Public Notification and Closures

Applicability
This procedure is applicable to all antimycin A application projects.

Purpose
The purpose of this administrative protocol is to provide guidance for public notification prior to applications of antimycin A and for notification of closure of the action area to public entry during treatment.

Procedure

Policy on Pre-Treatment Media Notification (applicable to federal, state, local, and tribal agencies)
General press release: General press releases are sent to media outlets (i.e. newspapers and radio and television stations) within the watershed and adjoining areas a minimum of one week in advance of treatment. Press releases are provided to the media for voluntary publication or for broadcast to the public. This should include a map of the project area and the length of the period of closure to the public.

Policy on Closure and Posting of Treatment Sites as Closed to Public Entry
The project area will be closed to public entry and used during the application. This closure will minimize if not eliminate exposure of the public to antimycin.

Potential posting locations adjacent to the proposed project area:
Designated public access sites
- Public fishing sites
- Public parks
- Boat launch sites
- Public trail access

Non-designated sites routinely used by the public
- Road crossings
- Known trails crossing or terminating on public lands

Commercial sites
- Public businesses in immediate treatment area

Government agency offices near the treatment area
All treatment area access points

Time period: Access sites are posted the evening before treatment is initiated.   Postings are removed the day after completion of the treatment for streams and one week after treatment for lakes or ponds.

Accepted posting materials:
Description of area closed to public entry
Press releases
Laminated treatment notice and project area map and information sheet (Appendix B.4)

AOP 5 Safety Training and Use of Personal Protective Equipment

Applicability
These procedures address the training and use of Personal Protective Equipment (PPE) to ensure applicator safety.

Purpose
The purpose of this training is to ensure that personnel are provided with and have knowledge of and experience with safe handling and use of antimycin A during applications, including use of personal protective equipment. Conformance to the training standards and operating procedures outlined in this document guarantees that personnel conduct operations in safe and legal methods.

Procedure
Training with regard to Standard Operating Procedure and General Safety
- Ensure personnel are familiar with facilities and Station Safety Plan.
- Ensure personnel are familiar with areas of the facility requiring either safety glasses or chemical splash goggles for entry.
- Personnel must be familiar with safety procedures of all chemicals used during treatment.
- Antimycin A can be used only by persons who are properly trained.
- Chemicals are stored in a secured area.
- Ensure personnel have received all relevant safety training for implementation of an antimycin A treatment (e.g., electrofishing procedures and safety, ATV usage, boat safety and operations).

Training related to Field Operations (where applicable)
- Ensure personnel are familiar with health precautions in the use of antimycin A.
- Personnel are trained about use of PPE.
- Personnel are trained in pesticide spill procedures.
- Ensure personnel are familiarized with all safety and hazard considerations for an individual treatment (e.g., MSDS, First Aid awareness).

AOP 6 Quality Assurance (QA) Measures Utilized in Antimycin A Application

Applicability

These QA measures apply to procedures used in the chemical control of target fish species for a variety of management goals.

Purpose

Conformance to the training standards and operating procedures outlined in this document guarantees that personnel conduct operations by similar, proven, and accepted methods regardless of project location. The QA measures described will ensure that data generated by workers are accurate and reliable.

Procedure

This synopsis outlines the steps taken to assure and to document those day-to-day functions in the application of antimycin A for control of target fish species are conducted properly. The primary areas addressed in this document include training in standard procedures used in field work during antimycin A application treatments. The **Training** section outlines training offered to workers. The **Documentation** section describes applicable record keeping procedures.

Training

The following training is offered to antimycin A applicators. Only training which supports working skills necessary to successfully complete an antimycin A treatment are listed. Other forms of training which support skills not directly related to antimycin A control are not included.

- Toxicity testing
- Dye application
- Discharge measurement
- Certification of pesticide applicators
- Antimycin A application
- Potassium permanganate application

Training Session Content

Certification of pesticide applicators: Certified pesticide applicator(s) associated with a treatment project must have passed a current state certification examination prior to the initiation of a project. A minimum of one certified applicator must be present on site during all phases of a treatment project.

Toxicity testing: Senior staff participates in and supervise bioassay procedures to determine toxicity to target species prior to project implementation. Additional personnel receive limited introduction. Flow-through test procedures are demonstrated and explained. Data records are extensive, so attention is paid to completing all forms accurately.

Dye application: A training session for dye application to determine water travel time between drip stations and/or known points is presented to field personnel. Application of dye is described for the purposes of determining water travel time within the project area.

Discharge measurement: Personnel responsible for conducting measurements of stream discharge will be proficient in the use of the flow meter to be used and in the collection of discharge data. During the treatment, personnel who have no experience in the collection of discharge data will be teamed with experienced personnel and trained in the proper use of the meter and the collection and recording of discharge data. Additional instruction on conducting function checks on the meter, completing calculations of stream discharge, and selecting locations to conduct discharge measurements will also be given to inexperienced personnel.

Antimycin A application: Personnel who apply antimycin A are instructed in the operation of all equipment used in applications. An experienced operator demonstrates the equipment and procedures used for applications to trainees at a drip station location. Trainees are instructed in setting up drip stations, handling antimycin A, keeping records, washing cans, glass or plastic containers, and using all safety equipment. After instruction, the operator trainees are required to demonstrate their abilities to properly operate an antimycin A drip station during an application. Experienced applicators are stationed at antimycin stations adjacent to trainees and they or the project manager visits each trainee periodically each day of treatment.

Potassium permanganate application: Prior to the operation of the potassium permanganate ($KMnO_4$) station, an experienced operator demonstrates the equipment and procedures used for potassium permanganate applications to trainees at the deactivation site. Trainees are instructed in setting up the deactivation station, weighing and mixing $KMnO_4$, keeping records, washing containers, proper and secure storage of $KMnO_4$ and using all safety equipment. After instruction, the operator trainees are required to demonstrate their abilities to conduct potassium permanganate applications. A minimum of one experienced applicator must be at the site at all times during the operation of the $KMnO_4$ station

Documentation

Training

A record of training is maintained for each person participating in antimycin A applications. This record, kept by the Project Manager for each project, lists training provided to all antimycin A application personnel.

Data Collection and Reporting

All data collected during field operations is recorded on standardized data forms. Specific data collection forms include Discharge Measurement (Appendix B.5), Antimycin A Daily Application Form (Appendix B.9), Potassium Permanganate Daily Application Form, ,(Appendix B.10,), and Daily Antimycin and Potassium Permanganate application Summary Sheet (Appendix B.11). In addition, a series of forms are used to record data from on-site toxicity tests. The forms are organized to allow the data to be easily transferred to computer data files. Transcription of the data is conducted as time allows, if possible, on the day of data collection. Forms are stamped "ENTERED" to prevent duplicate entries into data files. Original data forms are retained after data entry.

Data are recorded in ink; however, pencil is acceptable if data forms are used in wet conditions. Entry errors are crossed out with a single pen stroke, corrected, and initialed.

AOP 7 Storage, Transportation, and Spill Containment of Antimycin A during a Treatment Project

Applicability
Procedures apply to all activities related to the storage, transportation, and use of antimycin A during a treatment project.

Purpose
The purpose of this procedure is to provide instruction for the safe handling and storage of antimycin A in the field.

Procedure

General Storage
- Antimycin A stockpiles are secured in locked buildings.
- Antimycin A is stored in locked, covered vehicles in treatment areas having vehicle access.
- Antimycin A is stored in secured areas in treatment areas lacking vehicle access.

Specific Storage
A unit of antimycin A is packaged as discreet components of active ingredient and diluent in two glass bottles, padded with thick paper sheeting and held in metal, 3.75L pails. Inventory logs are maintained to record the use of antimycin A.

In the field, antimycin A bottles, will be transported to the base camp or base of operations in the original 3.75L pails, or other appropriate containers (e.g., hard panniers). At this point the project supervisor may elect to mix the antimycin A needed for the next day's treatment or the bottles can be removed from the pails with original padding in place and placed in plastic bags, and transported to drip station sites in padded backpacks or padded, plastic panniers.. If the first option is selected, a mixing station must be set up with absorbent pads to absorb any material that may be spilled. If the project supervisor decides to mix the antimycin A on site then the chemical must be mixed over the pads used to wrap the bottles and mixing must take place at least 10 feet from the stream.

Transportation
Antimycin A is transported from the storage facility to the treatment site in a variety of methods ranging from motor vehicles, pack mules, watercraft or backpacks. Each mode of transportation is supplied with chemically absorbent materials (spill kit) and personnel are supplied with a two-way radio to allow rapid communication if problems arise. Passengers and foodstuffs are not allowed in compartments used to transport antimycin A.

Proper precautions are taken to evenly distribute and secure loads. Containers are secured to prevent shifting or tipping and are protected from the weather regardless of the mode of transportation. After application, empty containers are returned to the storage facility for disposal.

Container Disposal
Empty metal containers and padding are disposed of in an approved municipal landfill or offered for recycling.

Spills
Spills on land: In the event of a spill during storage or transport or at an application site it is of greatest importance that the spill is stopped at its source, the spilled material is contained and the Project Leader is notified. Shovels and other hand tools are used for immediate containment or channelization of the spilled antimycin A into a containment area. The following actions are taken, as necessary, to contain and clean up a spill on the ground:

1. Stop the spill at its source
2. Dike solution in pools
3. Absorb with clay, soil, or noncombustible, absorbent material
4. Deactivate materials resulting from an antimycin A spill to the extent possible.

Spills into water: If antimycin A is spilled near or into a waterway, containment is initiated to prevent or minimize movement into the waterway. If an antimycin A spill occurs into a stream not scheduled for immediate antimycin A treatment, a deactivation station should be deployed using potassium permanganate according to neutralization techniques described in TOP 16. Detoxification records must be completed for this incident. Accidental spills of antimycin A into a stream during treatment operations may occur during a period when the treatment project is already underway. In such an instance, monitoring is extended to ensure that the area of impact of the plume does not exceed previous projections.

AOP 8 Procedure for Management of Project Records

Applicability
Procedures apply to all records of treatments using antimycin A.

Purpose
To ensure continuity in the management and archiving of all project records regardless of location and to assure compliance with environmental regulations.

Procedure

Management of Routine Treatment Records

Hard copies of treatment data: Treatment data are given to the project manager daily and stored in camp until he can return them to his office for storage. Original data are stored in a series of folders organized by stream which contain all data collected during each stream treatment (i.e. water chemistry, discharge measurements, antimycin A application records, etc.) and summaries of data sets collected daily during the treatment (i.e. Daily Antimycin and Potassium Permanganate Application Summary Sheet (Appendix B.11). The treatment supervisor is responsible for maintaining all treatment folders. Duplicates are made as required.

Computer records: Data are transferred to the station system at the end of the field season. Electronic treatment records are backed up and stored in a secure space. Data are retained in a secure space for the length of time required by NPS data management requirements or agency specific requirements.

AOP 9 Contingency Management for Environmental Changes

Applicability
Procedures apply to environmental contingencies that may arise during antimycin A application.

Purpose
To provide for standardized responses to changing environmental conditions that occur just prior to or after initiation of antimycin A application.

Procedure
Changes in environmental conditions may occur just prior to or after initiation of an antimycin A application. Changing environmental conditions primarily relate to climatic changes that can decrease water temperature, alter quantity of water to be treated within the project area, and/or hinder personnel performance due to inclement weather. A process for identifying and responding to environmental factors that may disrupt safe application or alter the effectiveness of antimycin A application are defined herein.

Pre-Application Planning
Scheduling of application activities will consider local weather patterns. Pre-application review of weather will include both long- and short-term forecast to determine likelihood of consistent environmental conditions during planned application duration. Reduced water temperature may increase the exposure time necessary to effectively remove target fishes and may also increase the distance at which antimycin A remains toxic. Change in stream flow may result from rain and associated runoff into the project area, thereby requiring additional measurement of flow to ensure effective application according to label directions.

Response to Changing Environmental Conditions during Application
- A significant decline in air temperature may result in a decrease in water temperature. Water temperature declines will slow fish metabolic rates and may increase the length of time before antimycin A effects are observed.
- Increases in stream flow will require recalculation of the amount of antimycin A needed to achieve the appropriate treatment concentration.
- Inclement weather may detrimentally affect personnel performance in field operations.

Treatment Termination and/or Postponement
- Postponement prior to initiation of application will occur if inclement weather results in prolonged unstable environmental conditions.
- Termination after initiation of application will occur if inclement weather results in unstable environmental conditions.
- Rescheduling a treatment will occur if prolonged instability in environmental conditions is predicted for the duration of planned treatment.

AOP 10 Final Report

Applicability
The development of a Final Report for each antimycin A treatment project is highly recommended as it provides a concise administrative history of the project.

Purpose
To provide for a standardized document for the Final Report at the conclusion of an antimycin A project. This report is normally completed once follow-up monitoring indicates that all non-native fish and project objectives have been completed (about one year after treatment).

Procedure
At the completion of the antimycin A project, the project manager will prepare a “Final Report.” The primary objective of the Final Report is to document the intended and actual outcomes of the antimycin A application. The outline for completing the required Final Report is presented herein.

Final Report

Background
Objectives: Explain the overall purpose and objectives of the project.
Rationale: Explain the rationale for the project.
Fish Species Management Strategy: Explain the overall fish species management strategy and how it relates to the objectives and rationale of the project.
Legal Authority: explain the legal authority for conducting the application including citations of applicable statutes.
Project Team: identify the project manager and key team members along with corresponding roles, responsibilities, and a summary relevant experience.

Treatment Overview
Application Dates: List the start and end date of the project.
Treatment Area: Clearly identify the water bodies or portions thereof to which antimycin A was intended to be and, if different, actually applied.
Project Area: Clearly identify the intended and, if different, actual location(s) where antimycin A was formulated (i.e., mixed and measured for use), the areas affected by the treatment (i.e., where antimycin A and potassium permanganate was measurably present in the environment), the location(s) of the deactivation site(s), the location of the barrier that will prevent invasive species from re-entering the treatment area, and potential public access ways.
Treatment Area Characterization: Summarize the results of the gradient and discharge analysis.
Pre-Treatment Bio-Monitoring: Summarize the results of the pre-treatment bio-monitoring and how they were used to define the treatment area.
Equipment Used: Describe the equipment used to conduct the application.

Regulatory Compliance
FIFRA: Describe the steps taken to comply with the requirements of FIFRA.
CWA: Describe the steps taken to comply with the requirements of CWA.

NEPA: Summarize the steps taken to comply with the requirements of NEPA.
ESA: Summarize the steps taken to comply with the requirements of ESA and append the Services' final determination and, if necessary, how reasonable and prudent measures/alternatives were implemented to the Final Project Plan.

Public Involvement

Public Concerns: Describe substantive public concerns.
Response: Describe project manager's response to each public concern.

Results and Discussion

Treatment Results: Describe the extent to which the application accomplished the objectives.
Bioassay Results: Summarize the results of any bioassays conducted prior to, during, and after the application.
Bio-Monitoring Results: Summarize the results of the pre- and post-application bio-monitoring.
Discussion: Describe any conclusions that can be drawn from the treatment including effectiveness of the treatment, best practices for future applications, and any conclusions that can be drawn regarding non-target species recovery.

Appendixes

Final Project Plan
"Safety and Personal Protective Equipment" meeting sign-in sheet
Protocol Deviation Forms

Upon completion, the project manager will distribute and properly file the Final Report along with other project documentation in accordance with applicable record keeping policies.

AOP 11 Protocol Deviations

Applicability
A Protocol Deviation Form will be completed for each deviation from an AOP/TOP/SOP for each antimycin A treatment.

Purpose
To provide for a standardized document for tracking deviations from the administrative, technical, or other standard operating procedures in this document.

Procedure
Throughout the project, the project manager will document on the Protocol Deviation Form (Appendix B.10) any deviation from the standard operating procedures identified in this manual. Extenuating circumstances that may permit the project manager to act in this manner include those that require deviating from standard operating procedures to accomplish any of the following:

- protect worker/bystander safety;
- prevent release of antimycin A beyond the treatment area;
- prevent violation of Federal or other regulation(s);

For each deviation, the project manager will document the following information:

- the TOP, AOP, and/or SOP from which the deviation occurred;
- a description of the cause and nature of deviation event;
- the date and time the deviation began;
- the date and time the deviation ended or was corrected;
- the date and time the deviation was identified;
- the location of the deviation within the treatment area;
- a description of the action taken to correct the deviation; and
- signature of the Project Manager on the deviation form.

It is a violation of Federal law to apply antimycin A in a manner inconsistent with its labeling. A protocol deviation that violates the antimycin A product label, while properly documented, does not absolve the violator(s) from potential administrative or legal action under FIFRA.

Technical Operating Procedures

TOP 1 Determination and Characterization of Treatment Area for Streams

Applicability
Procedure applies to the determination of the area to be treated during the project and collection of physical stream attributes to assist with defining and characterizing the treatment area.

Principle
Prior to an antimycin treatment, it is necessary to determine the distribution of target fish species within the watershed to ensure success of the project. Identification and location of selected physical stream attributes will help locate potential trouble spots and assist with project planning.

Equipment Required
- 7.5' USGS topographic maps of project area
- GPS unit
- Measuring tape or hip chain
- Surveyor flagging
- Aluminum tree tags
- Electrofishing gear, minnow traps, seines, or entanglement nets
- Dip nets

> ***Lessons Learned!***
> It is important that only qualified staff experienced in the use of backpack electrofishing gear verify all distribution information so that no areas are inadvertently missed. One untreated area can compromise the entire project!

Procedures
Upstream Limits

Upper target fish distributions should be verified early in the initial planning process and re-checked within a few weeks of the treatment date. Backpack electrofishing units are the method of choice to determine upstream distribution as they offer the greatest opportunity for capture in small high gradient streams. However, other appropriate sampling techniques may also be used. The main stem of the stream and all tributaries must be surveyed. Experienced personnel should conduct pre-treatment surveys. Once upper distribution limits are found, GPS coordinates and elevation should be recorded and the site marked (*e.g.* flagging or rock cairn) for future reference. In most cases, upper treatment stations are 50-200m upstream of the upper known distribution of target species to ensure target species are eliminated.

Figure 5. A cascade barrier to the up-stream movement of salmonids in Great Smoky Mountains National Park.

Downstream Limits

In all proposed treatment streams, a downstream physical barrier (*e.g.* waterfall, cascade, constructed barrier, dry stream reach, or thermal barrier) to upstream movement of target fishes should be located (Figure 5).

Data Requirements

The GPS coordinates of all distributional information and location of downstream physical barrier should be placed on topographical maps and a Geographic Information System (GIS) so that the project area can be clearly defined (Figure 6).

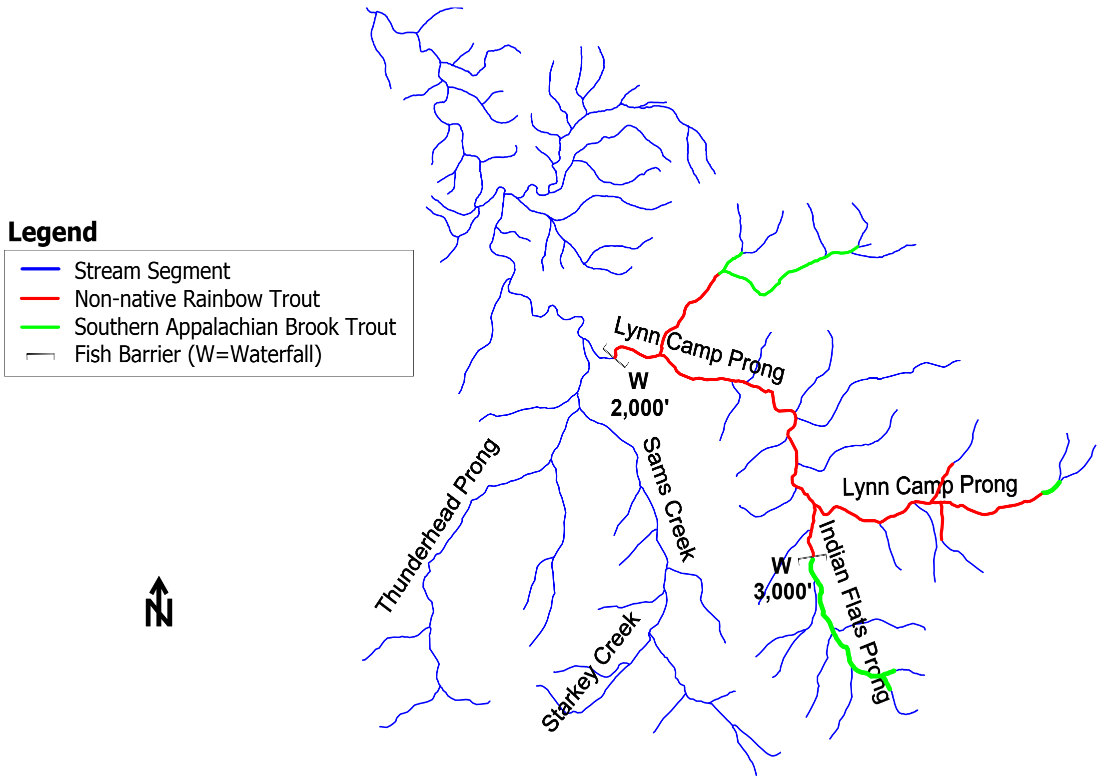


Figure 6. An example of a study area map depicting distribution of native and non-native fish species prior to removal of non-native fish.

Tagging 100m Sites Throughout Treatment Area

Once the target species distribution is determined, sequentially numbered markers (aluminum or plastic flagging tape) should be placed on trees or on stakes along the stream bank every 100m (or other pre-determined frequency), starting at the downstream physical barrier and continuing upstream to the upper limits of target species distribution. Relevant information (*e.g.* tag tree species, side of stream, elevation) should be recorded for each marker. Once markers are placed along entire treatment reach, the vertical drop between each marker location, total length of treatment reach, and total vertical elevation drop from top to bottom of the entire treatment reach can be determined. A schematic

Lessons Learned!
Many times, physical validation of barriers is unnecessary given a large waterfall or cascade >5m vertical (Figure 5), a thermal barrier, or when streamflow becomes subterranean. In other cases, plunge pools, side channels, stair-step pools, and other complex habitat make visual barrier validation difficult. In such cases, barrier validation is necessary using the "mark-n-move" technique (TOP 3).

depicting all these data (Figure 7) is useful for project planning and crew instruction during antimycin application.

The stream schematic is also helpful in determining amount of antimycin needed and length of stream that can be feasibly treated each day. In addition, markers provide excellent points of reference for workers throughout the treatment and elevation changes between markers are useful for determining daily treatment station placement (TOP 11).

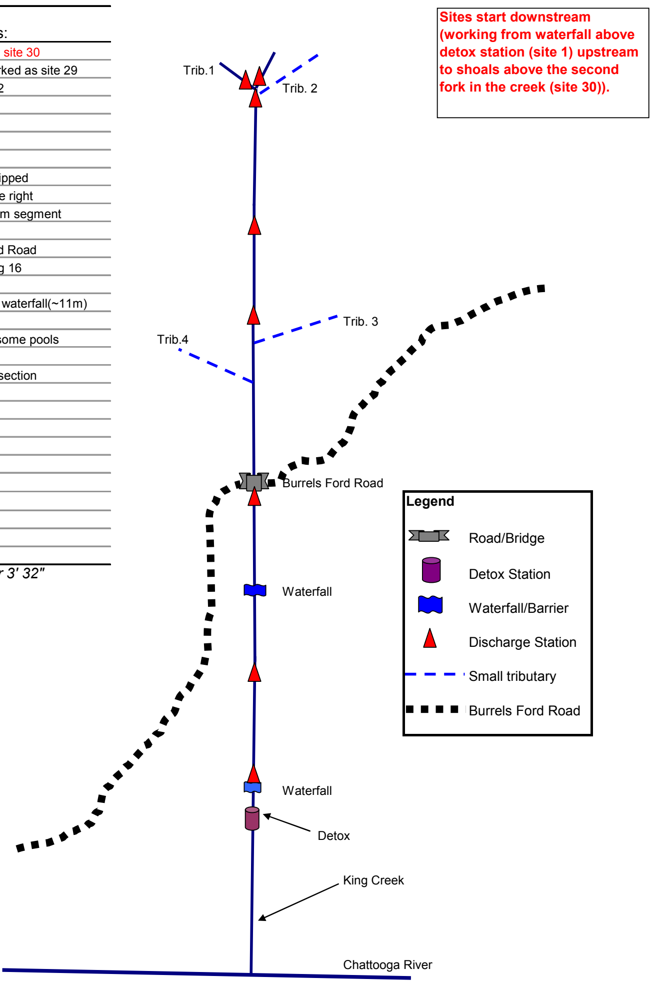
Measurement of Mean Stream Gradient (%) For Each Site

Mean stream width (m) and gradient (%) should be calculated for each section within the treatment area. The first stream width is collected at the bottom end of the site followed by additional wetted stream widths every 10m upstream to the upper end of the site. Mean stream width (m) is generated by averaging all the wetted stream widths (m) for a given site. Stream gradient (%) is generated using a clinometer to measure the gradient between each 10m wetted stream width, beginning at the bottom of the site and proceeding upstream to the top of the site. Mean site gradient (%) is then calculated by calculating the mean for all gradient measurements within each site. Once stream gradients are calculated, they can be graphed to identify potential trouble spots (Figure 4). Mean stream gradients >8%, which typically indicate areas where the antimycin degrades rapidly (a function of increased oxidation associated with increased turbulence) and dispensing station intervals may need to be less than where gradient is less steep. Other similar techniques may also be used to determine stream gradient for the project area.

King Creek Brook Trout Restoration Project
August 15-20, 2005

King Creek Gradient Profiles
June 21-22, 2005
June 27-28, 2005

Site #	Elevation	Flow Time	Discharge	Mean % Gradient	Notes:
30	2326	7	1.2	3.5	**Marked as site 30
29	2313	7	1.2	3	Trib. 1 enters - marked as site 29
27	2303	7	2.0	3.1	Trib. 2
26	2293	6		3.1	
25	2290	7		2.9	
24	2285	7		2.7	
23	2280	8		3.3	
22	2277	7		6	Tag 21 skipped
20	2257	10		3.5	Trib.3 to the right
19	2218	7		2.9	Trib. 4 at last 10m segment
18	2211	7	2.7	2.6	
17	2198	4		4.9	Burrels Ford Road
16B	2192	6		6.2	Extra Tag 16
16	2178	6		5.4	
15	2170	7	5.6	9.5	site length 120m & a waterfall(~11m)
14	2153	10		4.9	
13	2144	10		4.5	small falls and some pools
12	2122	11		5.1	
11	2102	9		6.1	waterfall in section
10	2096	9		2.8	
9	2086	9		2.9	
8	2069	4		2.6	
7	2063	4		2	
6	2062	8	7.3	2.4	
5	2050	6		1.9	
4	2033	9		3.1	
3	2030	5		2.6	
2	2028	7		2	
1	1995	8	8.9	7.3	
		212			*Note: 212 minutes or 3' 32"*



Detox
- 15 gal of $KMnO_4$/water applied per hour
  - Need 946 ml/min, OR
  - *Need 237 ml/15sec*

example:
Detox Station cfs = 3.0 cfs
- $KMnO_4$ for 4ppm for 1 hr = 1224g/hr
  - Mix 2,448 g in each 30-gal drum
  - Apply at 15gal/hr

Antimycin Use (mls concentrate for 8 hours)

Stream CFS	Time	8 ppb
0.5	8 hr	32.5
1.0	8 hr	64.9
1.5	8 hr	97.4
2.0	8 hr	129.9
2.5	8 hr	162.4
3.0	8 hr	194.8
3.5	8 hr	227.3
4.0	8 hr	259.8
4.5	8 hr	292.2
5.0	8 hr	324.7
5.5	8 hr	357.2
6.0	8 hr	389.7
6.5	8 hr	422.1
7.0	8 hr	455.6

Antimycin Calculation
1. Calculate ***Ac ft of water*** =
CFS x 449(GPM/CFS) x 60(min/hr) x 8 (total hr)
325,800 (gal/Ac ft)

Total Ac ft x 12.3 = mls required for 1 ppb

Total Ac ft x 49.2 = mls required for 4 ppb

Total Ac ft x 98.4 = mls required for 8 ppb

Also:
12.3 ml/ac ft = 1 ppb
49.2 ml/ac ft = 4 ppb
98.4 ml/ac ft = 8 ppb

$KMnO_4$ Use

Stream CFS	Time	1 ppm g/hr	2 ppm g/hr	4 ppm g/hr
0.5	1 hr	51	102	204
1.0	1 hr	102	204	408
1.5	1 hr	153	306	612
2.0	1 hr	204	408	816
2.5	1 hr	255	510	1,020
3.0	1 hr	306	612	1,224
3.5	1 hr	357	714	1,428
4.0	1 hr	408	816	1,632
4.5	1 hr	459	918	1,835
5.0	1 hr	510	1,020	2,039
5.5	1 hr	561	1,122	2,243
6.0	1 hr	612	1,224	2,448
6.5	1 hr	663	1,326	2,652
7.0	1 hr	714	1,428	2,856

Figure 7. An example of a project area map for a stream slated for treatment. The map includes stream discharge, mean stream gradient, elevation, key projection locations and application rate tables.

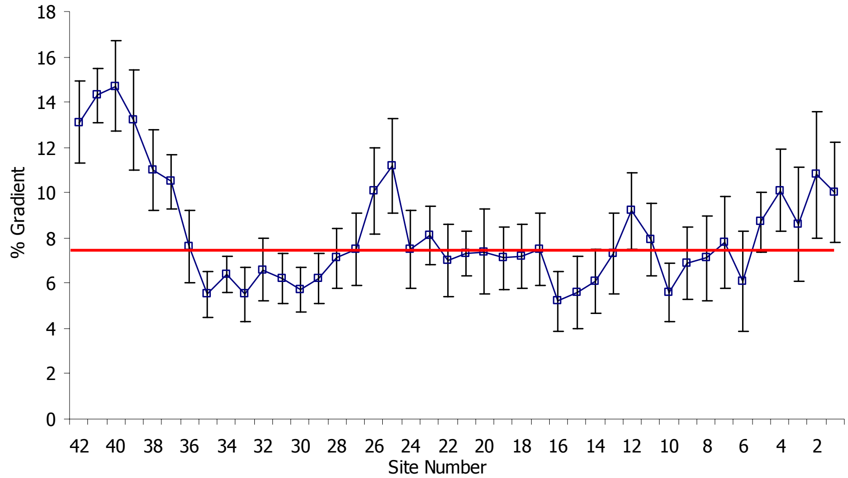


Figure 8. Mean stream gradient profile of Sams Creek, Great Smoky Mountains National Park. Bars represent standard error (SE). Note the red line at roughly 8% represents the line above which identifies sites in which the effective vertical travel distance of antimycin declined. Site 42 is the upper most treatment site (3,460 ft.) and site 0 is located at the lower barrier (2,180 ft.).

TOP 2 Determination and Characterization of Treatment Area for a Lake

Applicability
Procedure applies to the determination of the lake area and volume to be treated if one exists in the treatment area.

Principle
Prior to treatment with antimycin, it is necessary to determine the surface area, volume (acre-feet) inflows and outflow of the lake or pond to be treated.

Specific information to be obtained prior to treatment should include:

- Total surface area and volume of the lake
- Maximum depth, thermal stratification and temperature/oxygen/pH profile
- Identification of all inlets and outlets
- Inlet and outlet discharge, temperature and pH
- Distribution of fish within the inlets
- Distribution of fish within the lake (fish below thermocline)
- Presence of sensitive species
- Location of a permanent fish barrier below the lake
- Species of fish present, and spawning/fry emergence

Equipment Required
- 7.5' USGS Topographic map of the project area
- GPS unit
- raft and boat motor
- depth finder
- water quality kit (pH, oxygen, temperature)
- gill nets, or other appropriate entanglement gear
- field book

Procedures
For initial planning purposes, the area and volume of the lake may be extrapolated from a USGS map prior to going to the site. However, to accurately determine the amount of antimycin required for the lake treatment, it is necessary to accurately determine the total volume (in acre-feet of water) of the lake/impoundment prior to the treatment. It is recommended that site a visits be made to the project area at the same time of the year that the project is proposed, in order to obtain as much information on water volume/inflows and outflows, water chemistry and fish distribution. On the year the project is to be conducted, a site visit should be completed just prior to application, in order to make appropriate adjustments for current conditions.

TOP 3 Validation of A Potential Fish Barrier

Applicability
Procedures apply to determining if a natural feature (*i.e.* waterfall or cascade) is a barrier to upstream movement of undesirable fish. This TOP applies only to potential barriers for which their ability to preclude upstream movement of undesirable fish is questionable.

Principle
To determine if a questionable natural or constructed feature (*i.e.* waterfall or cascade) is a barrier to upstream movement of undesirable fish.

Equipment Required
- Backpack electrofishing units
- Safety equipment for electrofishing
- Data sheets and/or field data logger
- Water quality instruments
- Tape measure or hip chain
- Scissors for fin clips
- Measuring boards, scales and data boards
- Holding cages
- Fish anesthetic
- USGS topographical maps

Procedures
Experience in GRSM has shown that the best method for determining if a presumed barrier actually blocks movement of undesirable fish is to conduct a 'mark-and-move' study. Data from previous 'mark-and-move' studies has shown displaced fish will return to their home range if possible. Target fish are collected upstream of the presumed barrier and transported below the feature. If marked fish are later found above the feature, it is not a barrier to fish movement.

Identify and map the location of the feature on USGS topographic map (7.5' map is optimal). The UTM or Lat-Long location of the feature should be recorded. Establish one to two 100-m sites downstream of the obstruction and two to three 100-m sites upstream of the obstruction. Conduct three-pass electrofishing sampling in each site. The length and weight of each fish collected should be recorded on field data sheets or entered into a data logger. Each fish collected should receive a fin clip (i.e. adipose for salmonids) that identifies the fish as being collected upstream or downstream of the feature. If the study is conducted over >6 months or on species without an adipose fin, PIT or VIE tags should be used. Release all marked fish collected in upstream sites in the downstream sites. Between 6 months and 1 year later, return to the site and sample all previously sampled locations. Record length, weights and mark of all fish collected at each site. From these data, determine if fish collected in upstream sites returned upstream to the area from which they were originally collected. If marked fish are collected upstream of the obstruction, it is not a barrier to upstream movement and the stream segment should not be considered for restoration unless the feature can be rendered impassable.

TOP 4 Pre- and Post-Treatment Aquatic Macroinvertebrate and Fish Monitoring

Applicability

Procedure applies to conducting aquatic macroinvertebrate and fish sampling prior to and immediately after treatment to evaluate impacts of antimycin application to non-target organisms within and downstream of treatment area. Should data from current and future projects demonstrate only short term impacts and complete recovery within months, implementing this TOP should be considered on a case by case basis.

Principle

Prior to and immediately following antimycin application, aquatic macroinvertebrate and fish collections are conducted to evaluate the effects of antimycin and potassium permanganate within and downstream of the treatment reach. These data are also useful for public and professional project evaluation, identifying potential resource impacts, and adjusting protocols for future projects.

Equipment

Aquatic Macroinvertebrates

- Surber or Hess aquatic macroinvertebrate sampler for quantitative sampling (or similar sampler)
- Kick nets and seines for Rapid Bio-assessments
- Specimen containers (ziplocks, whirl paks, or nalgene jars)
- Collection labels
- Sorting tray
- Forceps
- Preservative (alcohol)

Fish

- Backpack electrofishing gear
- Waders and electrical gloves
- Dip nets
- Buckets
- Block Nets
- Measuring tape or hip chain
- Measuring board
- Scales

PROCEDURES

Aquatic Macroinvertebrates

Prior to treatment, aquatic macroinvertebrate and fish assemblages should be sampled within the treatment area as well as downstream of the antimycin deactivation dispensing station. Pre-treatment aquatic macroinvertebrate sampling provides data that can be compared to post-treatment data to characterize short and long-term impacts as well as recovery (Figure 5). Pre-treatment fish sampling provides a realistic, measurable population density goal for the post-treatment population. Pre- and post-treatment fish sampling downstream of the deactivation site also provides data to assess impacts of potassium permanganate.

Etnier (2005) recommended a minimum of six macroinvertebrate sampling sites within the treatment area: two in treatment area, two in the deactivation reach, and two in control (reference) sites outside the treatment and deactivation area. Each site is sampled bi-annually in the spring (May) and fall (September) within one month of planned treatment. Spring samples afford the majority of the invertebrate fauna while the fall sample will capture potential short-term treatment impacts. Each site should be sampled within 1 month post-treatment and quarterly thereafter for one to two years depending on project objectives. A similar approach may also be used for different regions of the country, but the project manager should decide which method will best meet project objectives during the initial project planning phase.

Figure 9. —Dr. David Etnier sorting a pre-treatment aquatic macro-invertebrate collection from Sams Creek, Great Smoky Mountains National Park.

PROCEDURES

Fish

A minimum of four 100m fish sampling sites should be established prior to treatment: two in the treatment area, one in the deactivation reach, and one in a control area. Three-pass depletion or mark-recapture techniques should be used to quantify fish abundance at all sites. Data from pre-treatment surveys can be used as benchmarks to evaluate recovery of the desired species. Species abundance data from the deactivation and control sites can be compared to that from pre- and post-treatment sites within project area to characterize impacts of potassium permanganate in non-target areas. Post-treatment surveys should continue until pre-determined population goals are met.

TOP 5 Conducting Stream Discharge Measurements

Applicability
Procedure applies to determination of stream discharge (Q) prior to application of antimycin or potassium permanganate to streams (Murphy and Willis 1996).

Principle
Prior to an antimycin treatment it is necessary to measure stream discharge (ft^3/sec or m^3/sec) at various sites throughout the project area to calculate application rates of antimycin and potassium permanganate. At a minimum, discharge data must be collected at the site(s) where treatment is initiated, immediately downstream of each tributary stream that has surface water, and at the deactivation site. Discharge measurements should be made at the mouth of all tributary streams that are treated. If a rain event occurs during a treatment, discharge must be measured before resuming antimycin application and potassium permanganate dispensing.

Equipment Required
- Marsh-McBirney® , Pigmy® flow meter, or equivalent meter
- Topset rod with vernier scale
- Measuring tape
- Data forms (*e.g.* Appendix B.3.)

Potential Interferences
Ideally, the discharge measurement site should be located where stream bottom contour is smooth and relatively solid (not necessarily flat), there is perceptible flow from bank to bank, and no instream obstructions. Conditions detrimental to accurate discharge measurement include obstructions such as logs and boulders, aquatic vegetation, undercut banks, shallow water, riffle areas, dead water areas, eddies, soft substrate, bridges and culverts, multiple stream channels.

Safety
Discharge measurement in deep or swift water may be hazardous. An uneven bottom, soft, loose or slippery substrate, turbidity, and obstructions may increase the danger. Select areas where the stream bed is firm and provides good footing. Avoid potentially dangerous situations such as deep pools or waterfalls immediately downstream of the gauging site.

Procedures
General Principles
Stream discharge (Q) may be defined as the volume of water moving through a cross section of stream per unit of time and is expressed as cubic feet per second (cfs) or cubic meters per second (cms). The procedure described here is the velocity-area technique whereby the computed discharge of a stream is the product of the cross-sectional area and the average velocity.

The cross section of the stream at the measurement site is divided into sufficient sections so that the depth measurements will provide an accurate profile of the bed, and the velocity measurements are made sufficiently close to obtain an accurate representation of velocities in each of the sections. The width of individual sections will largely depend on the overall width of the stream, the unevenness of the stream bed and the variation of velocities across the channel. It is important to space the sections more closely where the depths and velocities are more variable in order to define accurately the discharge for any given section.

In most streams the ideal discharge site cannot be found. In this situation it is recommended that three discharge measurements be taken in a 20-50 m section of stream. If the three measurements are similar, average the values to determine the discharge for that site. If one value is significantly different from the other two, take a fourth measurement, determine if it is close to the two closest measurements. If so, determine the use theses values to determine the discharge for the site.

The number of cells will vary dependent on stream width. For streams with a width of ≤ 1m, a minimum of five cells is recommended. For streams with width >1m, 10 cells are recommended. No more than 10% of the discharge should be in one cell.

Velocity is determined for each cell. Velocity should be measured at 0.6 the depth from the surface. In larger streams where depths >1m, velocity is measured at 0.2 and 0.8 the depth from the surface. This is based on both mathematical theory and on observations from numerous vertical velocity curves. The mean of the two velocities is used for that cell. The standard equation to calculate stream discharge is:

$$\mathbf{Q = \Sigma (V \cdot D \cdot W)}$$ where,

Q = stream discharge (ft3/sec or m3/sec)
V = mean velocity of cell (ft/sec or m/sec)
D = depth of cell (ft or m)
W = width of cell (ft or m)

Figure 10. — Measurement of stream discharge using the cross sectional area method. *Note* personnel standing downstream of the meter.

Site Selection Criteria

The discharge measurement site should be in a safe location, with a firm bottom and depth of less than 1 meter. The measurement cross section is perpendicular to the general direction of flow and stream bed cross section is as uniform as possible and free from instream flow obstructions.

Conducting the Measurement

Stream discharge is measured by using a flow meter attached to a topset rod that has depth measurement capability. To begin a measurement, a tape measure (English or metric) is placed across the stream (Figure 10). Anchor one end at the initial point and proceed across the stream at right angles to the direction of the current.

While wading across the stream, an overall impression of the depths and velocities can be obtained. This is a good time to look for rocks and debris which can be removed from the stream bed to improve accuracy of measurements. On smaller streams it may be possible to construct small dikes to cut off sections of shallow flows and dead water. After any modifications to obtain more accurate flow estimates, be certain to allow sufficient time for conditions to stabilize before proceeding with measurement.

Based on wetted perimeter width, the cross-section is typically divided into 10 equidistant cells (see above). Measure begins on left bank (looking upstream). The first measurement is made one-half cell width. The second measurement is made 1 cell-width from the first (*i.e.* middle of second cell) and so on across the stream's wetted width. At each cell's center point, measure velocity, depth, and visually characterize substrate (*e.g.* sand, gravel, or cobble). Depths are measured by reading the level of the water surface on the rod when the base plate rests on the stream bed. The current meter is set to the correct depth to obtain and record velocity. This procedure is repeated until the stream is traversed and all cells measured. The total discharge for the site is the sum of discharges of each individual cell.

The position of the operator with respect to the current meter is important when making a discharge determination by wading. The operator should stand to the side and downstream of the meter to avoid influencing the measurement of velocity. Studies indicate that the position that has minimal effect on the operation of the current meter is when the operator stands facing either shore and is no less than 0.4 m (1.25 ft) downstream and to the side of the current meter.

Data Requirements

A depth measurement is required for each side (or one in center) of a cell and a velocity measurement is taken at the center of each cell. The stream discharge data sheet (Appendix B.3) is an example that can be used to record discharge data.

TOP 6 Determination of Stream Water Travel Time Using Fluorescent Dyes (Fluorescene & Rhodamine)

Applicability

This procedure determines time required for antimycin to travel from one point to the next in treatment.

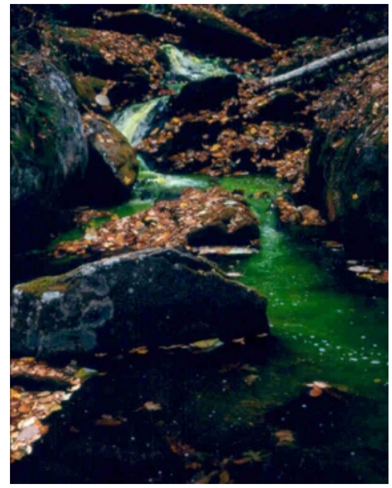


Figure 11. Fluorescene dye traveling downstream during a flow study to establish project timelines..

Principle

Water travel time determines the length of time required for antimycin to travel from the upstream most antimycin station to the deactivation site each day. Travel times are also useful in developing time lines to bring treatment in a tributary stream and the main stream at the same time.

Equipment Required

- Goggles
- Rubber Gloves
- Rhodamine® WT (20% solution) or Uranine (fluorescene)
- Graduated cylinder
- Transport containers with Zip-loc bag
- Watch or stopwatch
- Flagging/tags
- Measuring tape or hip chain

Potential Interferences

There can be significant loss of Rhodamine® WT to adsorption on clay.

Safety

No special safety precautions.

Procedures

Determine the section of stream for which water travel times are needed and proceed to the upstream end of the segment. Water travel time should be calculated using previously marked stream segments (see TOP 1). Record elevation and segment number where dye travel time estimation will begin. Measure 100ml of dye into a graduated cylinder and pour dye into the stream at the site marker. Record the time the dye was introduced into the stream. Follow the leading edge of the dye downstream recording the time the dye first appears at each tag or flagging at every 100m increment (Figure 11) or at known points. In addition, observers should make detailed notes of any abnormal flow areas along the stream corridor (*i.e.* side channels, meadows, beaver complexes, or anywhere the dye does not appear to mix with stream water). Knowledge of the location of these areas is critical to project success and this information is critical for project planning.

Over time, the dye plume will begin to dissipate and become difficult to see. If it becomes difficult to determine when the dye plume is approaching, you may refresh the plume by adding an additional 100ml of dye and noting the time and location it was added. Continue following the dye plume downstream recording arrival times at each 100m tag downstream to the deactivation site. Once completed, travel times for each 100m segment can be added to study area maps to assist with project planning (TOP 1, Figure 7). In some cases, it may be desirable to collect water travel time from the deactivation site to other areas of interest downstream, such as stream confluences, hatcheries, public areas, or other significant landmarks.

If the stream reach to be treated is longer than 3 to 5 km, water travel time may be calculated in a subset of measured segments. The gradient profile prepared in TOP 1 should be used to determine how many discrete water travel time measurements are necessary to obtain a good estimate of water travel time through the entire treatment reach.

TOP 7 Closure of Treatment Area and Public Notification of Closure

Applicability
Closure is required to preclude non-project personnel from entering the project area.

Principle
Exclusion of non-project personnel from project area eliminates potential for disturbance of project equipment and precludes public contact with the piscicide or the potential consumption of treated fish or water during project implementation.

Authorities
A. Reference will be made to appropriate agency authority for administrative closure of the action area. For example, the authority used for the National Park Service, is Title 36, Code of Federal Regulations, Section 1.5 (Appendix B.4.).

B. Action areas within private property must be posted to ensure adequate public notification.

Procedures
The first step is to advise the public of the planned closure 2 or 3 weeks prior to the treatment. The next step is actual closure of the project area to the public during project implementation. A map delineating the project area, and all access locations to be closed during the project, will be prepared for federal and state sponsored projects. This map will become part of public notice documents. Signs advising the public of project area closures should be posted at all project access points. Access points include, but are not limited to, roads, trails or boat ramps. These locations should be marked on the project area map. Signs will advise the public of closure, duration of closure, and purpose of closure (Appendix B.2). It is recommended that signs advising the public of the upcoming area closure are posted at least 14 days prior to project implementation. Project areas located on private property must be posted to no trespassing to ensure that non-project personnel do not enter the project area.

The land management agency/organization responsible for conducting the project will post the closure schedule and duration. Closures should cite relevant agency responsibilities and mandates (*e.g.* the authority used for the National Park Service, is Title 36, Code of Federal Regulations, Section 1.5) (Appendix B.2).

Public service announcements (except for private lands) should be provided to local media (print and broadcast) outlets in vicinity of project at least 14 days prior to project implementation. Statements should include description of project area, purpose of project, duration of closure, and agencies involved in the project.

Upon completion of the project, all signs for stream applications will be removed within 2 days and within 7 days for lake or impoundment projects.

TOP 8 Conducting a Pre-Treatment Safety Meeting

Applicability

Procedure applies to conducting a safety meeting for all project staff prior to treatment.

Principle

Prior to an antimycin treatment, it is necessary to conduct a safety meeting to ensure that personnel are provided with and have knowledge of and experience with safe handling and use of antimycin A during applications, including use of personal protective equipment. Conformance to the training standards and operating procedures outlined in this document ensures that personnel conduct operations in safe and legal methods. This meeting will identify the project leaders, review safety concerns, identify who issues daily work assignments, and communicate other project topics.

Procedures

Prior to treatment, the project manager(s) should have a meeting with all project staff to review safety and to provide an overview of the project. This meeting covers several key issues and ensures that everyone understands key public and personnel safety concerns, project leaders and contacts, work schedules and assignments, and other important housekeeping items. All safety and project overview attendees should sign a sign-up sheet or safety meeting checklist that can be found in Appendix B.6. Personnel from non-governmental organizations (NGO's) must be signed up as volunteers if they participate in any phase of the project.

Figure 12. A pretreatment antimycin A application safety meeting to discuss project supervision, safety, technique, assignments, and timelines.

Typical antimycin projects involve personnel from several different agencies and pre-approved personnel from NGO's (Figure 12). Begin each meeting with introductions of project staff and prior antimycin experience. Those with prior working knowledge of the treatment process are very useful in assisting others during treatment. After introductions, identify key project leaders, their roles and responsibilities during the project.

If there is a chance members of public may wander into the project area, appropriate procedures to escort them from the project area should be discussed. Specific personnel to deal with such situations should be identified. Other assignments that should be discussed include identifying project leaders, safety officer, antimycin station staff, sprayer operators, deactivation station staff, sentinel fish personnel, biotic and abiotic data collection personnel, and other personnel. Work schedules should be reviewed daily to be sure all staff fully understands their assignments. Personnel safety gear for all staff should be identified and its use reviewed. Personnel safety gear will vary by project assignment.

Communication methods and protocols, both among project personnel and with off-site agency personnel should be reviewed to ensure all personnel know equipment operation and protocols.

Personal and project first aid stations should be identified and discussed to ensure all project personnel are familiar with procedures in case of accident or injury. Material Safety Data Sheets (MSDS) forms and other relevant safety informational materials on treatment compounds should be made available to all project personnel and be available [on site] throughout the project.

The project manager/safety officer, or other appropriate personnel, should review all safety issues or concerns that might be specific to project area. For example, if pack animals are used to transport gear, personnel should be advised of correct behavior around pack animals. Or, if venomous snakes are in project area, personnel should be advised on avoidance and identification methods.

TOP 9 Setup of a Bioassay to Determine a Target Antimycin A Concentration

Applicability

Procedure applies to the determination of the lethal concentration (ppb) of antimycin needed to eliminate all target fish in a 24-hour period. This procedure also applies to the determination of the effective concentration (ppm) of potassium permanganate needed to effectively deactivate antimycin based upon the water chemistry of the project area.

Principle

Application of any pesticide requires a thorough knowledge of the correct application rates in order to effectively treat the target organism (s) and eliminate waste. A bioassay (laboratory or field) is a tool used to define the lethal dosage (LD_{100}) of a piscicide in the environment where the project will be conducted. The Fintrol® label clearly identifies the recommended lethal concentrations of antimycin A required to kill select fish species, however, lethal concentrations for many other species are not provided. Additionally, varying environmental conditions (*e.g.* pH, alkalinity, temperature, stream gradient) can and do affect antimycin efficacy in a stream or lake. A laboratory bioassay using water from the project area or a bioassay conducted on-site will ensure the correct concentration of antimycin is applied and that impacts to non-target organisms will be minimized.

Typical bioassays seek to address three key uncertainties: 1) the concentration in micrograms per liter (µg/L) or parts per billion (ppb) of antimycin and exposure duration needed to kill 100% of the target species in a 24 hour period; 2) the concentration of potassium permanganate in milligrams per liter mg/L or parts per million (ppm), applied to water to deactivate antimycin and minimize or eliminate non-target mortality downstream of the project area and 3) the mortality rates of target species exposed to the desired treatment concentration of potassium permanganate downstream of the project area.

Procedures

There are two methods used to determine the working concentration of antimycin needed for a given project: on-site bioassays and/or laboratory bioassays. On-site bioassays are preferred because stream water chemistry will be identical to that which will be encountered during actual treatment thus eliminating concerns that different environmental conditions may yield different results. If laboratory bioassays are used, investigators should use water from the project area if possible or attempt to mimic the water quality conditions of the target stream. Any project planned for a geographic area where antimycin has not been previously applied must complete a field or laboratory bioassay prior to project implementation. Once completed, the results should be applicable to similar habitat in the study area and bioassays do not have to be repeated. There are several methods for conducting on-site bioassays; methodologies for two streamside toxicity tests are described herein. The methodology and reporting needs for a laboratory trial are identical.

Find a suitable location along the stream which will allow enough space for 8-10 wash tubs or other suitable containers. This number of tubs will provide enough space for three replicates of each target concentration. Fill each wash tub with water (the water in the wash tubs will serve as a water bath for the test buckets during the bioassay). Within each wash tub, place three 5-gallon buckets filled with water (18.9L), place an aerator hose and air-stone in each bucket. Place ten target species in each bucket (a suitable surrogate species may be used). The larger-sized fish found in the population are preferred as these fish tend to be the least sensitive to the treatment. If possible, the test fish should be obtained from the area to be treated, but may be obtained from other sources if necessary. Cover each bucket with mesh netting or some other breathable material to reduce stress on the test specimens.

Figure 13. Setup of streamside bioassay tubs and buckets used to determine the target concentration of antimycin (ppb) and potassium permanganate (ppm)

Three experiments should be performed to evaluate toxicity of antimycin and potassium permanganate to target species. Test # 1 examines the toxicity of different concentrations of antimycin (ppb), test # 2 examines the concentration of potassium permanganate (ppm) needed to deactivate a given concentration of antimycin, and test # 3 examines the toxicity of potassium permanganate prior to deactivation. A control tub (with 3 buckets, each stocked with 10 test animals) should be maintained. Be sure to properly dispose of all test fish upon study completion (TOP 10). An example of the Sams Creek (GRSM) bioassay can be found in Appendix D.

Antimycin Toxicity

Prepare the stock solution #1 by mixing 5ml of antimycin concentrate (20% active ingredient) with 5ml dilutent. The final solution is 10ml at 10% active ingredients or 0.1 ml/ml. Next, mix stock solution #2 by mixing 1ml of stock # 1 in 1 liter stream water. The final solution is 0.0001ml/ml or 100 ppm vol/vol. Note: Stock # 2 is stable for only about 8 hours and must be prepared fresh thereafter.

For concentrations of 2, 4, and 8 ppb, mix and stir in 0.4, 0.8, and 1.6ml of stock solution #2 in each respective set of buckets. Be sure each bucket is appropriately marked and add enough stream water to fill the bucket to the 20 L. One wash tub will serve as a control and receive no antimycin stock solution. Once the appropriate amount of antimycin is added to each bucket, fish should be monitored with observations noted hourly over an eight hour period. Fish that survive the exposure period are placed in holding cages directly in the stream and total percent mortality is recorded at 24 and 48-hours after the initial 8-hour exposure period. Record and summarize results in a summary bioassay table (Table 2) to determine the proper concentration of antimycin and potassium permanganate.

Amount of Potassium Permanganate Needed to Deactivate Antimycin

The bucket setup for test #2 is set up the same as test #1, except three concentrations of potassium permanganate are used in addition to the antimycin. Begin by determining the effective concentration from test #1 (*e.g.* 8ppb) and adding the appropriate amount of antimycin (stock solution #2) in each bucket (*e.g.* 1.6ml) to get the effective concentration. Be sure to have one set of buckets as a control. Next, prepare the potassium permanganate stock solution by adding 1g potassium permanganate to 100 ml of stream water. The resulting stock solution equals 10g/L potassium permanganate. For 1, 2, 3, and 4 ppm of potassium permanganate, add 2, 4, 6, or 8ml of stock solution to the appropriate buckets. Be sure each bucket is appropriately marked and add enough water to fill the bucket for a total of 20L. Once the appropriate amount of, potassium permanganate has been added to each antimycin bucket, mix thoroughly and then let the mixture set for 30 minutes before adding fish. After the fish have been added to the buckets, mortality and behavioral observations are to be recorded hourly for eight hours. Fish that survive the exposure period should be placed in holding cages directly in the stream. Holding cages are checked at 24 and 48-hr intervals and number of dead fish recorded. Record and summarize results in a summary bioassay table (Table 2) to determine the proper concentration of antimycin and potassium permanganate.

Potassium Permanganate Toxicity

The bucket setup for test #3 is set up the same as tests #1 and #2, except only various concentrations of potassium permanganate are used. Be sure to have one set of buckets as a control. Next, prepare the potassium permanganate stock solution by adding 1g potassium permanganate to 100 ml of stream water. The resulting stock solution equals 10g/L potassium permanganate. For 1, 2, 3, and 4 ppm of potassium permanganate, add 2, 4, 6, or 8ml of stock solution to the appropriate buckets. Be sure each bucket is appropriately marked and add enough water to fill the bucket for a total of 20L. Once the potassium permanganate and fish have been added to the buckets, monitor each bucket of fish and record mortality and behavioral observations hourly over an eight hour period. Fish that survive the exposure period should be placed in holding cages directly in the stream and percent mortality recorded at 24 and 48-hours after the initial 8-hour exposure period. Record and summarize results in a summary bioassay table (Table 2 to determine the proper concentration of antimycin and potassium permanganate.

Table 2. Results of a field bioassay conducted on rainbow trout in a GRSM stream.

	% Mortality Through Time (hours)								% Mortality in holding cages	
Antimycin concentration	1	2	3	4	5	6	7	8	24	48
1 ppb	0	0	0	0	0	0	0	0	0	0
2 ppb	0	0	0	0	0	0	0	10	10	20
4 ppb	0	0	0	10	20	20	30	40	40	70
8 ppb	20	20	40	40	50	60	60	80	100	100
0 ppb (Control)	0	0	0	0	0	0	0	0	0	0

Alternatively, on-site bioassays may be conducted within the stream. In-stream bioassays should be conducted only if the test site is a sufficient distance from downstream terminus of project that no active antimycin will be carried downstream of project terminus. TOP 9 provides details on instream bioassays.

In addition to determining if label recommended concentration is appropriate for a project, in-stream bioassays provide information that may be used to determine optimal dispensing station placement, and thus more efficient and safe application of antimycin. For example, if all fish in a holding cage 200-m downstream of the dispensing station die within 48 hrs and a fish survives in the holding cage 300-m downstream of the station, dispensing stations should be placed at 200-m intervals.

TOP 10 Collection, Maintenance, and Disposal of Sentinel Fish

Applicability
Procedure applies to the collection, handling, and disposal of sentinel (bioassay) fish to monitor lethality of antimycin applied to stream or lake.

Principle
Sentinel fish are used to ensure that antimycin stations are placed at appropriate distance to maintain an effective concentration of antimycin to achieve complete elimination of fish between stations. All sentinel fish should be collected, handled, and disposed of properly in order to eliminate unwanted attraction of scavengers.

Sample Collection, Transportation, and Maintenance
Bioassay fish are either collected from treatment area or may be transported in from sources external to treatment area. Species of fish used as sentinels should be species targeted for removal, but congeners may be used.

Equipment Required
- Fish collecting gear (*e.g.* electrofisher, seine, minnow trap, and trammel nets)
- Live cars
- Dip nets
- Fish transport equipment
- Fish transport chemicals (to minimize stress)

Safety
Established safety standards for each type of collecting gear used should be followed.

Procedures
Collection
Sentinel fish may be collected using a variety of sampling methods (i.e. backpack electrofishing, seines, trap nets, etc.) but gear type should be appropriate for habitat. In stream systems, sentinel fish collection is typically accomplished using backpack electrofishing gear. As fish are collected, they are temporarily placed in buckets and then transported to and held in holding cages located outside the project area until needed. Test animals may also be held in coolers filled with water from the project stream. If coolers are used, they should be properly shaded and adequately aerated to reduce stress. Portable backpack storage tanks with aerators are ideal for transporting fish to and from collection, storage, and deployment sites (Figure 14).

Maintenance
Once the desired number of fish is collected, they may be transported to the holding site and held in large holding cages until ready for deployment. Holding sites must be located outside of the treatment area to avoid impacting or killing sentinel fish. For lakes and some stream systems, holding areas may be neighboring streams or lakes which already contain the target species. **DO NOT** use fishless or any other system where the target species is not already present to avoid

accidental introduction. It is best to avoid holding target fish more than 7 days prior to deployment in order to reduce stress and handling mortality.

All sentinel fish should be collected and transported in a manner that minimizes stress. Possible stress factors include temperature, dissolved oxygen, and handling/transport. To limit temperature stress, avoid changes in water temperature during transport or holding of more than 3°C over a 24-h period. To minimize critical drops in dissolved oxygen, change or aerate transport water often and do not place too many fish in transport container. Maintain dissolved oxygen concentration at greater than 5 mg/L or 60% of saturation. Limit transport stress by avoiding overcrowding fish in tanks, limiting transfers from device to device (*e.g.* buckets, trucks, and tanks), or by adding stress reducing compounds. Examples of stress reducing compounds include Stresscoat®, buffered tricainemethylsulfonate, and salt. Each of these reduces "hauling stress" and enhances survival of sentinel fish. All test animals should be transferred to stream water as soon as possible to maximize acclimation time. Test animals should be in stream a minimum of 24 hours before exposure.

Figure 14. A backpack fish transporter and aerator used to transport sentinel fish to and from holding cages and the treatment area.

Disposal

Sentinel fish should be buried off channel.

TOP 11 Sentinel and Wild Fish Observations for Daily Treatment Evaluation

Applicability

This procedure will determine where to physically place holding cages with sentinel fish during treatment and provides the project manager with a tool to evaluate the success of daily treatments. This information coupled with observations of wild fish in the stream will help ensure a thorough treatment of the entire target area for the day and project.

Principle

Currently, on-site procedures and instruments to detect antimycin in the waters of the project area are available but are not practical for real time monitoring of treatment concentrations. Therefore sentinel fish in holding cages (bioassays) and wild fish in the stream are used as bio-indicators to evaluate and monitor the efficacy of the treatment. As the time of exposure duration for antimycin increases, sentinel and wild fish undergo predictable physiological and morphological changes which trained observers can recognize and use to evaluate the progression of each days treatment.

Procedures

Prior to treatment, holding cages should be placed 5-10m upstream of each drip station in operation for that day's treatment. Additionally, holding cages should be placed at a predetermined interval (*i.e.* 100 or 200m) for an additional 1,000m downstream of the downstream lower most downstream treatment station each day of the project. Following this procedure will allow project managers to determine how far downstream of the daily treatment area antimycin eliminated fish and will help determine where antimycin stations need to set for subsequent days. In addition, one holding cage should be placed 5-50m above and one holding cage 100-300m below the deactivation site prior to treatment.

Ten fish should be placed in each holding cage, preferably using larger individuals from the target population or another source that is close to the project area. The date and time fish are placed in the holding cage must be recorded on flagging tape and attached to the cage. Young-of-year (YOY) fish may be used but are not preferred as they are more susceptible to the antimycin than adult fish. However, in situations where sub-adults or adults may not be available nor can be feasibly transported into the project area, YOY fish can be used. A daily log indicating holding cage placement should be maintained. This information is useful to project managers if they need to quickly locate project personnel incase of an accident or spill and will help ensure all project equipment is removed during project cleanup.

During treatment, drip station operators should check the holding cages hourly and record all observations on the antimycin A daily application form (Appendix B.9.). All dead fish should be removed from the cage, properly disposed of (TOP 10), and the date and time noted on the corresponding datasheet. Sentinel fish should remain in the cage until they have fully expired so that project managers can evaluate the progress of each day's treatment and the entire project. When all fish have expired and the project manager is confident that all non-native fish have been eliminated from upstream reaches, remove the cage from the stream. If subsequent treatments are planned, move the cages to the appropriate location downstream for the next day's treatment.

TOP 12 Determining Distance between Antimycin Dispensing Stations

Applicability
This procedure ensures that antimycin stations are placed at the appropriate locations to ensure a uniform concentration of the piscicide throughout the treatment area for the day. This procedure, in conjunction with the placement of sentinel (bioassay) fish, provides the project manager with daily on-site bioassays for the stream, lake or impoundment being treated. This procedure applies to streams being treated in new areas or regions where no data are available.

Principle
Antimycin dispensing stations placed at appropriate locations along the treatment reach ensure that each segment receives the appropriate dosage of antimycin per hour for the prescribed amount of time. The location of each station is determined by the physical characteristics of the area being treated and information obtained from the previous days treatment.

Equipment Required
- Goggles
- Gloves
- Antimycin dispensing buckets w/ accessories
- Antimycin A (formulated end product)
- Graduated cylinders
- Stopwatches
- Exposure containers
- Measuring tape
- Flagging

Safety
Standard field safety procedures are followed. No special safety procedures are required.

Procedures
Determining Vertical and/or Linear Distance between Drip Station(s)
Select a location within the treatment area which can be used to determine the effective distance between antimycin dispensing stations. One of two locations is recommended. The first location is just above the upper end of the target species distribution. This area is recommended as it will determine the effective lethal distance and serve as the initial phase of the project. A second option is to select a reach with higher stream gradients, given that the effective distance of antimycin is typically lower in higher gradient areas. Selection of a reach ≥1 km upstream of the project terminus will enable natural deactivation of antimycin, precluding the need for artificial deactivation.

Once the location is selected, place holding cages with sentinel fish (TOP 10) at 100m intervals for 1km downstream of the station. Adhere to the procedures outlined for station operation in TOP 12 and begin treatment. The station operator and project managers must keep accurate records on fish condition in each holding cage (TOP 10) at one hour intervals.

The target distance between stations (linear or vertical) is determined by the distance from the antimycin dispensing station to the last holding cage in which 100% mortality occurs in 24 hours. This distance will be used to guide the placement of stations for the remainder of the project. Keep in mind that the distance between stations may vary because of the effects of stream gradient, the amount of organic material in the stream, water chemistry or other factors. Daily observations of sentinel fish in holding cages will help the project managers refine the placement of antimycin stations for each day's treatment.

TOP 13 Operation of a Dispensing Station to Apply Antimycin to Streams

Applicability
This procedure ensures that dispensing stations are properly set-up and operated to ensure a uniform concentration of antimycin throughout the treatment area for the day and to ensure necessary data are recorded.

Principle
A properly set dispensing station will deliver the appropriate amount of antimycin for a specified time period.

Safety
Antimycin A is a restricted use pesticide. The pesticide label (Appendix E) and Material Safety Data Sheets (MSDS) (Appendix F) safety precautions must be followed.

Potential Interferences
Several potential interferences have been identified that reduce or eliminate the effectiveness of antimycin A on target fish. These interferences include, but are not limited to, temperature, pH (>8.0), alkalinity, elevated organic load, (e.g. deciduous leaves), and high stream gradient (*i.e.* >8%). (See pertinent literature in Appendix A for additional information.)

Equipment Required
There are several methods for dispensing antimycin at a constant rate to the stream. The particular method selected by an applicator will be based upon on-site conditions, applicator experience and preferences, available equipment, and transport considerations. In addition, to the specific dispenser detailed below, information on other dispensing methods is detailed in literature referenced in Appendix A.

- Drip bucket (18.9L)
- Hose (6')
- Drip pan
- Hammer (3 lb)
- Rebar – 3/8"x2' (2)
- Leveling blocks 1"x1"x1'(2)
- Pitcher (2.5L)
- Torpedo level
- Socket driver or screwdriver
- Mesh filter
- Funnel
- Paper clip

Figure 15. A Operational antimycin dispensing station on Sams Creek, GRSM during treatment in 2000.

Procedures
Antimycin is best applied to the stream from a container (5-gallon or 18.5L containers are frequently used) and delivery device (*e.g.* Farnum float pan or Mariotte bottle principle) that dispenses toxicant at a constant rate (Figure 15). Other devices that provide

a constant flow are also appropriate. According to the label, the maximum single treatment concentration is 25 µg a.i./L. The maximum number of treatments per year is three (3) per project area. The minimum reapplication treatment interval is one (1) day. The maximum annual treatment concentration is 50 µg a.i./L per project area. The maximum daily treatment duration is 8 hours.

Setup

Begin by verifying that all the necessary equipment listed in the equipment list above or for the particular dispensing apparatus chosen are available. After each dispensing location is identified, find the best spot to set the antimycin dispensing bucket. An ideal location is a level stream bank located 1-2m above a shallow mainstream pool, run or riffle (Figure 16). In most cases, a fairly level stream bank is not available. In such instances, use the leveling blocks and any other available items to level the bucket on a rock, bank, or other surface (Figure 16). Be sure the bucket is level and can support 18kg (40 lb) without tipping over. Once the bucket is level, attach the hose to the base of the bucket (outlet) and place the bucket lid on so no debris enters the bucket.

Identify a suitable location just in the stream thalweg just below the drip bucket to set the drip pan (Figure 16). Suitable locations are those which are of shallow to moderate depth (2-20cm) with substrate preferably composed of medium to small gravel or smaller. Hammer both pieces of rebar into the substrate within hose length of the drip bucket. Mount the drip pan to the rebar and attach the hose. If necessary, prop the hose up with branches or other available material so there are no low spots for air to settle into before the water reaches the drip pan.

Sometimes when the bucket is filled with water, air can become trapped in the hose. To remove air from the hose, raise the bucket 0.5-1.5m off the ground and open the valve on the bucket to allow the water to flow through the hose and purge all the air from the line. Once all the air is purged, close the valve on the bucket and set it back on the blocks. Check to be sure the drip pan and bucket are level and the station is ready for operation.

Placement of Upper Antimycin Dispensing Station(s)

Antimycin dispensing station placement should begin just upstream of the upper most end of the target fish distribution. In most cases, placement of the first station should be 100-200m upstream of the upper fish distribution location to ensure the antimycin is completely mixed in the water column when it reaches the target area. When treating tributary streams follow the same procedure. In this situation, use dye travel times to determine when to begin treatment on each tributary so that treated water from each stream segment will “merge” at their confluences at about the same time. The practice of merging treated segments is important in order to maintain exposure levels throughout the treatment area and reduce the opportunity for lapses or gaps which may offer temporary sanctuaries for the target species.

Figure 16. An operational antimycin drip station on Bear Creek, GRSM during the 2003 treatment.

Treatment
Roughly 15 minutes before starting the treatment, prepare the drip bucket by filling it half full of water. The container should be filled with filtered (*e.g.* using a sieve or cheese cloth) water from the stream. Using the graduated cylinder, measure out half of the prescribed antimycin and nonoxyl-9 to the bucket and mix thoroughly with a stirring stick or clean twig. Fill the graduated cylinder with stream water three times and empty the rinseate into the bucket. Place the "dirty" graduated cylinder in the stream to rinse and secure it with a stone or small twig. After adding half the antimycin, add the remainder of the water to fill the bucket to the level needed for four hours (*e.g.* for 4 hours at a rate of 4L/hr, fill to 16L) and mix the solution again. The mixture is now ready (charged) for deployment.

The amount of antimycin needed for each dispensing unit is calculated by the following formulas:

(3**) CFS x 449 (GMP/CFS) x 60(min/hr) x 4 or 8 (Total hours)**
325,800 (gallons/acre foot)

(4) **Acre Feet of Water x 98.4 mls = mls required for 8 ppb**

Approximately 100ml of a tracer dye should be added to the stream when treatment is initiated each day at the first charged station. Treatment will either begin at a specified time (uppermost dispenser) or when the fluorescene dye reaches the next downstream drip station indicating treated water is present. To begin treatment, simply open the valve on the bucket and the antimycin should travel through the hose to the float pan and enter the stream. If there is no stream of antimycin entering the stream, check the drip hole in the drip pan with the paper clip and also check the hose for air. Once filled with treatment solution (charged), each container should be checked at least every 30 minutes to ensure the proper dispensing rate of diluted antimycin (*i.e.* L/hr or gal/hr). Each container must be under the direct control of an applicator, with one applicator monitoring no more than two antimycin stations. Containers should be charged sequentially in a downstream direction. If the unit is running fast or slow, adjust the float inside the drip pan up or down to either speed up or slow down the application rate. Once adjusted, tighten the set screw on the float to ensure a consistent application rate. Using the antimycin application data sheet (Appendix B.7), record bucket stage, water temperature, and sentinel fish observations hourly for the duration of the treatment. Additionally, note the time and amount of antimycin and water that is added to the bucket during the treatment.

Approximately three hours after a dispensing station was turned on, one gallon of the mixture should remain in the bucket. At this point, pour the remaining antimycin into the bucket. Rinse the antimycin bottle three times with stream water, emptying the contents into the bucket. The bottle and graduated cylinder can then be placed in the stream under a stone or limb for additional rinsing or placed in the original Zip-loc bag. Enough water most be added to the bucket to bring the level up to 20L (about 16L). Mix the solution and place the lid back on top of the bucket to keep debris out. At the end of the day, place the goggle and gloves in the Zip-loc bag and return all equipment to the designated cleaning area. Once the treatment is complete, rinse the bucket and hose system in the stream. Collect all the soiled safety gear, graduated cylinder, and antimycin bottle and safely transport them to the washing area.

Evaluate the status of sentinel fish in the holding cages downstream and upstream of each antimycin dispensing station hourly during application and at the 24 hour milestone. Record the physical condition, appearance, and mortality for the fish in each cage on the antimycin station datasheet. The placement of the following days dispensing stations will be based on vertical and/or linear distance between the drip station and the live car in which all fish have died 24 hours after initiation of dispensing. Using this vertical and/or linear distance (m) (TOP 12) and the study area gradient/elevation map (Figure7), place each drip station no more than the determined distance below the drip station immediately above it. The total target treatment segment (km) and/or manpower will determine the number of stations set each day. Caution should be used not to set more stations than personnel can manage. Typically, one person can easily manage two stations along a stream segment given no terrain limitations. The vertical and/or linear distance between each additional downstream drip station should be similar to the effective distance determined during the "on-site bioassay".

TOP 14 Application of Antimycin to a Lake

Applicability

This procedure ensures that antimycin is properly dispensed and a uniform concentration throughout the treatment area for the day and to ensure necessary data is recorded.

Principle

Fish do not detect and avoid antimycin, which provides the applicator an advantage in removing fish from standing waters. However, it is important to use proper dispensing techniques to ensure an even distribution antimycin. In most cases, the injection of antimycin into an outboard motor prop wash through a Venturi pump or boat bailer system will deliver the appropriate amount of antimycin evenly throughout the lake surface. Aerial application of antimycin is not recommended, except for limited spraying of shallow surfaces around the lake perimeter.

Alpine lake treatments have been successful in removing trout with as little as 2 ug/l (25 mls/acre-foot), based upon total volume of lake and applied to the surface of the lake. However, application rates for alpine lakes with low inflow rates and low turn-over rates (inflows equal total lake volume in 30 to 60 days), have ranged from 2-5 ug/l, with 4-5 ug/l used for most alpine lake applications. Treatment rates need to be as high as 8 ug/l in lakes and ponds with pH nearing 8.0, and high turn-over rates (inflows equal total lake volume in one to seven days). Independent of the application rate, planning and application needs to focus on obtaining an even distribution of the chemical throughout at least the surface of the lake, and means of treating the lower water column, if it is demonstrated that fish are concentrated throughout all depths. A battery operated bilge pump can be used to dispense antimycin into deeper areas of the lake.

Selection of the date of treatment should be based upon the spawning of the target species. Overall application should occur prior to spawning, or long enough after spawning to allow fish to develop to at least the swim-up stage.

Safety

Antimycin A is a restricted use pesticide. The pesticide label (Appendix E) and Material Safety Data Sheets (MSDS) (Appendix F) safety instructions must be followed.

Potential Interferences

Several potential interferences have been identified that reduce or eliminate the effectiveness of antimycin A on target fish. These interferences include, but are not limited to, pH (>8.0), alkalinity, elevated organic load, deciduous leaves, and algal blooms.

Equipment Required

Lake treatments will use the stream treating apparatus and kits to treat the inlet and outlets, and sprayer kits to spray the edge of the lake, bogs, shallow areas and seeps. In addition to the equipment previously listed, the following equipment is needed for back-country lake renovations projects:

- Good raft, with floor, oars, oar locks and motor mount
- Outboard motor (>4 hp) gas and oil
- Holding tank for mixing water and chemical, with lid to reduce spill and fumes

- Venturi pump/battery bilge pump system
- Buckets
- Boat/motor tools, including starting fluid, and raft repair kit
- Latex or Nitrile gloves
- Goggles
- Life jackets
- Dip nets
- Rain jackets
- Dip nets
- Sand bags (for blocking outlet flows)

Miscellaneous Equipment

In addition to the above equipment, the following equipment should be available:

- Dye
- Water bags (drinking water)
- Dip nets
- Shovel
- Flagging
- Block Nets
- Rope
- Extra drill bits (52-38)
- First Aid kit
- Radio
- Measuring board and weighing scales (measuring fish)
- Length frequency sheets
- Scales
- Live cages (net bags)
- pH meter
- Extra thermometers
- Extra bottle for antimycin split-out
- Marking pens
- Gill Net and setting rope

Procedures

Boat and Motor

The use of a raft/zodiac with a minimum of a 4 hp outboard motor is recommended for lake applications. Although projects have been completed with the use of electric motors, the use of a 4-5 hp gas motor for lakes in the 2-10 ha range, helps ensure mixing in a variety of conditions (high winds/no wind). Planning lake operations without the use of a gas motor should be considered only in rare circumstances.

Mixing and Application.

Antimycin should be diluted with lake water to at least 1/50 within an on-board holding tank, with the hold tank to include a lid to limit spills and fumes. The antimycin/water mixture should then be injected into the prop wash of the outboard motor, using a venture or battery-powered bilge pump to ensure effective mixing of antimycin across the surface of the lake. It is recommended that antimycin be applied around the perimeter of the lake, and across the lake in multiple grid patterns. A minimum of 0.5 hours per hectare should be spent applying diluted antimycin to help assure an even distribution. Approximately 9-10% of the total antimycin for the lake treatment should be sprayed around the edge of the lake to ensure a lethal concentration around the shoreline, using multiple applications (3 passes of 3%) to ensure thorough mixing. If it has been determined that fish occupy all depths of the lake, the bilge pump can be used to apply antimycin into the lower sections of the lake. In order to check the effectiveness of the prop wash injection system and shoreline spraying, the use of dye mixed with the antimycin mixture will help in adjusting the motor and highlight areas not sprayed in previous passes.

Inlet Streams

It is extremely important to survey and document the distribution of fish in the inlets to the lake system, using the procedures outlined in TOP 1. It is important to remember that some small inlet streams with limited flow may not contain adult fish, but fry may be present.

Generally, the procedures for treating small inlet streams that enter the lake must follow the procedures outlined in TOP 2. However, lakes with inlet flows colder than the surface temperature may attract fish to greater depths than found on the average throughout the lake. To address the attraction of lake fish to inlet streams, consideration should be given to treating inlet streams at about 4 ug/l for up to 24 hours to ensure complete removal of target species.

Outlet Stream

The procedures for treating the outlet stream must follow the procedures described in TOP 12.

Monitoring

Live cages with fish from the lake or nearby stream should be placed at various locations and depths to aid monitoring the progress of the project and the response of the target species as described in TOP 11.

Deactivation of Antimycin

If a barrier exists downstream of the lake and serves as the terminus of the project, deactivation procedures must follow those outlined in TOP 16. If the project area continues downstream for several kilometers from the lake, then the project should follow the procedures outline in AOP 9: Preliminary and Final Project Plan.

In some reservoirs, and even some natural lakes, it may be possible to block the outlet flow of the lake using existing valves or sandbags, thus allowing photo-oxidation and hydrolysis to neutralize the antimycin. This approach must be identified in the Final Project Plan. Project managers must have the necessary detoxification equipment on site in case the temporary dam fails or weather events increase flows. In this event, detoxification must follow the procedures in TOP 16. In addition, applicators must determine if antimycin is detoxified prior to releasing water from reservoir systems.

TOP 15 Use of a Backpack or Hand-Held Sprayer to Apply Antimycin A

Applicability
This procedure will outline the procedures used to safely apply antimycin with a backpack or hand-held sprayer.

Principle
Any areas identified during the dye travel studies where the dye does not mix well are areas that must be treated with a backpack sprayer. Typical areas treated with a backpack sprayer include backwater pools, seeps, side channels, beaver complexes, bogs, wet meadows, and springs along the stream corridor.

Equipment Required
- Goggles
- Rubber gloves
- Waders
- Backpack sprayer (3 gal) with coarse nozzle
- Graduated cylinder
- Pitcher
- Antimycin
- Fluorescene or rhodamine dye (100ml)

Safety
Goggles, gloves, and waders are required when dispensing antimycin using a sprayer. A long sleeved shirt or Tyvek® poncho is recommended to keep any spillage off the operator (Figure 13). To eliminate inhalation of vaporized antimycin, only sprayers with a coarse nozzle are approved for applying antimycin with a backpack sprayer. An 11 to 15L (3 to 4 gallon) backpack sprayer will weigh 11 to 15kg (24 to 33lb). Crew members assigned the duty of backpack sprayer operation should be in good physical condition and able to carry moderate loads (*e.g.* 10 to 20kg) without difficulty.

Procedures
Backwaters, seeps, springs and any other areas along the stream corridor where dye is not mixing should be treated with a backpack sprayer. Backpack sprayers must not be charged with more than 10% vol/vol solution of antimycin, and the total amount of antimycin used in backpack sprayers must not exceed 10% of total amount used daily in all dispensing stations.

Prior to daily treatment, determine the length of treatment reach and total amount of antimycin to be used for that day. The backpack sprayer applicator will spray the entire treatment area and will be assigned ≤10% of the total daily amount of antimycin (ml), which is determined by the project leader, and 50ml of dye. For ease of application, the sprayer typically begins at the upper end of the daily treatment zone and follows the initial dye release downstream in order to identify areas where mixing is not occurring. The sprayer will make two trips through the daily treatment zone dispensing ½ the total allotment of antimycin and dye during each trip.

Immediately after the initial release of antimycin and dye at the upper end of the daily treatment area, the sprayer will measure out half of the allotted antimycin and dye and place it into the sprayer tank. Fill the remainder of the tank with stream water and mix gently with a stirring stick. Securely replace the cap, being sure not to cross-thread the cap, and the tank is ready for application. After donning goggles, gloves, and waders, and backpack sprayer, the applicator begins treatment. To properly treat, follow the dye released by the upper drip station downstream, spraying any area where dye is not readily observed in the stream, such as backwater pools and side channels. The dye in the sprayer will assist the sprayer in assuring all areas are treated. Note problem areas and record location for second sprayer application. Apply moderate amounts of antimycin to all untreated areas keeping in mind the prescribed amount is needed to treat the entire treatment reach. Once the sprayer reaches the end of the daily treatment reach, return to the top of the daily treatment reach to refill and make the second pass through the same section. Once the second spraying pass is complete, return the sprayer to the cleaning area, rinse sprayer with clean water from an untreated tributary or other source, and hang the sprayer to air dry. Be sure to pressurize the sprayer and run clean water through the nozzle to prevent future clogging.

Figure 17.A backpack sprayer applies antimycin to back water eddies during an antimycin project. Note the operator is wearing all required safety gear for safe application of antimycin A with a sprayer.

TOP 16 Deactivation of Antimycin A in a Stream

Applicability
Procedure ensures that the deactivation station is located at the downstream terminus of the treatment reach and that active antimycin does not impact areas outside the project area.

Principle
Prior to treatment, the deactivation station is set up, calibrated, and prepared for operation to preclude any possibility of antimycin impacting areas downstream of the treatment area.

Equipment Required
The particular apparatus for dispensing potassium permanganate will vary, depending on location condition, accessibility, applicator experience and preference. The following equipment list is for one commonly used dispenser:

- Hanging Scale
- 5-gal bucket
- Small boat paddle
- Holding drums
- CDS drip boxes
- Garden hose
- Hose fittings
- Graduated cylinder (1 litre)
- Dust mask
- Goggles
- Chemical resistant gloves
- Long sleeve shirts
- Waders
- Shoes/boots and socks
- Potassium permanganate

Figure 18. The potassium permanganate deactivation station used to neutralize antimycin during a native fish restoration project in GRSM.

Procedures
Locate Suitable Deactivation Setup Area
Once the barrier and study area have been clearly identified, it is important to survey the area to locate a suitable location for the deactivation site. The deactivation site does not have to be located directly below the barrier, but should be located within 100m of the barrier to avoid non-target impacts and should have some of the following characteristics (Figure 18 & 19):

1) level area large enough to hold potassium permanganate dispensing units (typically 5 to 55 gallon containers)
2) level area with at least 8 feet vertical difference from stream
3) fairly accessible for staff from trail or road to transport equipment in/out
4) stream morphology such that you can safely work in the stream to install and calibrate equipment on an hourly basis

> ***Lessons Learned!***
> Once a deactivation site is identified, clearly mark drum and drip box setup areas with flagging so setup crews know where to place equipment when they return.

Setup of deactivation drums

Setup storage tanks or drums on previously selected level streamside surface. Be sure the surface is located ≥ 1 foot vertically above the drip boxes to ensure adequate flow to the drip boxes. If needed, level tanks using boards, rocks, or other means, being sure drums are stabile and will accommodate weight of the water without tipping over. *Safety note*: Water weighs ~1 kg per L, care should be exercised when using drums of ≥ 113.55L (i.e. ≥ 113.6 kg).

Figure 19. A Potassium permanganate deactivation station configuration used to detoxify antimycin during native fish restoration project in Crater Lake NP. Note large drums used for larger stream volume (~15cfs).

`Next, setup saw horses in the stream paralleling the direction of flow. Using wire or rope, secure planks (*e.g.* 1"x 8") about 25 cm apart across the top of saw horses. Secure drip boxes to planks and ensure entire structure is level (Figure 19). Once tanks and drip boxes are level, attach and secure hoses from tanks to drip boxes. If there is considerable distance between the tanks and drips boxes, a support system for the hose may be needed.

Test the fire pump and hose that will be used to fill the drums. Place a small intake hose in the stream and begin filling drums, being sure to use a filter on the intake to reduce uptake of debris. Be sure system works properly as the tanks will need to be filled many times during the operation of the deactivation station. Once the tanks are filled, open valves on drip boxes to test system for stability, leaks, and ensure no air is trapped in the hoses.

Figure 20. An example of the drip box system used to deliver potassium permanganate to a stream to neutralize antimycin A.

It is important to understand that the purpose of the water in the tanks is to act as a delivery mechanism for the potassium permanganate. Once the tanks are set to deliver a fixed volume of water per hour, you can add the amount of potassium permanganate required to deactivate antimycin for the stream discharge measured. Two tanks are an efficient means of delivering the potassium permanganate because: 1) a backup tank is available if primary tank malfunctions, and 2) an uninterrupted flow is maintained because the second tank can operate while the first tank is refilled (Figure 19).

To calibrate the drip boxes, open the valve on one tank and use a large graduated cylinder to measure the amount of water exiting the drip box over a 15 second period. For 56.775L (15 gallons) an hour, 946ml per minute, or 237ml per 15 seconds is needed. If 2 drip boxes are working from 1 tank, these rates are halved (Figure 20). For example, for 56.775L an hour from two drip boxes, about 119ml per 15 seconds must be dispensed from each drip box. If the drip rates are off, turn the valves accordingly to adjust the flow rates. Drip boxes should be re-calibrated every hour to ensure proper functioning.

Data Requirements:

Stream discharge at the site must be determined daily prior to the initiation of deactivation. The hourly amount (g) of potassium permanganate applied (ppm) during deactivation is based upon stream discharge (Table 3). For any given discharge, the weight of potassium permanganate added to each barrel is calculated and recorded on the potassium permanganate data sheet (Appendix B.8).

Table 3. — Antimycin and potassium permanganate application rate table based upon stream discharge (ft^3/sec) and desired application concentration.

	Antimycin			Potassium Permanganate		
Stream CFS	Time	8 ppb	Time	1 ppm g/hr	2 ppm g/hr	4 ppm g/hr
0.5	8 hr	32.5	1 hr	51	102	204
1.0	8 hr	64.9	1 hr	102	204	408
1.5	8 hr	97.4	1 hr	153	306	612
2.0	8 hr	129.9	1 hr	204	408	816
2.5	8 hr	162.4	1 hr	255	510	1,020
3.0	8 hr	194.8	1 hr	306	612	1,224
3.5	8 hr	227.3	1 hr	357	714	1,428
4.0	8 hr	259.8	1 hr	408	816	1,632
4.5	8 hr	292.2	1 hr	459	918	1,835
5.0	8 hr	324.7	1 hr	510	1,020	2,040
5.5	8 hr	357.2	1 hr	561	1122	2,243
6.0	8 hr	389.7	1 hr	612	1224	2,447
6.5	8 hr	422.1	1 hr	663	1326	2,650
7.0	8 hr	455.6	1 hr	714	1428	2,854
7.5	8 hr	487.9	1 hr	765	1530	3,058
8.0	8 hr	520.5	1 hr	816	1,632	3,262
8.5	8 hr	553.2	1 hr	867	1734	3,465
9.0	8 hr	585.9	1 hr	918	1836	3,669
9.5	8 hr	618.6	1 hr	969	1938	3,873
10.0	8 hr	651.2	1 hr	1020	2040	4,077

Cleaning and Maintenance of Deactivation Equipment

Deactivation operators should pay close attention to limit the amount of potassium permanganate sediment build up in hoses and drips boxes. Adding more than 26g potassium permanganate per 1L of water will cause excessive sedimentation and clogging in the drip boxes. Therefore, for 113.55L (30 gallon) drums, deliver 56.775L (15 gallons) per hour up to 26g/L and then switch to 113.55L (30 gallons) per hour delivery when application exceeds 26g/L. Prior to deactivation each day, or if the drip boxes or tanks begin to collect potassium permanganate sediment, flush the tanks and hoses with clean water prior to refilling the tanks for the next round.

TOP 17 Equipment and Safety Gear Cleaning

Applicability
Procedure ensures that all equipment and safety gear used daily during antimycin A treatment is properly cleaned and sanitized or disposed of.

Principle
To ensure project personnel are not exposed to contaminated equipment during treatment.

Equipment
- Alconox detergent
- Sponges
- Graduated cylinder brushes
- Chemical resistant gloves
- Buckets or tubs dedicated to washing equipment in the field
- Clothes line and clothes pins (if necessary)

Procedures
All bottles, gloves, goggles, and graduated cylinders used during antimycin A treatment or deactivation must be returned to a designated washing area to be cleaned daily. Designated washing areas may be a nearby wet laboratory or for remote projects, all antimycin mixing and glassware washing must be done in the field.

Fill two buckets (or wash tubs) with water adding Alconox to one of the buckets. Pre-rinse all equipment and safety gear with water and then place them in the detergent bucket. Wash all equipment and safety gear with either a sponge or brush and place in the rinse bucket. Allow all equipment and safety gear to fully dry on a drying rack or clothesline prior to re-use. Dispose of rinsates on the forest soil at least 100m from any water body or campsite.

Disposal
Original shipment bottles must be completely emptied. Prior to disposal or re-use, these bottles must be triple-rinsed with an oxidizing detergent (*e.g* Alconox®). If bottles will not be re-used for future applications, they should be disposed of in an approved sanitary landfill or offered for recycling.

TOP 18 Documenting Field Deviations from the Protocols

Applicability
Procedures apply to all actions taken preparing for and conducting an antimycin A application as defined in this document.

Principle
All deviations from the protocols including technical operating procedures (TOP's) and administrative operating procedures (AOP's) are recorded to ensure that noncompliance is identified, corrected (where possible), and explained.

Equipment Required
- Pen or pencil
- Protocol Deviation Form (Appendix B.12.)

Procedures
If at any time during the project, a deviation from the any of the stated AOP's or TOP's occurs, the project manager will record such using the protocol deviation form (B. 10). Identify the TOP, AOP, or SOP from which the deviation occurred and record it on the protocols deviation form (Appendix B.12.). Respond to the deviation appropriately and expeditiously to minimize continued/future deviations and potential impacts of the action. Report the deviations in the Final Report on the Application of Antimycin A (see AOP 11).

Steps to Document the Deviation
1. Indicate the TOP, AOP, or protocol(s) from which the deviation occurred.
2. Indicate the date and time the deviation was identified.
3. Indicate the date and time the deviation began.
4. Indicate the date and time the deviation ended or was corrected.
5. Indicate the location of the deviation within the treatment area.
6. Provide a description of the cause and nature of deviation event.
7. Provide a description of the action taken to correct the deviation.
8. Obtain the signature of the Project Manager on the deviation form.
9. Summarize the deviations and the corrective actions.
10. Identify lessons learned or best practices that could potentially prevent future deviations.

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Appendix B.1. Integrated Pest Management Legislation and Policy

Title 7 USC 136r-1 Federal Fungicide Insecticide and Rodenticide Acthttp://www.chemalliance.org/Handbook/background/back-fifra.asp

(http://uscode.house.gov/usc.htm) SEC. 303. Integrated Pest Management states: "The Secretary of Agriculture, in cooperation with the Administrator, shall implement research, demonstration, and education programs to support adoption of Integrated Pest Management. Integrated Pest Management is a sustainable approach to managing pests by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks. The Secretary of Agriculture and the Administrator shall make information on Integrated Pest Management widely available to pesticide users, including Federal agencies. *Federal agencies shall use Integrated Pest Management techniques in carrying out pest management activities and shall promote Integrated Pest Management through procurement and regulatory policies and other activities*".

Department of Interior Manual, Sec.517
Integrated Pest Management Policy: Including the Use of Pesticides and Biological Control Agents**.**

1.1 *Purpose* - The purpose of this document is to incorporate Integrated Pest Management (IPM) in all Department pest management activities. As defined in 7USC136r-1, "*Integrated Pest Management is a sustainable approach to managing pests by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental risks*."

1.2 *Scope* - This chapter applies to all Department and Bureau activities involving planning, procurement, prevention, design, detection, control, and management of native and nonnative pest species on DOI lands and properties.

Public Contracts and Property Management, Facility Management, 2001 Code of Federal Regulations (C.F.R.) Title 41, Volume 2, 102.74.35, directs executive agencies to provide IPM services.

Federal Register, July 19, 2000, Standard Concession Contract Language directs concession managers to use an integrated pest management program to manage weeds, harmful insects, rats, mice and other pests on Concession Facilities and that weed and pest management activities shall be in accordance with Applicable Laws and guidelines established by the Director."

National Park Service Management Policies 2006

4.4.5.2 Integrated Pest Management Program

The Service conducts an integrated pest management (IPM) program to reduce risks to the public, park resources, and the environment from pests and pest- related management strategies. IPM is a decision- making process that coordinates knowledge of pest biology, the environment, and available technology to prevent unacceptable levels of pest damage, by cost- effective means, while posing the least possible risk to people, resources, and the environment.

Appendix B.2. 11 Step Process to Developing and Implementing an Integrated Pest Management Strategy

1. Describe your site management objectives and establish short and long term priorities.
2. Build consensus with stakeholders-occupants, decision makers and technical experts (ongoing).

3. Document decisions and maintain records.

4. Know your resource (site description and ecology).

5. Know your pest. Identify potential pest species, understand their biology, and conditions conducive to support the pest(s) (air, water, food, shelter, temperature, and light).

6. Monitor pests, pathways, and human and environmental factors, including population levels and phenological data.

7. Establish “action thresholds,” the point at which no additional damage or pest presence can be tolerated.

8. Review available tools and best management practices. Develop a management strategy specific to your site and the identified pest(s). Tools can include: 1) no action, 2) physical, 3) mechanical, 4) cultural, 5) biological, and 6) chemical management strategies.

9. Define responsibilities and implement the lowest risk, most effective pest management strategy, in accordance with applicable laws, regulations, and policies.

10. Evaluate results; determine if objectives have been achieved; modify strategy if necessary (adaptive management).

11. Education and outreach. Continue the learning cycle, return to Step 1.

Appendix B.3. Example of an antimycin project managers planning and implementation checklist. The checklist provides a general outline of the key tasks and considerations of the project manager for any antimycin project.

____ Validate and Geo-Reference Barrier
____ Work through all NEPA requirements prior to project planning and implementation
____ Acquire proper permits from state water quality enforcement division (*e.g.* NPDES, 404, etc.)
____ Acquire Pesticide Applicator Permit from appropriate state (FIFRA requirement)
____ Meet with local resource groups to educate and solicit volunteer assistance where necessary
____ Determine species distribution in all waters above barrier (flag upper ends)
____ Determine if any special fishing opportunities will be offered prior to treatment
____ Purchase & build appropriate supplies & equipment for treatment
____ Test all equipment to be sure it works and application rates are set accordingly
____ Measure Out & tag treatment area
____ Determine & mark elevations for each site
____ Establish fish monitoring Sites
____ Establish discharge sites throughout treatment area
____ Construct site map of treatment area including site numbers, tributaries, elevations, dye times, discharge sites, fish sites, & landmarks
____ Print signs and other education material to support closure of treatment area
____ Meet with in-house and neighboring law enforcement officials to inform and request any support for project
____ Set location and take hose measurements for detox station
____ Determine how to transport all gear into treatment & detox area
____ Record preliminary round of discharge and dye time data
____ Implement OPEN FISHING portion of project (optional)
____ Conduct pre-treatment fish monitoring
____ Post "CLOSED TO PUBLIC" signs just prior to project start-up
____ Redo upper stream discharges at upper end of treatment area to prepare for first day

Appendix B.4. Example of a National Park Service close order sign including federal authorities and Superintendents signature under which such closures are authorized.

NOTICE

TRAIL CLOSURE

The following area is closed to public entry from 8:00 PM September 11, 2005 until 8:30 PM September 16, 2005.

The Middle Prong Trail will be closed from the junction with Panther Creek Trail up to its junction with the Lynn Camp Prong Trail and this trail will be closed to it's junction with the Mirey Ridge Trail. The Greenbrier Ridge Trail will be closed from its junction with the Lynn Camp Prong Trail to the Appalachian Trail from 6:00 PM September 11, 2005 until 8:30 PM September 16, 2005.

Please refer to the project map on the board that shows these closures.

During this time, a native brook trout restoration project will be conducted in a 1.5 mile segment of Indian Flats Prong.

This closure is necessary "for the maintenance of public health and safety… and aid to scientific research " [36 CFR 1.5. (a) (1)].

By Order of the Superintendent, per authority vested in Title 36, Code of Federal Regulations, Section 1.5 (a) (1)

________________________ **Date ________________**

Dale Ditmanson
Superintendent

Appendix B.5. Example of Great Smoky Mountains National Park stream discharge sheet.

Stream Discharge Datasheet

Stream: ____________________ Date: ____________________

Watershed: ____________________ Station: ____________________

Cell Width: (circle one) Fixed ______________ ft Variable ______________ ft

DISTANCE ON TAPE (feet)	DEPTH (feet)	VELOCITY (feet/second)	CELL VOLUME (cubic ft/sec)
			1
			2
			3
			4
			5
			6
			7
			8
			9
			10
			11
			12
			13
			14
			15
			16
			17
			18
			19
			20

grsmflow.xls

DISCHARGE (cfs) = ____________________

Appendix B.6. An example of a pre-treatment safety meeting checklist and personnel signup datasheet..

Safety Meeting Checklist & Sign-Up Sheet

Stream Name: ______________________________________ Date: __________________

Introduction & Project Command/Control

- ☐ Introduction of Team & Affiliation
- ☐ Project Coordinators and responsibilities

Antimycin Properties

- ☐ Properties of Antimycin
- ☐ PPE (Use, technique, cleaning procedures)
- ☐ MSDS location
- ☐ Exposure to eyes or skin

Antimycin Station Use & Safety

- ☐ Safety
- ☐ Station Setup
- ☐ Common Problems & Fixes
- ☐ Data Recording
- ☐ End of Day & Cleanup

Potassium Permanganate Station Use & Safety

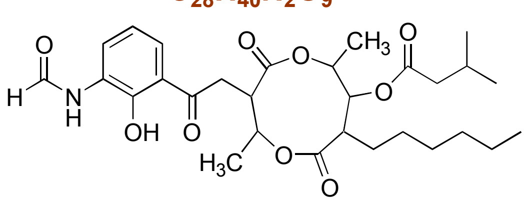
- ☐ Properties of $KMnO_4$
- ☐ PPE (Use, technique, cleaning procedures)
- ☐ MSDS location
- ☐ Exposure to eyes or skin

Potassium Permanganate Station Use & Safety

- ☐ Safety
- ☐ Station Setup
- ☐ Common Problems & Fixes
- ☐ Data Recording
- ☐ End of Day & Cleanup

Antimycin A_1

$C_{28}H_{40}N_2O_9$



	Attendee	Agency
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
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14		
15		
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Appendix B.7. Antimycin station equipment checklist and vendor contact information.

Antimycin Station Equipment Checklist

Item	Quantity	Item #	Vendor [1]	Cost per Unit	Notes
Plastic 5-gal Bucket	4	13058	1	$13.50	
Plastic 10-gal Bucket	4	25730	1	$25.10	
Male PVC Top Cap w/ Threaded Cap (1-1/4'	8		3 or 6		Used to hold funnel and fill bucke
Wood Blocks (2"x2"x1')	4		3 or 6		Keeps level
90° Through Hull Fitting (3/4")	4	SZ-01-2946	4	$1.99/ea	
Rubber Gasket (1" hold, 2-1/4" wide, 1/8")	4		3 or 6		
Two-Way Valve	2		3 or 6		
Mesh Reinforced Garden Hose (6')	80 feet		3 or 6		Reinforced hose is best
Hose couplings	12		3 or 6		
Hose Clamps (3/4-1")	24		3 or 6		
Garden Hose Male Thread	12		3 or 6		
Wooden Dowels (0.5"diam x 12" length)	8		3 or 6		Stirs water/antimycin in bucket
Farnam Floats Waterer for Dogs	8	30775	2	$18.95	
Wood or Plexiglass Lid for Waterer	8		3 or 6		Keeps debris out of waterer
Screwdriver or socket	1		3 or 6		Tightens rebar and hose clamps
Funnel (6")	1	95925	1 or 8	$11.50	
Mesh Filter or Red Devil Nylon Bag Straine	2		3 or 6		All bucket water should be filtere
Goggles or Safety Glasses	2	11566-2	2 or 7	$2.40/ea	
Gloves	2	105393L	2 or 7	$9.80/100	
Torpedo Level	8		3 or 6		Keeps waterer level
Paper Clips	36		Misc.		Used to unclog waterer outflow
Rebar (1/2" x 24")	24		3 or 6		
5lb Hammer	8		3 or 6		
Plastic Graduated Pitcher (2-ℓ)	12	02-543-36D	8	$81.36 per 12	
Graduated Cylinders (100ml)	8	47312	1 or 8	$4.70/ea	
Graduated Cylinders (250ml)	8	47313	1 or 8	$5.90/ea	
Amber Widemouth Bottles (250ml)	24/case	13091	1 or 5	$90.40	Widemouths easier to use & clear
Amber Widemouth Bottles (500ml)	12/case	28794	1 or 5	$80.60	Widemouths easier to use & clear
Ziploc Bags (1-gal)	96		3 or 6		For Gloves/Goggles & Bottles
Flourescent Dye	1-gal		8		
Handheld Thermometers	12		8		
Solo Sprayer (2.5 gal)	1		8		Smaller tank easier on slopes
Nalgene Bottles (250ml)	12	88333	1 or 5	$39.20	Transport dye for trials & stations
Bic Lighters	12		3 or 6		To warm NoNox-9
Misc. Garden Hose Gaskets, Fittings, Screen	Extras		Misc.		Always Handy

[*] January 2004 Costs

[1] *Vendor Information*

Index	Vendor Name	Phone Number	Web Site
1	Lab Safety Supply	(800) 356-0783	www.labsafety.com
2	Valley Vet	(800) 419-9524	www.valleyvet.com
3	Lowe's Home Improvement		www.lowes.com
4	Cabela's	(800) 237-4445	www.cabelas.com
5	Fisher Scientific		https://www1.fishersci.com/index.jsp
6	Local Hardware Store		
7	Northern Safety Supply, Inc.	(800) 631-1246	www.northernsafety.com

Appendix B.8.. Potassium permanganate equipment checklist and vendor contact information

Potassium Permanganate ($KMnO_4$) Station Equipment Checklist

Item	Quantity	Item #	Vendor [1]	Cost per Unit	Notes
$KMnO_4$ Powder (97%)	200 lb	CAIROX FF	1	$1.98/lb	Transport in smaller buckets
30 Gallon Drums	2	3972	2	$66.60/ea	
Wood Blocks (4"x4"x2')	4	-	3 or 6		
Small Boat or Kayak Paddle	1	-	Misc.		
90^o Through Hull Fitting (1-1/8")	4	SZ-01-2946	4	$2.99/ea	
Rubber Gasket	4	-	3 or 6		
Two-Way Valve	2	-	3 or 6		
Mesh Reinforced Hose (1-1/4 inch)	80 feet	-	3 or 6		Reinforced hose works better
Hose couplings (1-1/4 inch)	12	-	3 or 6		
Hose Clamps (1 to 1-1/2 inch)	24	-	3 or 6		
"T" Hose Fitting (1-1/4")	2	-	3 or 6		
Screwdriver or socket	1	-	3 or 6		
Utility Knife	1	-	3 or 6		
CDS Float Box (2-gal)	4	-	5	$68/ea	Use two at once & two for backup
Saw Horses	2	-	3 or 6		
Pressure Treated Boards (1"x6"x8')	2	-	3 or 6		
Malleable Wire	30 ft	-	3 or 6		
Dykes	1	-	3 or 6		
Mesh Filter	2	-	3 or 6		
Goggles or Safety Glasses	2	11566-2	2 or 7	$2.40/ea	
Gloves	2	105393L	2 or 7	$9.80/100	
Particulate Mask Filter	1	38024	2 or 7	$26.30/10	
Graduated Cylinders (500ml)	1	47321	1 or 8	$13.30	
Graduated Cylinders (1l)	1	47322	1 or 8	$16.60	
Waders (size to fit operator)	1	-	8		
Watch (w/second hand)	1	-	Misc.		
Pesola Hanging Scale (2.5kg x 20g)	1	93016	8	$39.90/ea	
Plastic Scoop (200ml)	1	77008	2	$18.20/ea	
Plastic 5-gal Bucket	1	-	8		
Small Gas Fire Pump	1	-	Misc.		A BIG HELP if available!
Hose for Small Gas Fire Pump (1-inch)	100 ft	-	Misc.		
Fire Pump Couplings (Pump to Hose)	as needed	-	Misc.		
Gas for Fire Pump 50:1)	1-gal	-	Misc.		
Electic Drill w/socket bits	1	-	Misc.		Optional - but VERY HELPFUL!

* January 2004 Costs

[1] *Vendor Information*

Index	Vendor Name	Phone Number	Web Site
1	CARUS Chemical Company	(815) 223-1500	www.caruschem.com
2	Lab Safety Supply	(800) 356-0783	www.labsafety.com
3	Lowe's Home Improvement		www.lowes.com
4	Cabela's	(800) 237-4445	www.cabelas.com
5	Schall Chemical Company	(719) 852-5938	
6	Local Hardware Store		
7	Northern Safety Supply, Inc.	(800) 631-1246	www.northernsafety.com
8	Forestry Suppliers	(800) 647-5368	www.forestry-suppliers.com

Appendix B.9. An example of an Antimycin A daily application form.

ANTIMYCIN DOSAGES AND TEMPERATURE FORM

Stream Name: ______________________

Operator: ______________________ Date: ______________________

Station #: ______________________

Flow (cfs): ______________________

Concentration: ______________________ Time Starting: ______________________

Duration: ______________________ Time Ending: ______________________

ml Antimycin/hr: ______________________

! ! *PLEASE RECORD* Time, Water Temp., Comments/Observations, and Fish Status hourly below ! !

Time	Water Temp	Antimy. added	Comments or Observations	Fish Status

*** Please bring your goggles, gloves, bottles, and this datasheet out with you at the end of the day!**

Appendix B.10. – An example of a potassium permanganate daily application form.

$KMnO_4$ APPLICATION AND TEMPERATURE FORM

Stream Name: ______________________

Operator(s): ______________________ Date: ______________

Stream Flow (cfs): ______________________

g KMnO4 per hr: ______________________

ppm KMnO4: ______________________ Time Starting: ______________

Duration: ______________________ Time Ending: ______________

Remember : Adjust flow rates so NOT to adding more than 100g KMnO4 per gal.
Stir mix every 30 minutes to prevent settling in drums and hoses.

! ! *PLEASE RECORD* Time, Water Temp., Comments/Observations, and Fish Status hourly below ! !

Time	Water Temp	$KMnO_4$ added	Comments or Observations	Fish Status

*** Please bring this datasheet out with you at the end of the day!**

Appendix B.11. An example of a antimycin and potassium permanganate application summary sheet.

Antimycin and $KMnO_4$ Application Table

Station	ppb	Flow (cfs)	TOTAL Hours	TOTAL cfs	Antimycin (ml)	***F***	Elevation	***E***	Range Temp (^{o}C)	Time Treated	pH	Staff	Equipment	Comments
Spray														
TOTAL														
KMnO4														

Notes:

Appendix B.12.

PROTOCOL DEVIATION FORM

Treatment/Project Name: ___________________________________

Description and Cause of Deviation: ____

____

____

SOP(s) Violated: AOP ___________________________________
TOP ___________________________________

Event Timing and Location
Start Date/Time of Deviation (e.g., 01/01/2007, 3:30 PM): ______________________________
End Date/Time of Deviation: ________________________________
Identification Date/Time of Deviation: ________________________
Location of Deviation (include site number or short description): ______________________

Reason(s) for Deviation (check all that apply):

- ☐ To protect worker/bystander safety
- ☐ To prevent release of antimycin A beyond the treatment area
- ☐ To prevent violation of Federal or other regulation(s)
- ☐ To maintain efficacy of treatment
- ☐ Other (describe):____

Action(s) Taken to Minimize Continued/Future Deviations and Potential Impacts

____

____

____

____

Project Manager Signature

____

Signature Date

____

Project Manager Printed Name

____

Appendix C. A summary of case history of antimycin and potassium permanganate dosage data of various fish species from throughout the country.

Bioassay Case Study 1: Sams Creek, Great Smoky Mountains National Park (GRSM), Tennessee. October 1998, pH 6.6-6.7, conductivity 15Ms/cm, temperature 14-15.5°C – Bioassays conducted in GRSM indicated 8ppb antimycin applied for 8 hours resulted in a 100% mortality rate of rainbow trout after 24 hours. Using 3ppm potassium permanganate and 8ppb antimycin, rainbow trout mortality was 0% after 48 hours.

Bioassay Case Study 2: Snake Creek, Great Basin National Park (GRBA), Nevada. July 2002, pH 7.8-7.9, conductivity 187 , temperature 7.8-7.9°C – Bioassays conducted in GRBA indicated 2,4, and 8ppb antimycin applied for 8 hours resulted in a 100% mortality rate of rainbow trout after 24 hours. Using 4ppm potassium permanganate and 8ppb antimycin, rainbow trout mortality was 30% after 24 and 48 hours.

Table C.1. List of antimycin treatment concentration and exposure period estimated to result in 100% mortality of various fish species across the United States. Note, exposure period reflects the time period when antimycin treatment area was maintained at the treatment concentration.

Family	Target Species	Scientific Name	Water Temp. (°C)	pH	Exposure Time (hrs)	LD_{100} (ppb)	Citation
Atherinidae	Brook Stickleback	*Labidesthes sicculus*	5	7.5	8	7.5	Gilderhus *et al.*1969
Catastomidae	White Sucker	*Catastomus commersoni*	5	7.5	8	7.5	Gilderhus *et al.*1969
	Northern Hogsucker	*Hypentelium nigricans*	10-18	8.3	15	5	Gilderhus *et al.*1969
Centrarchidae	Bluegill	*Lepomis macrochirus*	20	8.8	10	10	Gilderhus *et al.*1969
	Largemouth Bass	*Micropterus salmoides*	20	8.8	10	10	Gilderhus *et al.*1969
	Black Crappie	*Pomoxis nigromaculatus*	20-22	8.4	8	7.5	Gilderhus *et al.*1969
Cottidae	Mottled Sculpin	*Cottus bairdi*	10-18	8.3	15	5	Gilderhus *et al.*1969
Cyprinidae	Central Mudminnow	*Umbra limi*	20-22	8.4	8	7.5	Gilderhus *et al.*1969
	Central Stoneroller	*Campostoma anomalum*	10-18	8.3	15	5	Gilderhus *et al.*1969
	Common Carp	*Cyprinous carpio*	25	8.6	10	10	Gilderhus *et al.*1969
	Fathead Minnow	*Pimphales promelas*	20	8.8	10	10	Gilderhus *et al.*1969
	Blacknose Dace	*Rhinichthys atratulus*	10-18	8.3	15	5	Gilderhus *et al.*1969
	Longnose Dace	*Rhinichthys cataractae*	10-18	8.3	15	5	Gilderhus *et al.*1969
	Creek Chub	*Semotilis atromaculatus*	10-18	8.3	15	5	Gilderhus *et al.*1969
Percidae	Fantail Darter	*Etheostoma flabbellare*	20	8.8	10	10	Gilderhus *et al.*1969
	Johnny Darter	*Etheostoma*	5	7.5	8	7.5	Gilderhus *et*

		nigrum					*al*.1969
Petromyzontidae	American Brook Lamprey	*Lampetra appendix*	5	7.5	8	7.5	Gilderhus *et al*.1969
Poecilidae	Western Mosquitofish	*Gambusia affinis*	28.3	6.9	48	0.6	Lennon and Berger 1970
Salmonidae	Rainbow Trout	*Oncorhynchus mykiss*	14.0	6.6	8	8	Moore and Kulp 2005
	Brook Trout	*Salvelinus fontinalis*	7.9	7.8	8	8	Moore and Kulp 2005
	Brook Trout	*Salvelinus fontinalis*	9-16	7.4	10	8	Gresswell 1991
	Brook Trout	*Salvelinus fontinalis*	5-8	6.5-7.0	10	6	Rinne *et al.* 1981
	Brook Trout	*Salvelinus fontinalis*	12	7.5	5	7	Stevens and Rosenlund 1986

Appendix D. The antimycin toxicity report for Sams Creek, Great Smoky Mountains National Park in 1998.

Sams Creek Toxicity Test Report
Great Smoky Mountains National Park
October 26, 1998

Procedure

Toxicity tests were performed streamside, next Sams Creek, using water directly from the stream. Tests were conducted from October 19 - 22, 1998. Fish were collected and held approximately 15 hours prior to tests. Ten rainbow trout (*Oncorhynchus mykiss*) were exposed to concentrations of antimycin and $KMnO_4$ for eight hours and observed throughout this period. Fish that survived the exposure period were placed in holding cages directly in the stream and percent mortality was recorded at 24 and 48-hour intervals after initial 8-hour exposure period. Three experiments were performed to evaluate toxicity of antimycin and $KMnO_4$ to rainbow trout. Test # 1 examines the toxicity of antimycin, test # 2 indicates the amount of $KMnO_4$ needed to detoxify antimycin, and test # 3 examines the toxicity of $KMnO_4$.

I. Antimycin solutions.

A. Stock # 1. Mix 5 mls of antimycin concentrate (20% active ingredients) with 5 mls dilutant. Final solution is 10 mls at 10% active ingredients or 0.1 mls/ml.

B. Stock # 2. Mix 1 ml Stock # 1 in 1 liter Sams Creek water. Final solution is 0.0001-mls/ml or 100 ppm vol/vol. Note Stock # 2 is stable for only about 8 hours and must be prepared fresh thereafter.

C. Exposure Concentrations. For antimycin exposure concentrations tested the following amounts of Stock # 2 was added to five-gallon buckets to make 20 liters

Exposure Concentration	Stock # 2 added
2 ppb	0.4 ml
4 ppb	0.8 ml
8 ppb	1.6 ml

II. $KMnO_4$ solutions.

D. Stock Solution. Add 1 gram $KMnO_4$ to 100 mls water. The resulting stock solution equals 10g/l $KMnO_4$.

E. Exposure Concentrations. The following amounts of $KMnO_4$ were added to five-gallon buckets to make 20 liters.

Exposure Concentration	$KMnO_4$ stock solution added for 20 liters.
1 ppm	2 ml
2 ppm	4 ml
3 ppm	6 ml
4 ppm	8 ml

III. Test Fish.

Test fish were adult rainbow trout from Sams Creek. Mean length was 161.8 millimeters and mean weight was 41.6 grams. Ten fish were added to each 20-liter test solution. There was no significant difference between mean total length within each group of fish tested ($P>0.05$).

IV. Dilution Water.

Dilution water was Sams Creek water which ranged in pH from 6.7-6.6 and was unaffected by the additions of antimycin and $KMnO_4$. Temperatures ranged from 14.5-15° C and were maintained by a water bath of ambient temperatures from Sams Creek.

A. Test Results Test # 1. Toxicity of antimycin in Sams Creek water.

Antimycin concentration	% Mortality Through Time (hours)								% Mortality in holding cages	
	1	2	3	4	5	6	7	8	24	48
0 ppb (Control)	0	0	0	0	0	0	0	0	0	0
2 ppb	0	0	0	0	0	0	0	10	100	100
4 ppb	0	0	0	0	0	10	10	20	100	100
8 ppb	0	10	10	20	20	30	60	90	100	100

B. Test # 2. $KMnO_4$ demand in Sams Creek water.

Antimycin concentration	$KMnO_4$ concentration	% Mortality Through Time (hours)								% Mortality in holding cages	
		1	2	3	4	5	6	7	8	24	48
8 ppb	1 ppm	0	0	0	0	0	0	0	0	10	10
8 ppb	2 ppm	0	0	0	0	0	0	0	0	0	0
8 ppb	3 ppm	0	0	0	0	0	10	10	10	10	10
8 ppb	4 ppm	30	50	50	50	50	50	50	50	50	50
0 ppb	0 ppm (Control #1)	0	0	0	0	0	0	0	0	0	0
8 ppb	0 ppm (Control #2)	0	0	0	0	30	80	100	100	100	100

C. Test # 3. $KMnO_4$ toxicity in Sams Creek water.

$KMnO_4$ concentration	% Mortality Through Time (hours)								% Mortality in holding cages	
	1	2	3	4	5	6	7	8	24	48
1 ppm	0	0	0	0	0	0	0	0	0	30
2 ppm	0	0	0	0	0	0	0	0	0	0
3 ppm	0	0	0	0	0	0	0	0	0	0
4 ppm	20	20	20	20	20	20	20	20	30	40
0 ppm (Control)	0	0	0	0	0	0	0	0	0	0

VI. Discussion.

At 24 hours 100% mortality was reached in all fish exposed to concentrations of antimycin. In Test # 1, 90% mortality was the maximum within the 8-hour exposure period at 8-ppb antimycin. These results conflict with control # 2 (8-ppb antimycin: 0-ppm $KMnO_4$) in Test # 2 where 100 % mortality was reached within the 8 hour exposure period.

Two-ppm was the only concentration of $KMnO_4$, used to detoxify antimycin in Test # 2, to generate 0% mortality. One and 3-ppm $KMnO_4$, used to detoxify antimycin, produced only 10% mortality. This occurred after the initial exposure period in 1-ppm $KMnO_4$. Four-ppm $KMnO_4$

had 40% mortality. This shows that 4-ppm $KMnO_4$, initially, has some sort of toxicity. The same concentration caused initial mortality in Test # 3 also.

Two and three-ppm $KMnO_4$ produced 0% mortality in Test # 3. No concentrations of $KMnO_4$ caused 100% mortality while 4-ppm $KMnO_4$ caused 40% mortality. However, the highest mortality beyond 4-ppm $KMnO_4$ was 1-ppm at 30% mortality.

Appendix E. A copy of the Fintrol® (antimycin) product label

FINTROL CONCENTRATE
PRECAUTIONARY STATEMENTS
Hazards to Humans and Domestic Animals

WARNING: May be fatal if swallowed or absorbed through skin. Causes substantial but temporary eye injury. Causes skin irritation. Do not breath spray mist. Do not get in eyes, on skin or on clothing. Wear protective goggles. Wear chemical gloves. Wash thoroughly with soap and water after handling and before eating, drinking or using tobacco. Remove contaminated clothing and wash before reuse.

Environmental Hazards
This product is very highly toxic to fish
Physical or Chemical Hazards

Extremely Flammable: Keep away from fire, sparks and heated surfaces.

FIRST AID: IF SWALLOWED: Call a physician or Poison Control Center. Drink 1 or 2 glasses of water and induce vomiting by touching back of throat with finger. If person is unconscious, do not give anything by mouth and do not induce vomiting.

IF INHALED: Remove victim to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention.

IF ON SKIN: Wash with plenty of soap and water. Get medical attention.

IF IN EYES: Hold eyelids open and flush with a steady, gentle stream of water for 15 minutes. Get medical attention.

STORAGE AND DISPOSAL
Do not contaminate water, food or feed by storage or disposal.

Storage: Store only in original containers, in a dry place inaccessible to children and pets. Fintrol Concentrate will thicken if stored at temperatures below 65 F. Before use store overnight above 70 F. Fintrol Concentrate is stable for a minimum of 3 years when stored in unopened original glass bottles.

Pesticide Disposal: Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of federal law. If these wastes cannot be disposed of by use according to label instructions, contact your state pesticide or environmental Control Agency or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.

Container Disposal: Triple rinse (or equivalent). Then dispose of in a sanitary landfill or by other approved state and local procedures.

RESTRICTED USE PESTICIDE
Due to Aquatic Toxicity & Need for Highly Specialized Applicator training.
For retail sale to, and use only by, Certified Applicators, or persons under their direct supervision, and only for those uses covered by the Certified Applicators' Certification.

FINTROL® FISH TOXICANT KIT

(contains Fintrol Concentrate and Fintrol Diluent)

This can contains 1 bottle of FINTROL-Concentrate and 1 bottle of Fintrol-Diluent.

FINTROL CONCENTRATE (8 fl. Oz.)		
Active Ingredients		
Antimycin A	23%	w/w
Inert Ingredients		
Soy lipids	15%	
Acetone	62%	
	100%	w/w

FINTROL DILUENT (8 fl. Oz.)		
Inert Ingredients		
Diethyl Phthalate (surfactant)	30.5%	w/w
Nonoxynol-9 (detergent)	16.7%	
Acetone	52.8%	
	100.0%	w/w

AQUABIOTICS CORP. P.O. BOX 10578. Bainbridge Island, WA 98110
E.P.A. Reg. No 39096-2 E.P.A. Est. No 39096-WA-01

WARNING

Keep out of reach of children
See side panel for other Precautionary Statements.

DIRECTIONS FOR USE
It is a violation of federal law to use this product in a manner inconsistent with its labeling.
See "USE DIRECTIONS LEAFLET" for Fintrol (Antimycin A) Fish Toxicant Kit

FINTROL DILUENT
PRECAUTIONARY STATEMENTS
Hazards to Humans and Domestic Animals

CAUTION: Harmful if swallowed. Harmful if inhaled. Harmful if absorbed through skin. Causes moderate eye irritation. Avoid contact with skin and clothing. Do not breath spray mist. Do not get in eyes, on skin or on clothing. Wear protective goggles. Wear chemical gloves. Wash thoroughly with soap and water after handling and before eating, drinking or using tobacco. Remove contaminated clothing and wash before reuse.

Physical or Chemical Hazards

EXTREMELY FLAMMABLE: KEEP AWAY FROM FIRE, SPARKS AND HEATED SURFACES.

FIRST AID

IF SWALLOWED: Call a physician or Poison Control Center. Drink 1 or 2 glasses of water and induce vomiting by touching back of throat with finger. If person is unconscious, do not give anything by mouth and do not induce vomiting.

IF INHALED: Remove victim to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention.

IF ON SKIN: Wash with plenty of soap and water. Get medical attention.

IF IN EYES: Hold eyelids open and flush with a steady, gentle stream of water for 15 minutes. Get medical attention.

STORAGE AND DISPOSAL
Do not contaminate water, food or feed by storage or disposal.

Storage: Store only in original containers, in a dry place inaccessible to children and pets. Fintrol Concentrate will thicken if stored at temperatures below 65 F. Before use store overnight above 70 F. Fintrol Concentrate is stable for a minimum of 3 years when stored in unopened original glass bottles.

Pesticide Disposal: Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of federal law. If these wastes cannot be disposed of by use according to label instructions, contact your state pesticide or environmental Control Agency or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.

Container Disposal: Triple rinse (or equivalent). Then dispose of in a sanitary landfill or by other approved state and local procedures.

Appendix F. The Material Safety Data Sheet (MSDS) for Fintrol® (antimycin).

MATERIAL SAFETY DATA SHEET

Antimycin A in Acetone   Issued 01/01/99

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Material Name: Antimycin A in Acetone
MANUFACTURER: Aquabiotics Corp.
10750 Arrow Point Drive
Bainbridge Island, WA 98110
TELEPHONE NUMBER: 1-206-842-1708
FAX NUMBER 1-206-842-7266

2. COMPOSITION/INFORMATION ON INGREDIENTS

INGREDIENT NAME: Acetone *
CONCENTRATION: 80.0000%
CAS/RTECS NUMBERS: 67-64-1 / AL3150000
OSHA-PEL 8HR TWA: 750 ppm
STEL: 1000ppm
CEILING: N/L
ACGIH-TLV 8HR TWA: 750 PPM
STEL: 1000 PPM
CEILING: N/L
OTHER LIMITS 8HR TWA: N/A
STEL: N/A
CEILING: N/A
* Hazardous per OSHA criteria

INGREDIENT NAME: Antimycin A *
CONCENTRATION: 20.0000 %
CAS/RTECS NUMBERS: 1397-94-0 / CD0350000
OSHA-PEL 8HR TWA: N/L
STEL: N/L
CEILING: N/L
ACGIH-TLV 8HR TWA: N/L
STEL: N/L
CEILING: N/L
OTHER LIMITS 8HR TWA: N/A
STEL: N/A
CEILING: N/A
* Hazardous per OSHA criteria

3. HAZARDS INFORMATION

EMERGENCY OVERVIEW: Flammable Liquid and a marine hazard. Thc active component is toxic by ingestion and perhaps also by skin absorption. It is an eye, skin and respiratory irritant.

ROUTE(S) OF ENTRY: Skin: Yes
Inhalation: Yes
Ingestion: Yes

INGESTION RATING: Highly Toxic

SKIN ABSORPTION RATING: Possibly highly toxic

INHALATION RATING: N/D

CORROSIVENESS RATING: N/D

SKIN CONTACT RATING: Irritant

SKIN SENSITIZATION RATING: N/D

EYE CONTACT RATING: Irritant

TARGET ORGANS: Eyes, skin, respiratory tract, cardiovascular system, nervous system, kidneys, possibly fetus

CARCINOGENICITY RATING: NTP: N/L IARC: N/L OSHA: N/L
ACGIH: N/L
None

SIGNS AND SYMPTOMS: N/D. Inhalation of vapors or aerosol could irritate the eyes, nose and respiratory tract. Direct contact with skin or eyes could produce severe irritation. Systemic intake could produce a decrease in blood pressure, nausea, light headedness, dizziness, excitement, incoordination, weakness, loss of coordinated speech and drowsiness.

MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE: N/D. Available information suggests pre-existing eye, skin, respiratory, kidney, nervous system or cardiovascular ailments.

4. FIRST AID MEASURES

EYES: Remove from source of exposure. Flush with copious amounts of water. If irritation persists or signs of toxicity occur, seek medical attention. No known antidote. Provide symptomatic/supportive care as necessary.

SKIN: Remove from source of exposure. Flush with copious amounts of water. If irritation persists or signs of toxicity occur, seek medical attention. No known antidote. Provide symptomatic/supportive care as necessary.

INGESTION: Remove from source of exposure. Seek immediate medical attention. No known antidote. Provide symptomatic/supportive care as necessary.

INHALATION: Remove from source of exposure. If signs of irritation or toxicity occur, seek medical attention. No known antidote. Provide symptomatic/supportive care as necessary.

5. *FIRE FIGHTING PROCEDURES*

FLASH POINT: O F (for acetone)
FLASH POINT METHOD: Closed Cup
LOWER EXPLOSIVE LIMIT(%): 2.6% (for acetone)
UPPER EXPLOSIVE LIMIT(%): 12.8% (for acetone)
AUTOIGNITION TEMPERATURE: 869 F (for acetone)

FIRE & EXPLOSION HAZARDS: Flammable Liquid. Keep away from heat, sparks and open flame.

EXTINGUISHING MEDIA: Use "alcohol" foam, dry chemical or carbon dioxide. Water may be ineffective.

FIRE FIGHTING INSTRUCTIONS: Wear protective clothing and self-contained breathing apparatus.

6. *ACCIDENTAL RELEASE MEASURES*

SPILL OR RELEASE PROCEDURES: Recover product and place in an appropriate container for disposal. Ventilate and wash the spill area.

7. *HANDLING AND STORAGE*

HANDLING: Ground and bond all containers during transfer operations.
STORAGE: Tight container.
SPECIAL PRECAUTIONS: Wash hands and face after handling this compound.

8. *EXPOSURE CONTROLS/PERSONAL PROTECTION*

ENGINEERING CONTROLS: Use local exhaust.
RESPIRATORY PROTECTION: Air purifying respirator with organic vapor cartridge.
SKIN PROTECTION: Butyl rubber.
EYE PROTECTION: Full-face respirator.

OTHER PROTECTION: Wear saranex tyvek coverings with hood and shoe covers if contact may occur.

9. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE/PHYSICAL STATE: Brown to black liquid
ODOR: Acetone
BOILING POINT: 56.2 C (for acetone)
MELTING/FREEZING POINT: -94.6 C (for acetone)
VAPOR PRESSURE (mm Hg): N/D
VAPOR DENSITY (Air=1): N/D
EVAPORATION RATE: N/D
BULK DENSITY: N/D
SPECIFIC GRAVITY: 0.8 (for acetone)
SOLUBILITY: Miscible in water, alcohols, ethers and most organic solvents.
pH: N/D
VISCOSITY: N/D

10. STABILITY AND REACTIVITY

CHEMICAL STABILITY: Neutralize active component with bleach, potassium permanganate, or other strong oxidizer.
INCOMPATIBILITIES: Oxidizers.
HAZARDOUS DECOMPOSITION PRODUCTS: N/D
HAZARDOUS POLYMERIZATION: N/D

11. TOXICOLOGICAL INFORMATION

ORAL TOXICITY: N/D. LD50 = 30 mg/kg in rats for antimycin A. LD50 = 1738-10, 700 mg/kg in mice, rats and rabbits for acetone.

DERMAL TOXICITY: N/D. Cumulative lethal dosage for antimycin A in rabbits about 65-150 mg/kg in animals receiving one gram of a 5% suspension in carbowax twice daily for three applications. Death possibly the result of absorption through broken skin as marked inflammation present after second application. LD50 = 20,000 mg/kg in rabbits for acetone.

INHALATION TOXICITY: N/D. A 10% formulation of antimycin A in alcohol administered to rats and guinea pigs as an aerosol for 10 minutes a day for 5 days at a nominal concentration of 170 mg/m3 produced eye irritation with corneal lesions and respiratory irritation and damage. LCLo = 16,000 ppm/4H in rats and 467,300 ppm/1H in mice for acetone. Vapors can cause irritation of the respiratory tract.

CORROSIVENESS: N/D

DERMAL IRRITATION: N/D. No irritation found following dermal application of 0.5 gram of a 5% suspension of antimycin A in carbowax (25 mg antimycin A); however, exudation, edema and scab formation were found after the first two of six applications over three days. Acetone mildly irritating to rabbit skin. Repeated or prolonged contact can cause dermatitis.

OCULAR IRRITATION: N/D. Corneal opacity clearing in four weeks resulted following application of 0.1 gram of antimycin A to the eyes of guinea pigs Application of 0.5 grams of 5% antimycin A in alcohol to the eyes of rabbits resulted in slight redness. Acetone severely irritating, with corneal injury in rabbits. Vapors can cause eye irritation and burning. Can cause stinging if splashed in the eyes.

DERMAL SENSITIZATION: N/D.

SPECIAL TARGET ORGAN EFFECTS: N/D. Dietary administration of antimycin A at a dosage of 10 mg/kg/day for four weeks produced soft stools and reduced weight gain in rats. Dietary administration at a dosage of 0.5 mg/kg/day to rats prior to and during pregnancy resulted in reduced body weight of the offspring (about 10%). Infusion to dogs at a rate of 1mcg/kg/minute for 1 hour produced no adverse effects; however, infusion of 10 mcg/kg/minute produced decreased blood pressure, slowed heart rate and death. Acetone causes central nervous system depression at elevated vapor concentrations and irritation at lower concentrations. Produced kidney injury in rats at oral dosages of 500 mg/kg/day or more.

CARCINOGENICITY INFORMATION: N/D

12. ECOLOGICAL INFORMATION

ECOLOGICAL INFORMATION: Marine hazard. Used in conjunction with a surfactant to kill fish.

13. DISPOSAL CONSIDERATIONS

WASTE DISPOSAL METHODS: Dispose of product in accordance with federal, state and local regulations.

14. TRANSPORTATION INFORMATION

DOT STATUS:	Regulated
PROPER SHIPPING NAME:	Flammable Liquids, toxic, n.o.s. (Acetone, Antimycin A), 3, UN1992, II
HAZARD CLASS:	3
UN NUMBER:	UN1992
PACKING GROUP:	II
REPORTABLE QUANTITY:	5000/2270

IATA/ICAO STATUS:	Regulated
PROPER SHIPPING NAME:	Flammable liquid, toxic, n.o.s., (Acetone, Antimycin A)
HAZARD CLASS:	3
UN NUMBER:	UN1992
PACKING GROUP:	II
REPORTABLE QUANTITY:	5000/2270

IMO STATUS:	Regulated
PROPER SHIPPING NAME:	Not Authorized
HAZARD CLASS:	N/D
UN NUMBER:	N/D
PACKING GROUP:	N/D
REPORTABLE QUANTITY:	N/D
FLASH POINT:	O F (for acetone)

15. REGULATORY INFORMATION

TSCA STATUS: Exempt

CERCLA STATUS: N/D

SARA STATUS: N/D

RCRA STATUS: N/D

PROP 65 (ca): N/D

16. OTHER INFORMATION

LEGEND: N/A =
N/D = Not Determined
N/L = Not Listed
L = Listed
C = Ceiling
S = Short-term

The information and recommendations contained herein are based upon tests believed to be reliable. However, Aquabiotics Corp does not guarantee their accuracy or completeness NOR SHALL ANY OF THIS INFORMATION CONSTITUTE A WARRANTY, WHETHER EXPRESSED OR IMPLIED, AS TO THE SAFETY OF THE GOODS, THE MERCHANTABILITY OF THE GOODS, OR THE FITNESS OF THE GOODS FOR A PARTICULAR PURPOSE. Adjustments to conform with actual conditions of usage may be required. Aquabiotics Corp. assumes no responsibility for results obtained or for incidental or consequential damages arising from the use of these data. No freedom from infringement of any patent, copyright or trademark is to be inferred.

The U.S. Department of the Interior (DOI) is the nation's principal conservation agency, charged with the mission "to protect and provide access to our Nation's natural and cultural heritage and honor our trust responsibilities to Indian tribes and our commitments to island communities." More specifically, Interior protects America's treasures for future generations, provides access to our nation's natural and cultural heritage, offers recreation opportunities, honors its trust responsibilities to American Indians and Alaska Natives and its responsibilities to island communities, conducts scientific research, provides wise stewardship of energy and mineral resources, fosters sound use of land and water resources, and conserves and protects fish and wildlife. The work that we do affects the lives of millions of people; from the family taking a vacation in one of our national parks to the children studying in one of our Indian schools.

NPS D-1966, January 2008

National Park Service
U.S. Department of the Interior

Natural Resource Program Center

National Resource Program Center
Water Resources Division
1201 Oakridge Drive, Suite 250
Fort Collins, CO 80525

www.nature.nps.gov

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