Supplement to the Environmental Assessment for an Apache Trout Enhancement Project: Considerations for Addition of CFT Legumine™ (Rotenone) and Sodium Permanganate treatments to the Previous NEPA Decision of 2004

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Adapted from "Supplement to the Environmental Assessment for Gila Trout Restoration in the Upper West Fork Gila River, Catron County, New Mexico: Considerations for addition of rotenone to the previous NEPA decision of 2003" by Jerry Monzingo and Amy Unthank

Background

The Decision Notice and Finding of No Significant Impact (DN/FONSI) for the 2004 Environmental Assessment (EA) of the Apache Trout Enhancement Project (referred to hereafter as the Apache Trout EA) was signed by James Anderson, Acting Forest Supervisor, on March 2, 2004. The decision was to authorize actions to help recover Apache trout populations through removal of non-native fish, salvaging of native fish and macro-invertebrates, and improvement of existing and construction of new fish barriers. Non-native fish would be removed using electro-fishing and the application of a piscicide, Fintrol® (antimycin A). A second DN/FONSI for the Apache Trout Enhancement Project specifically authorized piscicide use inside of wilderness areas and established, or candidate, research natural areas (RNAs) was signed by Abel Camarena, Acting Regional Forester, on March 29, 2004.

Within and outside the wilderness areas the chemical applicator would be experienced in antimycin A use and certified by the Arizona Department of Agriculture. Detoxification of streams would be implemented following piscicide application with an approved neutralizing agent, potassium permanganate. If necessary, a secondary detoxification station would be used prior to Apache trout reintroduction and the effectiveness of fish removal would be evaluated. If needed, streams would be chemically treated again using the same protocol. Downstream water users and homeowners within 3 miles of the project area would be notified; signs would also be posted at trailheads and stream access points.

Purpose and Need for the Supplement to the Apache Trout EA

The 2004 decision for the Apache Trout EA includes the piscicide antimycin A as a method of non-native fish removal in the stream renovation process. Two chemicals, rotenone and antimycin A, are commonly used by fisheries managers to remove non-native fish. Rotenone is manufactured in a number of formulations by two chemical companies, whereas antimycin A is only available as Fintrol®, produced and distributed by Aquabiotics Corporation.

Bioassays completed in 2007 in preparation for the renovation of the South Fork of the Little Colorado River for Apache trout revealed that the strength of the Fintrol® piscicide was variable and in some cases, compromised (Meyer and Lopez 2008). Biologists with the Arizona Game and Fish Department (AZGF) sent several samples of Fintrol® to the Upper Midwest Environmental Sciences Center, United States Geological Service, in LaCrosse, Wisconsin, for analysis. Results indicated a large amount of variance in the strength of the active ingredient, with 4.6 percent being the maximum strength of the Fintrol® samples submitted by AZGF compared to the full strength which is 23 percent by volume (Meyer 2008). Much of the Fintrol® purchased for use in Apache and Gila trout renovation projects during the last 3 years is suspected to be poor quality, and biologists are trying to locate Fintrol® made and purchased prior to 2005. At this time no new Fintrol® has been forthcoming from Aquabiotics Corporation.

During the analysis of effects disclosed within the Apache Trout EA and its DN/FONSIs, it was expected that Fintrol® would be available at full concentrations. Rotenone was not originally considered because older formulations included noxious chemicals such as

petroleum and benzene requiring additional protective gear and hazards for field personnel (Unthank 2008). A formulation of rotenone, CFT Legumine™, which is newer to the United States, has been used in Europe for over a decade and is designed to reduce or eliminate petroleum hydrocarbon solvents such as toluene, xylene, benzene, and naphthalene. The reduction of these petroleum solvents reduces concerns over issues such as water quality, chemical odor, public health, and applicator safety as well as decreased detectability of the chemical by fish (Monzingo and Unthank 2008). CFT Legumine™ was the formulation chosen by the California Department of Fish and Game and the Forest Service after analysis of ecological and human health risks in a joint environmental impact report/impact statement for treatment of Lake Davis (Fisher 2007). Currently, AZGF has authorization from the Arizona Department of Agriculture to use CFT Legumine™ as a piscicide (Carter pers. comm. 2009). The EPA registration of CFT Legumine™ is no longer current, but the stock of CFT Legumine™ in possession by AZGF is still permitted by the EPA (USEPA 2008), and therefore the State of Arizona, for use.

This supplement provides new information on the status and use of the two existing piscicides and two neutralizing agents, and reviews the adequacy of the existing EA to determine if the use of CFT Legumine™ and the neutralizing agent sodium permanganate is consistent with the previous analysis.

Project Description and Significant Issues of the Apache Trout EA

In cooperation with AZGFD and USFWS the project decisions allow for restoration of Apache trout to fourteen streams within the Black River and Little Colorado River systems. Specific actions include construction/renovation of barriers to prevent movement of non-native fish into renovated streams, removal of non-native fish, and re-introduction of Apache trout. The significant issues identified in the EA were: 1) water quality, 2) the effects of renovation and neutralization chemicals as well as fish barrier construction/remodeling, and 3) the effects of fish barrier construction/remodeling on stream dynamics. In addition to addressing these significant issues in relation to CFT Legumine™ use, this supplement will also address the issues in relation to the neutralizing agent sodium permanganate.

Apache Trout EA Proposed Actions with Supplemental Information

The Proposed Action is identical to that described and analyzed in the Apache Trout EA of 2004 and authorized under the DN/FONSI of 2004, supplemental actions that would be additionally included are 1) the use of rotenone in the formulation of CFT Legumine™, as one choice for chemical renovation of streams within the Black River and Little Colorado River systems, and 2) sodium permanganate as a neutralizing agent for rotenone. Fintrol®, should it become available at the needed quality, will also remain available as an option for renovation to restore native fish in the project area. Antimycin A and rotenone are both effective in removal of fish. However, they differ in effectiveness in some habitat types. Therefore, one may be used in a given stream, the other used in a different stream, or both may be used in the stream at some time based on habitat types within a treatment area.

Apache Trout EA Alternatives with New Information

The alternatives considered in the 2004 DN (no action and proposed action) have not been changed with the exception of the use of rotenone, and the neutralizing agent, sodium permanganate, for eliminating non-native fish. Mitigation and monitoring measures will remain identical and any additional biological opinion terms and conditions to reduce impacts to Endangered Species Act species will be part of project implementation. These will be provided by the US Fish and Wildlife Service upon completion of formal consultation on the additional use of rotenone and sodium permanganate. A Biological Assessment and Evaluation has been prepared to determine effects from the additional use of these agents on these and other species. Those effects are summarized in the Environmental Consequences section below. Consultation for Apache trout, Little Colorado spinedace, loach minnow and its critical habitat, Chiricahua leopard frog, and Mexican spotted owl, has been initiated and the decision for this supplement will not be signed until consultation is completed

Environmental Consequences

This section discusses the environmental effects of CFT Legumine™ to various resources and other considerations first, followed by the environmental effects of sodium permanganate.

(1) The Addition of CFT Legumine™

Piscicide Background: Rotenone is a naturally occurring substance derived from roots of certain tropical plants in the Leguminosae (bean) family; antimycin A is a chemical structure isolated from the bacteria *Streptomyces*. Both products interfere with mitochondrial electron transport and are potent inhibitors of aerobic respiration in fishes and other gill-bearing animals. In a comparison of antimycin A and rotenone, rotenone has to be applied at a relatively higher dose (parts per million [ppm] vs. parts per billion [ppb]), has longer degradation time when not detoxified by potassium or sodium permanganate, and can be detected by fish (and thus evaded without proper mixing). Extensive research has been conducted by the U.S. Fish and Wildlife Service (USFWS) and the U.S. Environmental Protection Agency (EPA) on rotenone to determine the safety of rotenone use in fisheries management. The results of this research demonstrated that when rotenone is applied according to the label instructions it is an environmental and human safe product (USEPA 2006). The American Fisheries Society has published a rotenone use manual in an effort to have rotenone continue to be available for fisheries management because of rotenone's usefulness in complete eradication on non-native fish (Finlayson et al. 2000).

The proposed application of rotenone for this supplemental decision would be a 5 percent formulation of CFT Legumine™ applied at a rate equal to or less than 1 ppm (<0.05 ppm pure rotenone) based on bioassays, and would be neutralized with sodium or potassium permanganate at each barrier. The application methods, the mitigation and monitoring measures and the method of neutralization are the same for both antimycin A and

rotenone (Apache Trout EA, page 11). However, research has shown that dissolved electrolytes and suspended organic matter have a major influence on the amount of potassium permanganate required to neutralize a given concentration of rotenone (Engstrom-Heg 1971, 1972). The rotenone use manual recommends using a ratio from 2:1 to 4:1 (potassium permanganate: formulated rotenone) for neutralization compared to the 1:1 ratio for antimycin (Finlayson 2000; CDFG 1994). Two neutralization stations will be utilized for rotenone treatments as with antimycin, however placement will differ. For rotenone, a primary detoxification station will be at the fish barrier and the secondary will be set a half hour travel time downstream (determined by discharge). As in the EA (page 11), the secondary station will be activated only in the event of incomplete detoxification at the primary station and effectiveness of detoxification will be determined using a bioassay of live, caged fish. As with antimycin A (Apache Trout EA, page 11), the application of rotenone would occur under the supervision of a certified pesticide applicator and with biologists experienced in the application of both antimycin A and rotenone.

Vegetation: There will no change in the effects to vegetation as analyzed (Apache Trout EA, page 21). Effects to vegetation, such as trampling, increased water tables, and increased trail use, from the Apache Trout Enhancement Project would come from activities associated with barrier construction/enhancement and backfilling. The addition of CFT Legumine™ would not alter these effects.

Soil: There will no change in the effects to soils as analyzed (Apache Trout EA, page 24 and 25). Effects to soils, associated with the Apache Trout Enhancement Project, would come from activities related to barrier construction or improvement. The addition of CFT Legumine™ would not alter these effects.

Water and Watershed:
Water Yield and Quality: There will be no change to water yield or hydrograph as analyzed (Apache Trout EA, page 26-34). Some impacts to water quality would occur with CFT Legumine™, including longer degradation time of the chemical (on the order of a few days if not neutralized by potassium permanganate or sodium permanganate, as opposed to hours for non-neutralized antimycin A applied to streams). As with antimycin A, detoxification at temperatures below 50°F may require longer contact time between the treated water and the application of potassium permanganate (CFT Legumine™ label). Rotenone readily binds with organic matter and is unlikely to reach groundwater (Dawson 1991). It is uncommon to find rotenone in stream sediments (CDFG 1994). Rotenone, specifically CFT Legumine™, has a number of other ingredients including methyl pyrrolidone, diethylene glycol, monoethyl ether, fatty acid esters, and polyethylene glycols which help the product mix with water. A number of trace compounds have also been detected, including naphthalene, substituted benzenes, and hexanol (Fisher 2007). None of the compounds identified are considered persistent in the environment nor will they bio-accumulate. They also rapidly biodegrade, hydrolyze, and/or are broken down by sunlight. None of the constituents identified in extensive lab analysis (Fisher 2007) appear to be at concentrations that suggest human health risks through water or ingestion exposure scenarios, and none of the regulatory criteria were exceeded in estimated exposure concentrations in the study conducted for treatment of

Lake Davis in California. New Mexico Game and Fish Department detected no rotenone, benzenes, toluenes, naphthalenes, or other inerts during standard water quality sampling downstream of CFT Legumine™ treatment sites in Comanche and Costilla Creeks in 2007 and 2008 (Patten pers. comm.). In comparison to the Noxfish™ formulation, another rotenone formulation not analyzed for this project, substituted benzene concentrations were 0.091 ppb in CFT Legumine™ to 23.14 ppb in Noxfish™ (Fisher 2007). Less benzene would improve operating conditions for workers and is better for long-term stream health.

Wetlands, Riparian, and Aquatic Habitat and Biota: As provided in Appendix A previous studies of the effects of antimycin A and rotenone on aquatic macroinvertebrates indicates varied impacts, with some species being highly sensitive. The formulation of rotenone proposed for use in this project area (CFT Legumine™), a formulation chosen partly based on recent human health and ecological risk assessments done by California Department of Fish and Game (GDFG) and the U.S. Forest Service (USFS) in 2004 (CDFG and USFS 2005) for various formulations of rotenone (Fisher 2007), is expected to show similar varied effects in aquatic invertebrate communities, with the reduction of certain groups of macroinvertebrates, but no long term elimination of existing taxa. Based on rapid biodegradation and/or photolysis of the constituents in CFT Legumine™, no additional ecological impacts are expected beyond those previously described for antimycin A (Apache Trout EA, page 34). As noted in the existing EA, aquatic invertebrate communities may be affected by the use of piscicides, but they also undergo natural variation in community composition due to other events such as fires and high run off.

As previously analyzed there will be no effects to wetland, riparian, or aquatic habitat, other than those analyzed under Water Quality (Apache Trout EA, page 27). Additional information is provided below in Appendix A specific to water quality and rotenone.

Wildlife:

Threatened and Endangered Species: : A Biological Assessment and Evaluation (BAE) was submitted to U.S. Fish and Wildlife Service in 2002 and the Service provided a Biological Opinion (B.O.), dated April 19, 2002), for five species for which formal consultation was required, the Apache trout, Little Colorado spinedace, loach minnow critical habitat, Bald eagle, and Mexican spotted owl. Consultation was reinitiated in May 2003 for Mexican spotted owl (withdrawal of timing restrictions) and Bald eagle (new nesting pair) and the Service provided a B.O. (July 2, 2003). Consultation was reinitiated again in December 2003 for changes in the proposed action (additional renovations, variable barrier locations, and backfill option) and the Service provided another B.O. (February 23, 2004).

During this timeframe, the status of the Chiricahua leopard frog changed from proposed to listed as threatened. The Service provided a B.O. (July 19, 2002) concurring with the "not likely to adversely affect" determination in an earlier conferencing opinion (April 14, 2002). Consultation was reinitiated in February 2004 for the Chiricahua leopard frog

(change in likelihood of occurrence) and the Service included this species in its February 23, 2004 B.O.

Specific project actions that likely adversely affect the Mexican spotted owl and the (now sensitive species) Bald eagle are related to disturbance by the presence of humans during treatment and not related to the type of piscicide used. As such, no additional impacts from the use of rotenone are expected beyond those previously described for antimycin A (Apache Trout EA, page 34-35).

Therefore, previous determinations for the Mexican spotted owl and Bald eagle, as well as for the Mexican gray wolf, jaguar and Southwestern willow flycatcher, would not change with the use of CFT Legumine™.

Sensitive, Management Indicator and Other Species: Individual species affected by the use of antimycin A are aquatic macroinvertebrates including the Three Forks springsnail and California floater, the water penny beetle and ameletus mayfly, and the northern water shrew. These species would be affected in the same manner with the use of rotenone as described for antimycin A, see Appendix A. However, as with antimycin A (Apache Trout EA, page 38), while individuals of these species may be affected by the use of rotenone, its use will not result in a loss of species viability or lead to a trend for federal listing.

In addition, impacts to these species would be reduced as follows. The 2/15/02 Specialist's Report for Aquatic Forest Sensitive [and MIS] Species states (page 9) that "…as part of the proposed action, aquatic macroinvertebrates will be salvaged pre-treatment and be reintroduced post-treatment to mitigate possible macroinvertebrate losses during piscicide treatments." The Report also recommends mitigation to reduce impacts to the California floater, water penny beetle and other sensitive aquatic species. This would be the survey and collection and temporary relocation to untreated stream reaches or held at the Pinetop Fish Hatchery until successful renovation is completed. After this they would be returned to stream reaches they were collected in (page 10).

Therefore, previous determinations for the above species, as well as all other sensitive, management indicator, and other species would not change with the use of CFT Legumine™.

In summary, the review of all biological documents, the effects determinations, mitigation measures (including survey and removal/replacement), and reasonable and prudent measures with their terms and conditions concludes that these actions are still adequate to protect the species within the action area of project effects relative to the use of antimycin. However, due to increased concerns for some threatened species, the Apache trout, Little Colorado spinedace, Chiricahua leopard frog, Mexican spotted owl, and loach minnow and its newly designated critical habitat, consultation will be reinitiated.

Air: There will be no changes in the effects to air resources as analyzed in the Apache Trout EA (page 40) with the use of CFT Legumine™ instead of Fintrol®. Effects to air quality would come from trucks hauling materials to sites (Apache Trout EA, page 40). This would not change with the addition of CFT Legumine™.

Heritage Resources: There will no change in the effects to heritage resources as analyzed (Apache Trout EA, page 40). No historic properties or cultural resources have been found near the proposed river segments or along access routes. No concerns have been expressed by Native American tribes or other parties concerning traditional uses or significant places within the project area (Apache Trout EA, page 40).

Recreation and Economic Impact: Recreational fishing concerns were analyzed in the Apache Trout EA (page 42). This analysis found that most of the streams designated for renovation are difficult to access for fishing and therefore renovation would have little impact on recreational fishing. Two of the streams designated for renovation, the West Fork Little Colorado River and West Fork Black River, receive the majority of the fishing pressure. While there would be some loss of fishing opportunities and guiding income during renovation, re-introduction of Apache trout will provide new opportunities for fishing and guiding for Apache trout. Other measures such as implementation over a gradual timeframe, stocking, and angling opportunities downstream of the renovation barriers would also help mitigate the effect of fishing closures during renovation. The degradation time of CFT Legumine™ will not increase the length of fishing closures therefore the analysis from the EA would not change with the use of CFT Legumine™ instead of Fintrol® as the piscicide used for renovation.

(2) The Addition of Sodium Permanganate for as a Neutralizing Agent for Stream Treatment

Neutralization Agent Background: The EA includes provisions for neutralization of the antimycin A stream treatment with potassium permanganate (Apache Trout EA, page 11 and DN, page 3), which is a strong oxidizing agent commonly used in both rotenone and antimycin A fish projects. Potassium permanganate for piscicide neutralization is activated by the mixing of a powdered form in water to reach a desired aqueous concentration or comes in a liquid formulation. Either formulation can then be applied to the treated water to provide a desired concentration for neutralization. Recent renovations of Fossil Creek and Bonito Creek by AZGF using sodium permanganate as a neutralizing agent for both rotenone and antimycin A led to the conclusion that sodium permanganate was a more effective neutralizing agent than potassium permanganate (Lopez 2008a.). In 2008 the Arizona Department of Agriculture issued a letter to the AZGF stating that sodium permanganate may be used with CFT Legumine™ for neutralization. The new formulation of rotenone includes sodium permanganate as a neutralizing agent on its Material Safety Data Sheet (MSDS) label. Sodium permanganate is used as a substitute for potassium permanganate in industrial uses when a strong oxidizer is required.

In review of the MSDS it has **no significant difference from potassium permanganate in terms of its substitution as an oxidizer**. It has no differences in health hazard rating, first aid measures, or conditions for storage and handling; but does have a higher

solubility in water, which allows much less of the material to be used for the stream neutralization as compared to potassium permanganate. Furthermore, sodium as the spectator ion in the chemical oxidation process is more common in natural stream settings than is potassium (Weedman et al. 2005)

Vegetation: There will no change in the effects to vegetation as analyzed (Apache Trout EA, page 21). Effects to vegetation, such as trampling, increased water tables, and increased trail use, from the Apache Trout Enhancement Project would come from activities associated with barrier construction/enhancement and backfilling. The addition of sodium permanganate would not alter these effects.

Soil: There will no change in the effects to soils as analyzed (Apache Trout EA, page 24 and 25). Effects to soils, associated with the Apache Trout Enhancement Project, would come from activities related to barrier construction or improvement. The addition of sodium permanganate would not alter these effects.

Water and Watershed:

Water Yield and Quality: There will be no change to water yield or hydrograph. Some impacts to water quality would occur with sodium permanganate as they would with potassium permanganate as discussed in the EA, Appendix D. While permanganate can be toxic to fish under sustained lab conditions (Apache Trout EA, page 68), it breaks down quickly under stream conditions, including the interaction with antimycin A. Additionally, potassium permanganate reduces the half-life of antimycin A from 4.6 to 9.5 hours to 7 to 11 minutes. Both potassium and sodium permanganate have a low estimated lifetime in the environment (MSDS). The lifetime is short because of the reaction with the piscicide and the organic matter within the stream. During the Fossil Creek Native Fish Restoration Project, AZGF staff observed no effect to fish that were below the neutralization station where sodium permanganate was used to neutralize antimycin A (Weedman et al. 2005)

Additionally, smaller amounts of sodium permanganate solution are needed for neutralization because of the higher concentration of sodium. In concentration, potassium permanganate has only been available in concentrations of 2.5 percent whereas sodium permanganate is available in concentrations of up to 40 percent in the liquid solution (Lopez 2008b). The smaller amounts of sodium permanganate necessary for neutralization would be beneficial for improved work conditions on small and large flow streams. Greater concentration in solution means less mixing by field staff allowing for safer work conditions in remote locations (Lopez 2008b).

Wetlands, Riparian, and Aquatic Habitat and Biota: As previously analyzed there will be no effects to wetland, riparian, or aquatic habitat other than those analyzed under Water and Watersheds-Environmental Consequences (Apache Trout EA, page 28).

Wildlife:
Threatened and Endangered Species: Impacts to the aquatic species and their critical habitat as analyzed in the BAE and Apache Trout EA (page 37) would remain the same with the addition of sodium permanganate as a neutralizing agent. Bioassays conducted by AZGFD found that sodium permanganate had a similar effect to potassium permanganate when used as a neutralizing agent (Meyers and Lopez 2008). Therefore, previous determinations would remain the same for the threatened and endangered aquatic species identified in the Apache Trout EA (page 37). Specific project actions that likely adversely affect the Mexican spotted owl and the (now sensitive species) Bald eagle are related to disturbance by the presence of humans during treatment and not related to the type of neutralizing agent used. As such, no additional impacts from the use of sodium permanganate are expected beyond those previously described (Apache Trout EA, page 34-35). Therefore, previous determinations for the Mexican spotted owl and Bald eagle, as well as for the Mexican gray wolf, jaguar and Southwestern willow flycatcher, would not change with the use of sodium permanganate.

Sensitive, Management Indicator and Other Species**:** Individual species that could be affected by the use of sodium permanganate are aquatic macroinvertebrates including the Three Forks springsnail, the water penny beetle and ameletus mayfly, and the northern water shrew. As noted above, sodium permanganate has no significant difference from potassium permanganate in terms of its substitution as an oxidizer and sodium in the oxidation process is more common in natural stream settings than is potassium (Weedman et al. 2005). As with potassium permanganate (Apache Trout EA page38), while individuals of these species may be affected by the use of sodium permanganate, its use will not result in a loss of species viability or lead to a trend for federal listing. Therefore, previous determinations for the above species, as well as all other sensitive, management indicator and other species would not change with the use of sodium permanganate as a neutralizing agent.

In summary, the review of all biological documents (including specialist reports, BAEs and B.O.s), effects determinations, and mitigation measures (including survey and removal/replacement), concludes that these actions are still adequate to protect the species within the action area of project effects relative to the use of sodium permanganate. However, due to increased concerns for some threatened species, the Apache trout, Little Colorado spinedace, Chiricahua leopard frog, Mexican spotted owl, and loach minnow and its newly designated critical habitat, consultation will be reinitiated.

Air: There will be no changes in the effects to air resources as analyzed in the Apache Trout EA (page 40) with the use of sodium permanganate instead of potassium permanganate.

Heritage Resources: There will no change in the effects to heritage resources as analyzed (Apache Trout EA, page 40). No historic properties or cultural resources have been found near the proposed river segments or along access routes. No concerns have been expressed by Native American tribes or other parties concerning traditional uses or significant places within the project area.

Recreation and Economic Impacts: Recreational fishing concerns were analyzed in the Apache Trout EA (page 42). The EA analysis found that most of the streams designated for renovation are difficult to access for fishing and therefore renovation would have little impact on recreational fishing. Two of the streams designated for renovation, the West Fork Little Colorado River and West Fork Black River, receive the majority of the fishing pressure. While there would be some loss of fishing opportunities and guiding income during renovation, re-introduction of Apache trout will provide new opportunities for fishing and guiding for Apache trout. Other measures such as implementation over a gradual timeframe, stocking, and angling opportunities downstream of the renovation barriers would also help mitigate the effect of fishing closures during renovation. This analysis from the EA would not change with the use of sodium permanganate instead of potassium permanganate as the neutralizing agent used during renovation.

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APPENDIX A: Comparison of Effects to Non-Target Organisms and Human Health for Rotenone and Antimycin

from "Supplement to the Environmental Assessment for Gila Trout Restoration in the Upper West Fork Gila River, Catron County, New Mexico: Considerations for addition of rotenone to the previous NEPA decision of 2003" by Jerry Monzingo, USDA Forest Service, Gila National Forest

Aquatic macroinvertebrates

Rotenone- After laboratory based tests, Chandler & Marking (1982), concluded that: apart from an Ostracod (*Cypridopsis sp*.), aquatic invertebrates are much more tolerant of rotenone than most fishes and amphibian larval stages. In their study the most resistant organisms exposed were a snail (*Helisoma sp.*) and the Asiatic clam (*Corbicula manilensis*) for which the LC50 96h concentrations were 50 times greater than those Marking & Bills (1976) reported for the Black bullhead (*Ictalurus melas*), one of their most resistant fishes. Sanders & Cope (1968) also conducted lab tests examining the effect of rotenone to the nymph or naiad stage of a stonefly (*Pteronarcys californica*). They found that the LC50 24h was 2,900 µg/L and the LC50 96h was 380 µg/L. These values are greater by an order of magnitude to those found by Marking & Bills (1976) for the black bullhead (*Ictalurus melas*) indicating that aquatic invertebrates are much less sensitive to rotenone than fish. Larger, later instar naiads were less susceptible to given concentrations of toxin than were smaller, earlier instars of the same species (Sanders & Cope, 1968). Field studies examining the effect of rotenone on aquatic macroinvertebrate communities have provided varied results. Whereas some workers noticed dramatic, long-term effects (Mangum & Madrigal, 1999; Binns, 1967), others observed rotenone has a negligible effect on most aquatic macroinvertebrates (Demong, 2001; Melaas, 2001). Most researchers would agree, however, that the effects of rotenone are less pronounced and more variable to macroinvertebrates than the effects of the chemical on zooplankton. Like the range of sensitivities demonstrated by various fish species to rotenone, different species of aquatic macroinvertebrates also exhibit a range of tolerances (Mangum & Madrigal, 1999; Chandler & Marking, 1982; Engstrom-Heg et al., 1978) again perhaps based on their oxygen requirements.

Invertebrates in the orders Ephemeroptera (Mayflies), Plecoptera (Stoneflies), and some members of Trichoptera (Caddisflies) are highly sensitive and have been completely eliminated by rotenone treatments in the past (Mangum & Madrigal, 1999). These sensitive species tend to be highly mobile and short life cycles, and may thus have the ability to repopulate depleted areas rapidly through dispersal and oviposition (Engstrom-Heg et al., 1978). Certain escape behaviors such as burrowing into benthos, associating with aquatic vegetation or the ability to trap air bubbles with appendages may reduce rotenone exposure to many benthic invertebrates. Of note, many studies have shown rapid population explosions of invertebrates following initial reductions in their biomass from rotenone treatment (Neves 1975, Cook and Moore 1969).

Antimycin- Reduction in abundance of certain groups of aquatic invertebrates is likely following antimycin treatments (Minckley and Mihalick, 1981). However, no invertebrate taxa are likely to be eliminated by antimycin treatments and abundance typically recovers with one to two years (Mangum1985, Mangum 1986, Jacobi 1988, Brooks and Propst 2001). Toxicity tests using antimycin found that Cladocera, Copepoda, Amphipoda, Ephemeroptera, and Trichoptera may experience marked declines in abundance following antimycin treatments and that Amphipoda are particularly sensitive (Schnick, 1974). Antimycin typically does not adversely affect Protozoa, Rotatoria, Nematoda, Nematomorpha, Annelida, Ostacoda, Decapoda, Plecoptera, Odonata, Hemiptera, Coleoptera, Diptera, Gastropoda, and Pelecypoda (Schnick 1974).

Amphibians

Rotenone- Rotenone is toxic to amphibians, but generally less toxic than to fish. Rotenone may be absorbed into both skin and respiratory membranes, but skin may prevent more of a barrier due to a greater distance for the chemical to diffuse across (Fontenot et al., 1994), and a smaller surface area relative to gill structure. Indeed, Fontenot et al. (1994) reported that amphibian larvae with gills are most sensitive to rotenone. In standard laboratory 24-hour and 96-hour aquatic rotenone toxicity tests, the LC_{50} values for tadpoles and larval amphibians have ranged between 5 µg/L and 580 µg/L (24-hour tests and 25 µg/L to 500 µg/L in 96-hour tests (Fontenot et al. 1994, Chandler 1982). The adult Northern Leopard Frog demonstrated a much greater resistance with LC_{50} concentrations ranging from 240 µg/L and 1,580 µg/L (24 hours) and 240 µg/L and 920 µg/L (96 hours). This highlights the fact that tadpoles and other larval forms of amphibians that utilize gills for respiration are just as sensitive to rotenone as fishes while adult forms, no longer having to utilize gills, have a much lower susceptibility to rotenone. Larval amphibians appear to have resistance roughly equivalent to the most tolerant fish species. Rotenone is variably toxic to amphibians, depending on their mode of respiration (i.e. gills, skin, buccopharyngeal, or lungs). Differences in sensitivity occur among taxa and lifestages. Adults that are obligatory aquatic or have high rates of cutaneous respiration are more sensitive as well.

Antimycin-Field studies of Fintrol® application found no effect on frogs or tadpoles (genera not specified) at an application rate of 10 ppb (Berger 1965, Berger 1965b, Berger 1966a, Berger 1966a). Frogs and tadpoles in these studies were exposed for an indefinite amount of time (i.e. longer than 96 hours). The field tests were conducted in ponds and Streams. Water temperatures ranged form 6°C to 21°C (43 to 70°F) and pH ranged from 7.9 to 8.8. Other field tests found no effect of 10 ppb antimycin over an indefinite period of time on Ranidae tadpoles (Gilderhus et al. 1969). Lab exposure studies found Fintrol-Concentrate to have no effect on leopard frog (R. pipiens) at concentration up to 48 ppb (Lesser 1972). However, bullfrog tadpoles were killed within 24 hours when exposed to antimycin at a concentration up to 40 ppb (Berger 1966c; Walker et al. 1964).

Mammals

Rotenone-Mammalian acute oral toxicity LD_{50} values for rotenone range from 39.5 mg/kg for female rats to 1,500 mg/kg for rabbits. For most lab mammals, rotenone is much more toxic when introduced intravenously or inhaled rather than taken orally. For example, the average oral LD_{50} for rats is 60 mg/kg compared with just 0.2 mg/kg for rotenone introduced directly into the bloodstream. Efficient breakdown of rotenone by the liver, oxidation of rotenone in the gut, and slow absorption in the stomach and intestines account for this significant difference in toxicity (Narongchai et al. 2005, Ling 2003). This explanation may also account for the significant difference in rotenone sensitivity between mammals and fishes, and not from a difference in the primary site of action between fishes and mammals (Fukami et al. 1969).

Antimycin- Extremely high levels of antimycin are required to produce toxic effects in carnivorous mammals similar to those that may occur in the project area. The oral LD_{50} for domestic dog is 5,000 mg antimycin/kg body weight. Thus, a domestic dog weighing 4.5 kg (10 lbs) would have to consume 57,900 kg (127,800 lbs) of antimycin-killed fish, using Ritter and Strong's (1966) maximum tissue concentration of 388 µg/kg. Field trials of 10 ppb antimycin treatments found no effects on raccoons.

Birds

Rotenone- Rotenone has a very low toxicity to wildfowl, and birds are extremely unlikely to be affected by 'normal' usage in fisheries management practices (Ling, 2003). Avian acute toxicity LD_{50} values range from 130mg/kg for the nestling English song sparrow (Cutcomp 1943) to 2200mg/kg for an adult mallard duck (USEPA 1988). In general, young birds are about 10 times more sensitive to rotenone poisoning (DFG 1994) and, like mammals, birds have a much-reduced tolerance to rotenone when it is introduced intravenously. Ling (2003) also examined rotenone poisoning and sublethal toxicity in birds as a result of consuming fish or even fish management baits. Ling concluded that "rotenone is slightly toxic to wildfowl, and birds are extremely unlikely to be affected by normal fisheries management programmes." For example, baits used to kill carp for management purposes have around 0.01 g of rotenone each. Ling calculated that a duck would need to consume approximately 200 baits to receive a fatal dose. It is very unlikely that birds would consume baits but they could consume fish killed by rotenone. The concentration of rotenone in poisoned fish is usually 25,000 times lower than that found in baits.

Antimycin- Vezina (1967) studied the toxicity of antimycin to mallard duck (Anas platyrhyncos) and found that ingestion of 2,900 mg/kg of antimycin was required to cause mortality of 50% of the test organisms. Using the highest residual concentration in dead trout of 338 µg/kg Reported by Ritter and Strong (1966), this translates to consumption of 7,474 kg (16,480 lbs) of dead trout. The amount of fish biomass that the treatment would generate (i.e. the total weight of all fish killed in the project area) would only be a fraction of this quantity. Field investigations found not effect on pelicans, cormorants, herons, surface-feeding ducks or diving ducks, or osprey from consumption of fish killed by antimycin (Berger et al., 1967; Gilderhus et al.,1969). Berger and Lennon (1967) found no effect on osprey, gulls, or terns exposed to antimycin in dead fish and water after antimycin treatment.

Human Health

Rotenone- Millions of dollars have been spent on research to determine the safety of rotenone before approval of use from the U.S. Environmental Protection Agency. Much of this research has been directed toward potential effects on public health. This research has established that rotenone does not cause birth defects (Hazelton Raltech Laboratories 1982), reproductive dysfunction (Spencer and Sing 1982), gene mutations (Biotech Research 1981;Goethem et al. 1981; NAS 1983), or cancer (USEPA 1981, Tisdel 1985). When used according to label instructions for the control of fish, rotenone poses little, if any, hazard to public health. The USEPA has concluded that the use of rotenone for fish control does not present a risk of unreasonable adverse effects to humans and the environment.

Antimycin- Antimycin is an organic compound, discovered in 1945, that was isolated form Streptomyces griseus at the Department of Plant Pathology at the University of Wisconsin (Leben and Keitt 1948, Dunshee et al. 1949, Lehninger 1979). Degredation compounds of antimycin include blatmycic acid and antimycin lactone. These degredation compounds have very low toxicity for either fish or mammals (Herr et al. 1967). Direct ingestion of normal quantities of water containing 10 ppb anticycin during the peak of the treatment would have no effect on humans or livestock. Oral LD_{50} values for mammals range from 1.0 mg antimycin/kg body weight for lambs to 55 mg antimycin/kg body weight for mice (Herr et al.,1967). Oral LD_{50} is defined as the amount of antimycin that, when administered orally over a specified period of time, is expected to cause death of 50% of the group of test animals. For example, if a person weighing 70 kg (154.3 lbs) drank 1.5 liters (0.39 gallons) from a stream during treatment, he would ingest 15µg of antimycin, or 0.00021 mg antimycin/kg body weight. A 70 kg person would have to drink 12,600 liters (3,329 gallons) of treated water during the six-hour period that antimycin is active in the project area to ingest the amount required to achieve the LD50 for the most sensitive mammal tested (Guinea pig, LD_{50} = 1.8 mg antimycin/kg body weight). This translates to a water consumption rate of about 2,100 liters (555 gallons) per hour, which is physically impossible. Similarly, a 363 kg (800 lbs) horse would have to ingest about 65,300 liters (17,250 gallons) of treated water to reach the oral LD_{50} value of 1.8 mg antimycin/kg body weight for Guinea pigs. Numerous studies have been conducted on the effects of antimycin at the cellular level. None have reported any carcinogenic effects.

Appendix A: Literature Cited

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