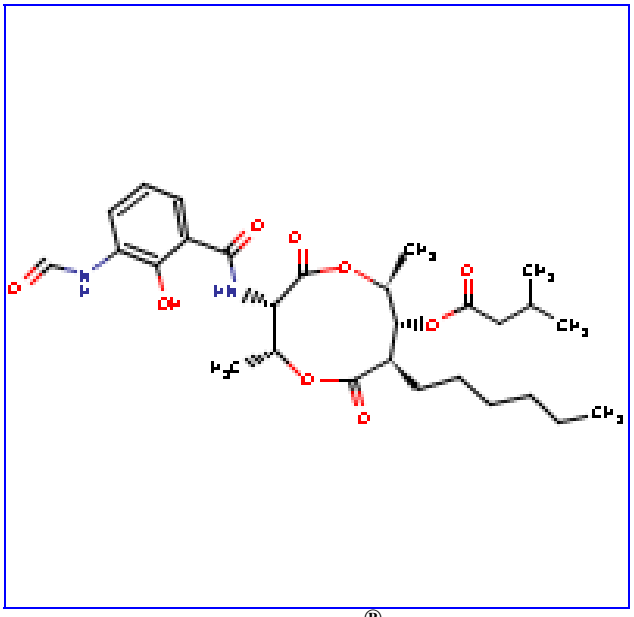
Office of Prevention, Pesticides, and Toxic Substances

Environmental Fate and Ecological Risk Assessment for the Reregistration of Antimycin A



Fintrol®
CAS 1397-94-0 PC Code 006314

Prepared by: Dirk Young, Ph.D., Environmental Engineer Thomas Steeger, Ph.D., Senior Biologist	U.S. Environmental Protection Agency Office of Pesticide Programs Environmental Fate and Effects Division Environmental Risk Branch IV 1200 Pennsylvania Ave., NW Mail Code 7507P Washington, DC 20460
Reviewed by: Elizabeth Behl, Branch Chief	

1 EXECUTIVE SUMMARY

Nature of the Chemical Stressor

Antimycin A (CAS 1397-94-0, under the tradename Fintrol®), is a restricted use pesticide derived as a fermentation product from Streptomyces mold and is registered for use as a piscicide. The chemical is primarily used to renovate recreational fish populations and to remove scaled fish from catfish fingerling and food-fish production ponds. Over the past decade antimycin has been used to restore Federally-listed threatened/endangered (listed) fish to their native habitats. Antimycin A is applied directly to water and is typically used at treatment concentrations of 25 µg/L or less; however, the current labels do not specify a maximum application rate.

Conclusions Regarding Exposure

Antimycin is applied directly to water and is maintained at a targeted treatment concentration for specified periods of time. Although environmental fate data are limited, the primary route of degradation appears to be base hydrolysis where under alkaline conditions the compound can degrade with half-lives of roughly 30 minutes. However, in at least one study, the compound persisted in an aquatic environment with a half-life of from 20 to 47 days. Field studies on downstream movement also show that the compound can extend and remain active to distances of at least 1.75 km beyond desired treatment areas when no effort is made to deactivate the compound. There are no direct measurements of antimycin sorption; however, indirect evidence suggests that antimycin will sorb significantly to soil and sediments and may reduce potential exposure outside of the immediate treatment area.

Conclusions Regarding Effects

On an acute exposure basis antimycin is moderately toxic to mammals, highly to very highly toxic to birds and very highly toxic to aquatic animals resulting in mortality. There are no data to evaluate the chronic toxicity of antimycin to animals and there are no data to evaluate the toxicity of antimycin to either terrestrial or aquatic plants.

Conclusions Regarding Potential Risks to Non-target Organisms

Based on the most sensitive species and typical treatment concentrations of 25 µg/L, acute risk levels of concern are exceeded for aquatic animals by factors of roughly 6,000X. Even for the least sensitive fish and aquatic invertebrates tested, treatment concentrations of 25 µg/L would exceed acute risk levels of concern. Although antimycin is moderately to very highly toxic to terrestrial animals on an acute oral exposure basis, treatment rates to water of 25 µg/L or less are expected to present a low risk of acute mortality for birds and mammals through ingestion of dead/dying fish or antimycin-treated water.

Because there are no chronic toxicity data, it is not possible to estimate potential chronic risk to aquatic or terrestrial animals. In situations where antimycin is applied to flowing water, the chemical is typically deactivated using potassium permanganate; however, deactivation is not currently required by the label. Additionally, in flowing water, antimycin would eventually be flushed through the system and would also be diluted by untreated tributaries. Also, in both lentic and lotic environments, antimycin is typically only applied once per year. Thus, the likelihood of chronic exposure in a lotic environment is low; however, in the absence of data, potential risk to nontarget organisms cannot be precluded. In situations where antimycin is not deactivated with permanganate and there is not dilution by untreated water, such as in lentic environments, the chronic risk to non-target aquatic organisms is uncertain.

Where antimycin is used to remove scaled fish from catfish fingerling and food-fish production ponds, treatment rates are considerably lower than 25 µg/L in order to not impair the health/survival of catfish. Therefore, the likelihood of either acute or chronic effects in non-target animals from the use of antimycin in aquaculture is low particularly since the treated water is typically retained within the aquaculture facility. To the extent that treated water is not retained but rather released into surrounding streams/rivers, the risk to non-target aquatic animals is uncertain.

Based on laboratory data, aquatic invertebrates appear to be as sensitive to antimycin as fish; however, field studies of antimycin in high-gradient mountain streams, where the compound was deactivated with potassium permanganate after the desired treatment time, suggests that aquatic invertebrates are not affected or are at worst temporarily affected. Sampling conducted several months to up to a year after treatment indicate that aquatic invertebrate abundance and diversity were similar to pretreatment levels. Even in first-order streams where antimycin was not deactivated with parmanganate, invertebrate drift and survival did not appear to be significantly affected during or immediately after 8-hr treatments at 10 µg/L. Whether these studies are indicative of aquatic invertebrate communities in all antimycin use areas is uncertain.

Effects on listed species may be an important consideration for site-specific applications. Antimycin is an important chemical used in recovery efforts for listed salmonids by eliminating competing species. The following table provides a summary of potential direct effects to listed taxa.

Listed Species Taxonomic Group of Concern	Direct Effects	Slope[a]	RQ
Freshwater Fish	Acute: mortality	15.5	2,778
Freshwater Invertebrates	Acute: mortality/immobilization	4.5	3,125
Saltwater Mollusc	Acute mortality	4.5	0.40
Aquatic Plants: Vascular Non-vascular	 no data no data	 — —	 — —
Birds	Acute: mortality/sublethal	4.5	<0.01* ---
Mammals	Acute: mortality	6.5	<0.01* --
Terrestrial Plants: Monocots Dicots	 Acute: no data Acute: no data	 — —	 — —

[a]Raw data were not provided so the default value of 4.5 is used.
*Dose-based value.

Uncertainties

The database on the environmental fate and transport properties of antimycin is incomplete. Since the compound is used exclusively as a piscicide, the terrestrial fate properties are relatively undocumented. Antimycin appears to degrade rapidly in the environment with half-lives on the order of

minutes to several weeks. Abiotic mechanisms (hydrolysis) appear to be the primary route of dissipation, but biodegradation may contribute to affecting the persistence of antimycin in the environment. The potential contribution of biotic routes of degradation to affecting the persistence is uncertain.

The database on the ecological effects of antimycin is incomplete. There are no chronic toxicity data for either terrestrial or aquatic animals; however, the likelihood of chronic exposure to antimycin following application to flowing water is likely low due to a combination of degradation, flow-through, deactivation and dilution. Additionally, there are no data for terrestrial or aquatic plants. The likelihood of exposure to terrestrial plants is considered low; however, aquatic plants will likely be exposed and the potential risk to these plants is uncertain.

The extent to which direct applications of antimycin to water as a fish control agent can be limited to the desired treatment area is dependent on the rigor of the protocols used to apply the chemical and the degree to which they are followed. Biological and chemical monitoring of the treatment area and deactivation of antimycin where feasible could track and limit exposure of non-target organisms. While many state and federal agencies follow standard operating procedures (SOPs) that limit exposure, the SOPs are not uniformly applied across all potential users. In addition, since the environmental fate database is incomplete, the extent to which antimycin will persist and move outside treated areas is uncertain.

Historically, efforts to monitor for antimycin in the environment have relied on fish bioassays. Quantitative analytical procedures capable of detecting toxic concentrations of antimycin have been limited, and those that exist are not practical in the field. Actual environmental concentrations during and after treatments with and without deactiviation with potassium permanganate are uncertain. While field bioassay data suggest that antimycin is no longer toxic following deactivation with potassium permanganate, there are no analytical data to confirm that all of the antimycin has been degraded or what transformation products result from the degradation.

Although antimycin may sorb to sediment, the extent to which the partitioning limits the toxicity of the pesticide is uncertain. There are no laboratory data on benthic macroinvertebrates with which to evaluate potential risks. A field study of aquatic invertebrates suggests, however, that both abundance and diversity of aquatic invertebrates exhibited transitory effects and many of these taxa are associated with the benthos.

This assessment evaluates potential risks from antimycin A treatment concentrations of 25 µg/L or less and primarily focuses on use in flowing water (lotic environments). To the extent to antimycin is used at higher treatment rates, the potential risks to non-target organisms are likely to increase. Additionally, the fate of antimycin in static water (lentic environments), e.g., high alpine lakes and aquaculture production ponds, is uncertain since the chemical is not typically deactivated with potassium permanganate and there is a potential for it to persist under certain environmental conditions such as low pH.

To the extent that risk managers require additional information to address uncertainties regarding potential risks to non-target organisms, additional data on the environmental fate and effects of antimycin would be required.

Acknowledgement

The Environmental Fate and Effects Division would like to acknowledge the U. S. National Park Service, the U. S. Geological Survey Biological Resource Division, the U. S. Fish and Wildlife Service, the New Mexico Department of Game and Fish, and the U. S. EPA Office of Research and Development (Athens, GA) and Office of Pesticide Programs' Biological and Economic Assessment Division (Bay St. Louis, MS) for their efforts to address uncertainties surrounding the environmental fate and ecological effects of antimycin.

2 TABLE OF CONTENTS

PROBLEM FORMULATION

The problem formulation establishes the direction and scope of an ecological risk assessment. According to the Guidelines for Ecological Risk Assessment[1], problem formulation consists of defining the problem and purpose for the assessment, and developing a plan for analyzing and characterizing risk. The critical components of the problem formulation are selection of the assessment endpoints, formulation of risk hypotheses, development of a conceptual model, and development of an analysis plan. The analysis plan and supporting rationale are aimed at determining whether the labeled uses of antimycin A as a piscicide could result in exposures that cause unreasonable adverse effects (risk) to non-target organisms including those Federally listed as threatened or endangered species (hereafter referred to as "listed"). This assessment was developed as part of the supporting information to determine the eligibility of antimycin A for re-registration. The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) requires that registered pesticide uses do not pose unreasonable adverse effects to the environment, and the Endangered Species Act requires that regulatory actions are not likely to adversely affect listed species or their habitats.

2.1 Stressor Source and Distribution

2.1.1 Source and Intensity

The source of the stressor considered in this ecological risk assessment is the application of antimycin A according to its label. Antimycin A was discovered in 1945 and is used as a general fish toxicant (piscicide); piscicidal applications are made directly to water. The label for Fintrol® Concentrate states that the effective treatment concentration is 25 µg/L for control of short nose gar (*Xenocara dolichopterus*), bowfin (*Amia calva*), goldfish (*Carrasius auratus*) and catfish (*Ictalurus* spp.) when the pH is 8.5 or more and when water temperatures are below 16°C (60°F); no maximum application rate is stated on the label however. Piscicidal applications, in general, are assumed to occur once a year since this type of application typically results in a complete fish kill[2].

2.1.2 Physical/Chemical/Fate and Transport Properties

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[3,4]—four "major" and four "minor"—that are distinguished by their chemical substituents. Preliminary estimates of the physical and chemical properties that determine the fate and transport of antimycin A are detailed in **Table 1** and were estimated using a quantitative structure activity-

[1] U.S. EPA. 1998. Guidelines for Ecological Risk Assessment. Risk Assessment Forum, Washington, DC. EPA 630/R-95/002F April 1998.
[2] Finlayson, B.J., R.A. Schnick, R.L. Cailteux, L. DeMong, W.D. Horton, W. McClay, C.W. Thompson, and G.J. Tichacek. 2000. Rotenone Use in Fisheries Management: Administrative and Technical Guidelines Manual. American Fisheries Society, Bethesda, Maryland.
[3] Abidi, S.L. and B.R. Adams. 1987. 1H and 13C resonance designation of antimycin A1 by two-dimensional NMR spectroscopy. Magn. Reson. Chem. 25: 1078-1080.
[4] Abidi, S.L. 1988. High-performance liquid chromatographic separation of subcomponents of antimycin A. J. Chromatogr. 447: 65-79.

based calculator, open literature, and registrant-submitted studies. There is a high degree of uncertainty surrounding the quantitative estimation of the environmental properties of antimycin. However, antimycin appears to sorb to sediment (potentially at a high level), degrade primarily by hydrolysis, not volatilize, not readily dissolve in water, and not bioaccumulate.

Table 1. Physical and chemical properties of antimycin A.

CAS Number	1397-94-0	
SMILES Notation O1C(=O)C(NC(=O)Cc2c(O)c(NC(=O))ccc2)C(C)OC(=O)C(CCCCCC)C(OC(=O)CC(C)C)C1		
Molecular formula: $C_{28}H_{40}N_2O_9$		
Property	**Value**	**Reference**
Molecular weight	548.6 g mol^{-1}	estimated - ASTER, 2004[5]
Melting point	149 - 150 °C	MERCK Index
Koc	10 – 10,000	estimated - ASTER, 2004; indirect estimation from submitted degradation study (MRID 45895901)
Water solubility (20 EC)	69 mg L^{-1} to insoluble	Estimation of 69 mg/L is based on ASTER, 2004; "insoluble" from Walker et al. (1971)
Vapor pressure (25 EC)	$2.31x10^{-15}$ mm Hg	estimated - ASTER, 2004
Henry's Law constant	$2.42\ x10^{-17}$ atm-m^3 mol^{-1}	estimated - ASTER, 2004
Hydrolysis half-life (25 EC)	Minutes to months, some (but inconsistent) evidence suggests dependance on pH; faster degradation as pH increases	estimated - ASTER, 2004; open literature; submitted studies (MRID 46023101)
Aerobic Aquatic Metabolism half-life	High uncertainty (possibly on the order of days)	submitted study (MRID 45895901)
Bioconcentration Factor (BCF)	350x	estimated - PBT Profiler

2.1.3 Pesticide Type, Class, and Mode of Action

Antimycin A is an antibiotic derived from the soil mold *Streptomyces*. Antimycin A uncouples oxidative phosphorylation by blocking the electron transport pathway to Complex III within the mitochondria.[6]

5 *Ibid* ASTER 2004.

6 Bettermann, A.D., J.M. Lazorchak and J.C. Dorofi. 1996. Profile of toxic response to sediments using whole-animal and in vitro submitochondrial particle (SMP) assays. Journal of Environmental Toxicology and Chemistry 15(3): 319 – 324.

Antimycin A is formulated as a liquid concentrate with a single formulation, Fintrol® Concentrate[7]. Antimycin A is applied to water by drip-feed device as part of a drip station, backpack sprayer, boat bailer, and sprayer.[8] Spraydrift from anitmycin A applications is not considered likely even when applied by backpack sprayers since hand-held wands are used to dispense the spray relatively close to the surface of the water.

2.1.4 Overview of Pesticide Usage

Antimycin A is used as a piscicide. According to a survey of fishery management agencies in the United States and Canada conducted by the American Fisheries Society (AFS) between 1991 and 2001, a total of 1,138 kg of antimycin were used by State/Federal resource management agencies[9]. In the latter half of the decade, antimycin use increased 75% due primarily to increased use in flowing waters. The average annual use was 5.05 kg (114 units) with each unit capable of treating 0.009 hm^3 at 5 ppb. The survey reported that the private sector used roughly 529 kg (1,166 lbs) and that private aquaculture facilities accounted for roughly three-quarters of the antimycin purchased by the private sector.

Also, according to the AFS survey, the primary use of antimycin has been to restore listed species, accounting for 33% of the water treated and 49% of the antimycin used. Renovation of fish populations was the second most frequent use of antimycin accounting for 29% of the waters treated and 27% of the antimycin applied. The third largest use was restoration of native fishes accounting for 26% of the waters treated and 19% of the antimycin used. In standing waters, 48% of the total antimycin used was to manipulate fish populations; in flowing waters, the majority (99%) of antimycin used was to restore native or listed species[10]. The primary use of antimycin in private aquaculture is for removal of scaled fish from catfish fingerling and food fish production ponds.

2.2 Receptors

2.2.1 Ecological Effects

Table 2 provides taxonomic groups and test species used to indicate the potential for ecological effects in screening-level risk assessment. Within each of these very broad taxonomic groups, an acute and/or chronic endpoint is selected from the available test data (see Section 2.3).

---

[7] EPA Registration No. 39096-2

[8] Special Review and Reregistration Division Use Closure Memo dated October 26, 2005.

[9] Finlayson, B.J., R.A. Schnick, R.L. Cailteux, L. DeMong, W.D. Horton, W. McClay, and C.W. Thompson. 2002. Assessment of Antimycin A Use in Fisheries and its Potential for Reregistration. Fisheries 27(6): 10 -18

[10] *Ibid* Finlayson *et al.* 2002.

Table 2. Taxonomic groups and most sensitive test species evaluated for ecological effects.		
Taxonomic group	**Example(s) of representative species**	**Endpoint used**
Birds[a]	Mallard duck (*Anas platyrhynchos*) Bobwhite quail (*Colinus virginianus*)	Chronic, NOAEC Acute, LD_{50}/LC_{50}
Mammals	Laboratory rat (*Rattus norvegicus*)	Acute LD_{50} and Chronic NOAEL/NOAEC
Terrestrial insects	Honeybees (*Apis mellifera*)	Acute Oral LD_{50}
Freshwater fish[b]	Rainbow trout (*Oncorhynchus mykiss*) Fathead minnow (*Pimephales promelas*)	Acute LC_{50} Chronic NOAEC
Freshwater invertebrates	Water flea (*Daphnia magna*) Midge (*Chironomus riparius*)	Acute LC_{50} and Chronic NOAEC Chronic NOAEC (Benthic)
Estuarine/marine fish	Sheepshead minnow (*Cyprinodon variegatus*)	Acute LC_{50}
Estuarine/marine invertebrates	Mysid shrimp (*Mysidopsis bahia*) Eastern oyster (*Crassostrea virginica*)	Acute LC_{50} Acute EC_{50}
Terrestrial plants[c]	Monocots – corn (*Zea mays*) Dicots – soybean (*Glycine max*)	Acute (Tier I, no EC_{50} estimated)
Aquatic plants and algae	Green algae (*Selenastrum capricornutum*)	Acute EC_{50}

[a]Birds are used as surrogates for terrestrial phase amphibians and reptiles (US EPA, 2004).
[b]Freshwater fish are used as surrogates for aquatic phase amphibians (US EPA, 2004).
[c]Four species of two families of monocots, of which one is corn; six species of at least four dicot families, of which one is soybeans.

2.2.2 Ecosystems at Risk

Antimycin A may be applied directly to both static (lentic) and flowing (lotic) waters in a manner that disperses the compound throughout the water column for maximum efficacy. Residues can then be managed to prevent release from the treated portion of the water body.

When antimycin A is applied directly to water, the ecosystem at risk consists primarily of that particular water body, but also includes the downstream outlet of the water body, if one exists. Steps may be taken to accelerate the natural degradation of antimycin A in order to reduce the risks of exposures occurring outside of the treatment area. Within the water body, compartments of concern are the water column, sediments, and pore water. Organisms of concern include fish, aquatic invertebrates, aquatic-phase amphibians, aquatic plants (algae and vascular), and birds or mammals that may rely on the water body as a habitat and/or drinking water source. Given the apparent propensity of antimycin to sorb to organic matter and depending on the flow regime and water quality characteristics, deposition of antimycin A-contaminated sediment into downstream flood plains, marshes, or estuaries may pose a concern. While antimycin A likely sorbs to sediment, it appears to have a low potential to bioaccumulate in aquatic organisms.

2.3 Assessment Endpoints

Agency guidelines define assessment endpoints as "explicit expressions of the actual environmental value that is to be protected."[11] Operationally, the environmental value is represented by an ecological entity and associated attributes or characteristics. The assessment endpoints for this ecological risk assessment are survival, growth, and reproduction of terrestrial and aquatic animals and plants. Specifically, this assessment addresses birds, mammals, reptiles, amphibians, fish, terrestrial and aquatic invertebrates, and terrestrial and aquatic plants. These endpoints are meant to reflect population sustainability and community diversity within ecosystems. These are standard assessment endpoints that are considered for the majority of pesticides with agricultural uses.

Measures of effect and measures of exposure are explicit toxicity and exposure measurements or estimates that are used to identify risks. Measures of effect are used to relate pesticide exposure to potential effects on the assessment endpoints, which are not explicitly evaluated in the assessment. Measures of exposure are typically values derived from chemical use information and standard fate, transport and exposure models. The toxicity and exposure values are used to generate a risk quotient, which is considered a screening-level estimate of risk only. Additional refinements may be triggered if the screen identifies potentially unacceptable levels of risk. Refinements may include the selection of assessment endpoints more directly relevant to populations or communities, spatially-explicit identification of risks, and the use of probabilistic methods for risk estimation.

Assessing risks to reptiles and amphibians represents unique challenges. Currently, data on direct toxicity to reptiles and amphibians are not required as part of the standard dataset submitted to the Agency for pesticide registration or re-registration and these data are only occasionally available from the literature. For ecological risk assessments in the Office of Pesticide Programs (OPP), if risks to birds are below the level of concern, then risks to reptiles are also assumed to be low. For amphibians, freshwater fish are considered a suitable surrogate for the aquatic life-stage and birds a suitable surrogate for the terrestrial life-stage.

2.4 Conceptual Model

2.4.1 Risk Hypotheses

The risk hypothesis that is presumed for the ecological risk assessment of antimycin A is based on the use and characteristics of this compound. Based on the fact that antimycin A is applied directly to water, it is presumed that aquatic environments are at risk. The following risk hypothesis is presumed for this screening-level assessment:

> *The use of antimycin A in accordance with the label results in adverse effects on survival and/or fecundity to non-target aquatic plants and animals. To the extent that terrestrial animals may feed off of dead or dying fish in the treatment area and/or ingest antimycin-treated water, terrestrial animals may experience adverse effects as well.*

[11] U.S. EPA. 1998. Guidelines for Ecological Risk Assessment. Risk Assessment Forum, Washington, DC. EPA 630/R-95/002F April 1998.

2.4.2 Conceptual Model Diagram

A conceptual model was developed for antimycin A to reflect its uses as a piscicide (direct application to water). The diagram in **Figure 1** includes the stressor (antimycin A), the source of the pesticide and/or transport pathway, exposure media, exposure points, biological receptors, and attribute changes (effects).

When used as a piscicide, antimycin A is deposited directly into a receiving water, which may be a stream, pond, or lake/reservoir. Once in the water column, non-target aquatic organisms may be exposed to antimycin A via three main exposure pathways and two minor pathways.

1. Antimycin A will initially be distributed in the water column of the receiving water, and organisms in the water column (aquatic invertebrates, fish, amphibians and plants) may be directly exposed through contact. Organisms can take up soluble antimycin A through the gills or integument. If sufficient concentrations exist, then individual toxic effects in aquatic invertebrates, fish, aquatic-phase amphibians and/or plants can result. This may result in reductions in populations of species within these taxa. This is expected to be the main route of exposure for antimycin A.

2. Antimycin A released into water will likely sorb to sediment and suspended solids. Receptors such as aquatic invertebrates, fish, and aquatic-phase amphibians may be exposed to sorbed antimycin A through incidental ingestion of sediment and/or suspended solids. This can result in changes in populations based on reduced survival, growth and reproduction.

3. Antimycin A may sorb to plant surfaces. Ingestion of plant materials with sorbed antimycin A can result in a similar exposure pathway as described for sediment and suspended solids. Nonvascular or vascular plants may also be exposed to antimycin A through direct contact or root uptake. This could result in changes in plant populations due to direct toxicity. However, since no plant toxicity data are available for antimycin A, potential risks to aquatic plants cannot be evaluated.

4. Antimycin A residues may accumulate in target and non-target organisms, although this is not considered likely given the low estimated bioconcentration factor. Other organisms that live in or around the treatment area may be exposed to antimycin A by consuming treated organisms.

5. Direct exposure of terrestrial animals/plants to antimycin is not likely since the chemical is only applied to water bodies. Indirect exposure to both mammals and birds is possible through the consumption of dead and dying fish and larger invertebrates and through ingestion of antimycin-treated water.

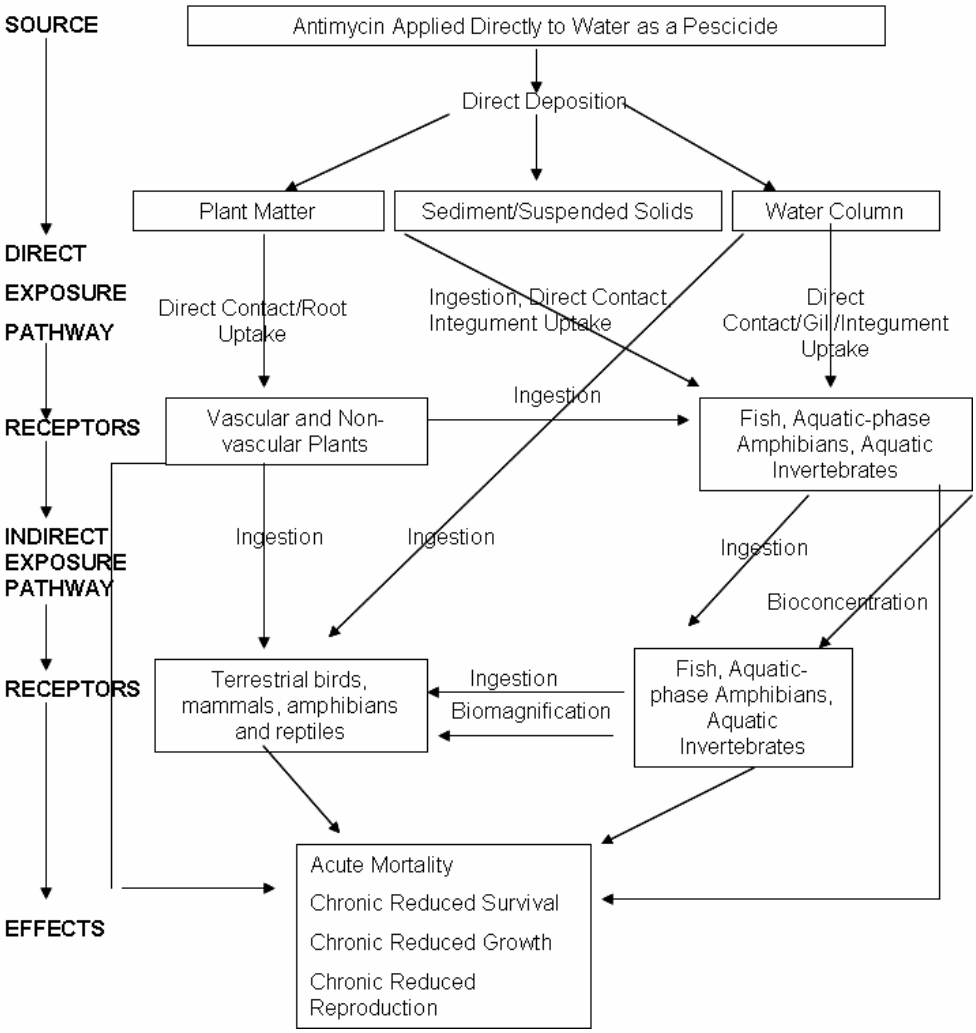


Figure 1. Conceptual model for antimycin A depicting source, direct and indirect routes of exposure, biological receptors and possible effects.

3 USE CHARACTERIZATION

Antimycin A is registered for use as a piscicide. As stated in the use closure memo (dated October 26, 2005), there is a single supported registration (Fintrol®; EPA Registration number: 39096-2) that contains antimycin A as an active ingredient. Antimycin A is applied to water by a

drip-feed device as part of a drip station, backpack sprayer, boat bailer, and sprayer. Drip stations are typically used in streams and rivers inaccessible to boat traffic; a photograph of a drip station is shown in **Figure 2**. 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 and rivers. Deeper water bodies may require the use of a pump mechanism (to ensure adequate mixing throughout the water column) where antimycin is dispensed through a perforated hose stretching the length of the water column or is delivered through the propeller wash.[12]

Figure 2. Antimycin A drip station used for applying product to high gradient (mountain) streams.

Environmental factors such as water body size, flow rate, pH, 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. For example, antimycin A is more effective in warm water; thus, less antimycin A may be required during the summer months than the winter months for the same treatment site, assuming other factors remain constant.

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 at roughly 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 to restore listed or indigenous species.[13]

In a selective kill, the water body is treated at 0.5 to 1.0 µg/L of antimycin A to eliminate only small, scaled fish. A common objective of a selective kill is to eliminate smaller fish to free up food and other resources for larger fish. Selective kills at higher concentrations are also used in catfish production to eliminate scaled fish that commonly reduce the catfish yields of

[12] Wormell, L. 2005. Use Closure Memo. Special Review and Reregistration Division Memo dated October 26, 2005.

[13] *Ibid* Wormell 2005.

commercial catfish farmers. According to the Fintrol® label, scaled fish in aquaculture ponds succumb to treatment at 5 to 10 µg/L of antimycin A, whereas catfish generally tolerate up to 20 µg/L.[14] Based on information provided by the registrant[15] the majority of Fintrol® use in [catfish] aquaculture is for food-fish production and not finger-production. According to aquaculture use information collected by the Southern Regional Aquaculture Center[16], Fintrol® use is roughly equally divided between food-fish and fingerling-production ponds to eliminate scaled fish at various points within the production cycle.

According to the Fintrol® label, in both complete and selective kills, dead fish may be collected and disposed of or left to biodegrade. Additionally, 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.

3.1 Detoxification

In situations where fish kills cannot extend beyond a certain point downstream, the Fintrol® label provides instructions for detoxifying antimycin A with potassium permanganate ($KMnO_4$) at a treatment concentration of 1 mg/L (part per million). Potassium permanganate (CAS No. 7722-64-7) is a strong oxidizing agent commonly used to purify drinking water and kill pond algae. According to the product label, antimycin may be considered detoxified when fingerling rainbow trout (*Oncorhynchus mykiss*) or juvenile bluegill sunfish (*Lepomis macrochirus*) survive for at least 48 hours in holding tanks (live-cars) placed 91 meters (100 yards) downstream from the site of potassium permanganate application. Depending on environmental conditions (*e.g.* total organic carbon) higher $KMnO_4$ treatment rates (*e.g.* 3 – 4 mg/L) may be required to deactivate antimycin A[17].

3.2 Usage and Use Rates

There is currently one active antimycin A registration (Section 3). There is currently no emergency exemption (Section 18) use or special local need (Section 24c) use. According to the registrant, less than 45 kg (100 lbs.) of antimycin A are used annually in the US. EPA's Screening Level Usage Analysis returned no data on agricultural or non-agricultural uses of antimycin A. Antimycin A is available as a soluble concentrate/liquid. Retreatment and reentry intervals are not specified on the current label. According to the registrant and based on sales data, antimycin is typically used for fishery management purposes between August and September while it is in aquaculture primarily in May through September[18].

Table 3 presents the antimycin A labeled use rates that are being considered for reregistration. The risk assessments will be based on the formulations, use rates, retreatment

14 *Ibid* Wormell 2005.

15 Personal communiction, Mary Romeo, President, Aquabiotics Corporation. 2006.

16 personal communication Dr. Craig Tucker, Director, National Warmwater Aquaculture Center and Southern Regional Aquaculture Center, 2006

17 Personal communication, Mr. Steve Moore, Supervisory Fishery Biologist, Great Smoky Mountain National Park, 2006.

18 Personal communication, Ms. Mary Romeo, Presidient, Aquabiotics Corporation, 2006.

intervals, and application methods presented in the table below.[19] As stated previously, the Fintrol® label indicates that rates as high has 25 µg/L are needed to be effective for control of certain fish when pH is 8.5 or more and water temperatures are below 16°C. Although maximum treatment rates are not stated on the label, this risk assessment is based on an upper-bound treatment rate of 25 µg/L applied once per year.

Table 3. Supported uses of antimycin A on use groups: aquatic food crops (D1), aquatic non-food outdoors (E1) and aquatic non-food industrial (F1).[20]

Use Site[a]	Max. Rate per App[b] (ppb)	Max. Rate Unit/Area UG[c]	Form[d]	Max.# Apps cc & yr[e]	Max. App Rate/ cc & yr[f]	Min. App Interval (days)[g]	Application Equipment[h] //Type[i]
NON-FOOD/NON-FEED USES							
lakes/ponds/reservoirs (without human or wildlife use)	25 "roughly"	ppb/5 gal F1	SC/L[j]	NS[k]	NS	NS	Backpack sprayer/ Boat bailer/ Drip-Feed device/ Sprayer //Water treatment
streams/rivers/ channeled water	25 "roughly"	ppb/5 gal E1	SC/L	NS	NS	NS	Backpack sprayer/ Boat bailer/ Drip-feed device/ Sprayer //Water treatment
swamps/marshes/ wetlands/stagnant water	25 "roughly"	ppb/5 gal E1	SC/L	NS	NS	NS	Backpack sprayer/ Boat bailer/ Drip-feed device/ Sprayer //Water treatment
FOOD/FEED USES							
commercial fishery water systems	25 "roughly"	ppb/5 gal D1	SC/L	NS	NS	NS	Backpack sprayer/ Boat bailer/ Drip-feed device/ Sprayer //Water treatment

[a] Use Site: The use site refers to the entity (crop, building, surface or article) where a pesticide is applied and/or which is being protected.

[b] Max.Rate per App: Maximum dose for a single application to a single site. System calculated.

[c] Max.Rate Unit/Area: Units and Area associated with the maximum dose (UG: Use Group codes)

[d] Form: The physical form of the end use product found in the container.

[e] Max. # Apps cc & yr: maximum number of applications.

[19] *Ibid* Wormell 2005

[20] *Ibid* Wormell 2005

[f] Max. App Rate/cc & yr: maximum amount of pesticide product that can be applied to a site in one growing season (/cc) or during the span of one year (/yr).

[g] Min. App Interval (days): minimum retreatment interval between applications in days (aggregated).

[h] Application Equipment: equipment used to apply pesticide (aggregated).

[i] Application Type: type of pesticide application (aggregated).

[j] SC/L: Soluble Concentrate/liquid

[k] NS: Not Specified (on label).

The extent to which direct applications of antimycin A to water as a fish control agent can be limited to the desired treatment area is dependent on the rigor of the protocols used to apply the chemical and the degree to which they are followed. Biological and chemical monitoring of the treatment area and deactivation of antimycin A where feasible could track and limit exposure of non-target organisms. While many state and federal agencies follow standard operating procedures (SOPs) that limit exposure, the SOPs are not uniformly applied across all potential users. Also, since the environmental fate database is incomplete for antimycin A, the extent to which antimycin A will persist and potentially move outside treated areas is uncertain.

Although not required by the label, relatively rigorous application procedures are typically followed by resource managers using this restricted use pesticide; **Appendix B** contains an example of a standard operating procedure followed by the State of New Mexico Department of Game and Fish. Additionally, state and federal resource managers participate in training courses such as *Rotenone and Antimycin Use in Fish Management*, offered by the U.S. Fish and Wildlife Service National Conservation Training Center (Shepherdstown, WV) to familiarize staff members with procedures for planning and executing fish management objectives using piscicides.

Although not required by the label, antimycin is typically deactivated following treatment of lotic systems. The label recommends that potassium permanganate be applied at a rate of 1 ppm to the outflow and that additional permanganate may be needed if the stream has a high permanganate demand, *i.e.*, organic carbon content. Drip systems of hose-and-clamp or carburetor types are recommended on the label to continuously disperse a solution of potassium permanganate into the water at the discharge outlet. Deactivation is maintained until all of the Fintrol-treated water has passed through the station and fish in holding containers (livecars) placed 91 meters (100 yds) downstream of the detoxification station remain alive for at least 48 hours.

4 ENVIRONMENTAL FATE CHARACTERIZATION

4.1 Background

Antimycin A is a relatively minor-use piscicide used primarily for direct applications to remote mountain streams, lakes and ponds and fishery operations. Several factors complicate the estimation of environmental concentrations of antimycin, among them non-strict application rates, sparse fate data, and insensitive detection methods. Because of these uncertainties quantification of antimycin exposure is speculative.

With regard to labeled use rates, the antimycin label provides only guidance and is not prescriptive with regard to the maximum amount of antimycin that may be applied to a water body. The label only refers to "rough" estimates of effective doses that are to be applied to water bodies, and it is left to the user to determine the actual dose. The label recommends that the amount of antimycin used should be determined by bioassays using the specific water to be treated. Such labeling makes it difficult to estimate how much antimycin may enter the environment since there is no maximum label rate.

Another difficulty is that there are comparatively little EPA-guideline fate data available for evaluation of the fate of antimycin A in the environment. The registrant has submitted only a hydrolysis and an aerobic aquatic metabolism study, both of which the EPA classified as providing only supplemental information and not fulfilling the data requirement. There are, however, non-guideline studies (primarily concerning hydrolysis rates) that offer some insight into the fate of antimycin in the environment. The combination of results from the submitted studies and the open literature studies gives a wide range of degradation rates for antimycin, further complicating the estimation of potential antimycin environmental concentrations.

Finally, analytical techniques for determination of antimycin are very limited for the concentration levels that are used in the environment. The only method currently available is a bioassay, in which detections would be non-specific (unknown if antimycin or some other toxicant is present) and would only be detected after detrimental effects occur (fish death).

Although the above issues hinder the ability to calculate "standard" EECs in a manner similar to other pesticide assessments, rough estimates that include the uncertainties associated with fate properties and application rates are possible. By considering the uncertainties, a large range of possible EECs and dissipation times are calculated as described below.

4.2 Fate Properties

Antimycin A is a complex of 4 structures that is reportedly soluble in polar organic solvents (e.g. ethanol, acetone, chloroform) and relatively insoluble in water (Walker et al., 1964). Most of the available fate studies in the open literature are hydrolysis studies, and there are no direct studies that measure the sorption properties of antimycin. There are only two

registrant-submitted studies—a hydrolysis study and an aerobic aquatic metabolism study. When the fate studies are viewed together, some conflicting information results in uncertainty.

One possible mechanism of antimycin degradation is base hydrolysis as suggested in the literature.[21, 22, 23] The supplementary registrant-submitted hydrolysis study[24] using H^3-labeled antimycin also suggests that base hydrolysis is a mechanism of degradation, but hydrolysis rates reported by the registrant's study are significantly higher than the earlier open literature studies (**Table 4**). The study[25] indicates that antimycin in an aquatic environment at 25°C has a half-life of about 15 days at a pH of 5, 3 days at pH 7, and 3 hours at pH 9. Lee *et al.* (1971)[26] reported much faster degradation rates using yeast assays, with half-lives of about 5.5 hours at pH 7, and 20 minutes at pH 9.5. Hussain[27] found degradation half-lives of about 46 hours for antimycin (A1) at pH 7.55 and about 2 minutes at pH 9. However, a recent study by Kenneke (2006)[28], which suggests that hydrolysis is an important mechanism of degradation, does not clearly show that the degradation rate is related to pH. Kenneke (2006)[29] estimated half lives of around 4 to 10 hours in unmixed systems; whereas in mixed systems half lives ranged from 1 to 10 hours. Hydrolysis rates from the literature are summarized in **Table 1**. In all cases hydrolysis half lives are on the order of minutes to hours.

Transformation products were not identified in the registrant-submitted study[30] ,Lee *et al*.[31] or Kenneke[32]. In an anecdotal report, Walker *et al*.[33] claimed that antimycin degrades by base hydrolysis and produced a diagram showing the mechanism of degradation along with assumed breakdown products. Hussain[34] made similar claims as to the degradation products. These products are blastmycic acid, antimycin lactone, and antimycic acid. No quantitative information regarding the relative concentrations of the transformation products is available.

The only other registrant-submitted environmental fate study is an aerobic aquatic transformation study[35] which indicated that antimycin A degrades with a half-life in the range of

---

21 Walker, C.R., R.E. Lennon, and B.L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 2 (Circular No. 186). 18 pp.

22 Hussain, A. 1969. Kinetics and mechanisms of hydrolysis of antimycin A in solution. Journal of Pharmaceutical Sciences 58: 316-320.

23 Lee, T.H., Derse, P.H., and Morton, S. 1971. Effects of Physical and Chemical Conditions on the Detoxification of Antimycin. *Transactions of the American Fisheries Society* 1971; 100: 13–17

24 Heim, D. 2003. Hydrolysis Determination for Antimycin A Complex. Project Number 47132. Unpublished study prepared by Analytical Bio-Chemistry Labs, Inc. 63 p. (MRID 460231-01)

25 *Ibid* Heim 2003.

26 *Ibid* Lee *et al*. 1971.

27 *Ibid* Hussain 1969.

28 See Appendix G for Kenneke 2006

29 *Ibid* Kenneke 2006.

30 *Ibid* Heim 2003.

31 *Ibid* Lee *et al*. 1971.

32 Ibid Kenneke 2006

33 *Ibid* Walker *et al.* 1964.

34 *Ibid* Hussain 1969.

35 Heim, D. 2003. Determination of Aerobic Aquatic Metabolism for Antimycin A Complex: Amended Final Report. Lab Project No. 47313. Unpublished study prepared by ABC Laboratories, Inc. 79 p (MRID 458959-01).

23 to 47 days in pH 6.5 water. This half-life appears to be substantially longer than the half-life due to hydrolysis alone at this pH (see above and **Table 4**), which adds some uncertainty to the half-lives reported for the hydrolysis study. However, since the aerobic aquatic metabolism study system included sediment, the longer half-life could be due to high sorption of antimycin to sediment which may shield antimycin from hydrolytic degradation (although this is purely speculative since sorption studies are unavailable).

Although the registrant did not provide sorption studies for antimycin, indications from the aerobic aquatic metabolism study are that antimycin A does sorb significantly to sediment. Rough estimates of sorption can be made from the aerobic aquatic metabolism study by considering the relative distribution of antimycin in the water and the sediment as measured in that study. If equilibrium between the water and the sediment were assumed throughout the aerobic aquatic metabolism study then K_d values would range from about 1 to 88 ml/g or K_{oc} values in the range of 84 to 10000 ml/g, which amounts to significant sorption.

In order to gain additional insight into antimycin, fate properties were also estimated with ASTER. **ASTER** (**AS**sessment **T**ools for the **E**valuation of **R**isk) was developed by the U.S. EPA Mid-Continent Ecology Division, Duluth, MN (MED-Duluth) to assist regulators in performing ecological risk assessments. ASTER is an integration of the AQUIRE (**AQU**atic toxicity **I**nformation **RE**trieval) toxic effects database and the QSAR (Quantitative Structure Activity Relationships) system, a structure-activity-based expert system. When empirical data are not available, mechanistically-based predictive models can be used to estimate ecotoxicology endpoints, chemical properties, biodegradation, and environmental partitioning. ASTER is designed to provide high quality data for discrete chemicals (when available in the associated databases), and QSAR-based estimates (when data are lacking). The QSAR system includes a database of measured physicochemical properties such as melting point, boiling point, vapor pressure, and water solubility as well as more than 56,000 molecular structures stored as SMILES (Simplified Molecular Input Line Entry System) strings for specific chemicals.[36] Based on ASTER model estimates, antimycin is not expected to be mobile in soil and sediment (log K_{oc}= 3.41) and the chemical has a relatively low potential for bioconcentrating in aquatic organisms (bioconcentration factor (BCF=350X). From ASTER, it appears that Antimycin A is not likely to be persistent in the environment and its low vapor pressure (2.31×10^{-15} mm Hg) and Henry's Law constant (2.42×10^{-17} atm-m^3 mol^{-1}) limit its volatility

[36] ASTER 2004. http://www.epa.gov/med/Prods_Pubs/aster.htm

Table 4. Antimycin fate properties		
Property	**Value**	**Source**
Molecular Wt	548.7	
Hydrolysis pH 3	9 hours	Kenneke (2006)
Hydrolysis pH 4	8.3 hours	Kenneke (2006)
Hydrolysis pH 5	10.5 hours	Kenneke (2006)
Hydrolysis pH 6	11 hours	Kenneke (2006)
Hydrolysis pH 7	7.1 hours	Kenneke (2006)
Hydrolysis pH 8	10 hours	Kenneke (2006)
Hydrolysis pH 9	3.4 hours	Kenneke (2006)
Hydrolysis pH 5	15 days	MRID 46023101
Hydrolysis pH 7	3 days	MRID 46023101
Hydrolysis pH 9	3 hours	MRID 46023101
Hydrolysis pH 4.5 – 5.5	>7 hours	Lee *et al.* (1971)
Hydrolysis pH 7 – 8	5.5 hours	Lee *et al.* (1971)
Hydrolysis pH 8.5	40 minutes	Lee *et al.* (1971)
Hydrolysis pH 9.5	20 minutes	Lee *et al.* (1971)
Hydrolysis pH 10	6 minutes	Lee *et al.* (1971)
Hydrolysis pH 7.55 (A1 only)	46 hours	Hussain (1969)
Hydrolysis pH 9 (A1 only)	2.2 hours	Hussain (1969)
Hydrolysis pH 10.1 (A1 only)	1 minute	Hussain (1969)
Hydrolysis (25^{o}C)	190 days	Estimated – ASTER, 2004
Water Solubility (20 ^{o}C)	69 mg L^{-1}	Estimated - ASTER, 2004
Koc	2500 ml/g	Estimated - ASTER, 2004
Koc	84 – 10000 ml/g	Estimated from aerobic study MRID 458959-01 (see text)
Aerobic Aquatic Degradation	20 to 47 days	MRID 458959-01
Bioconcentration Factor (BCF)	350x	Estimated – PBT Profiler
Henry's Law Constant	2.42 x10^{-17} atm-m^3 mol^{-1}	Estimated - ASTER, 2004
Vapor Pressure (25 EC)	2.31x10^{-15} mm Hg	Estimated - ASTER, 2004

4.3 Usage and Fate in Water Bodies

The antimycin label only gives "rough estimates" of the target concentration at which antimycin is to be used, and the label gives no upward limit on what concentration may be used. Rough estimates for target concentrations range from 25 ppb (for pH>8.5 and temperatures <60^{o}C) to 5 ppb (for pH<8.5 and temperatures >60^{o}C). The label suggests that the actual concentration that is to be used be confirmed with a bioassay. Because of the great latitude that the label gives, it cannot be determined *a priori* what amount of antimycin may be applied to a water body. For both stream and lake applications of antimycin, downstream movement of antimycin will occur; however, the extent is unknown. In many cases, application of antimycin

may coincide with the use of live-cars containing sensitive species that are placed downstream of the antimycin application, although this is not a label requirement. Use of live-cars with sensitive species would allow monitoring of the dissipation of antimycin effectiveness but is does not prevent antimycin from proceeding downstream, and there is some evidence that such downstream movement can be significant[37].

In one study, Tiffan and Bergersen[38] observed that antimycin was 100% effective at fish kills to at least 1.75 km downstream of a Colorado creek (pH = 6.3, 9-15^{o}C). Because there was a 100% fish kill at 1.75 km and no live-cars placed downstream of this distance, the effect of antimycin probably proceeded much farther downstream. In this creek, antimycin was applied to the creek at 8 ppb for 8 hours, which is a typical application. The reason that antimycin remained effective in this stream system is unclear (although the authors speculate stream gradient had an effect). In other streams examined by Tiffan and Bergersen[39], antimycin was effective to at least 0.5 km downstream.

4.4 Estimates of Water Concentration for Risk Assessments

When antimycin is applied to a water body (whether a lake, pond, or stream) the acute concentration that should be used for ecological assessments is the application concentration. The maximum application concentration is ambiguous but the label states that it is "roughly" 25 ppb. However, because of the significant uncertainties regarding the persistence and sorption properties of antimycin, predicted temporal concentration trends and chronic concentrations are also full of uncertainty. With this in consideration, ranges of estimates can be made by using the full range of possible degradation (hydrolysis) rates reported in **Table 4**. **Table 5** gives the range of chronic concentrations that may result following a 25 ppb application of antimycin to a water body (and with no consideration for sorption or other means of dissipation). Chronic concentrations vary considerably depending on the half-life assumed for antimycin. Such values apply to both static water bodies (*e.g.*, lakes) as well as flowing streams. In the case of streams, however, the chronic concentration would apply only if an organism were traveling downstream at the stream speed. Also in the case of streams, antimycin likely will dissipate due to hydrodynamic dispersion, sorption, and mixing with side channels; however, these factors cannot be readily determined.

One other factor that may be useful in assessing antimycin environmental exposure is the time that it would take antimycin concentrations to degrade below a certain level of toxicity. For example, **Table 6** presents the times that it would take antimycin to degrade from 25 ppb down to a concentration of 0.004 ppb (concentration where the most sensitive aquatic risk quotient would be equivalent to the acute risk level of concern of 0.5). These values apply to both streams and water bodies. For the case of streams the time would be associated with the downstream movement of antimycin, and if stream velocity were known these values could be associated with distance downstream of antimycin applications where antimycin remains toxic.

---

[37] Tiffan, K.F. and E.P. Bergersen. 1996. Performance of antimycen in high-gradient streams. North American Journal of Fisheries Management 16: 465-468.

[38] *Ibid* Tiffan and Bergersen. 1996.

[39] *Ibid* Tiffan and Bergersen 1996

In this regard, Tiffan and Bergersen[40] showed that antimycin can remain toxic in some circumstance for over 2 kilometers downstream of the antimycin application.

Table 5. Ranges of chronic concentrations depending on assumed half-life. Values in ppb. Initial concentration assumed to be 25 ppb. Higher initial concentrations would result in proportionally higher chronic concentrations.

Half-life	4 day	21 day	60 day	90 day	1 year
47 days	24	21	17	14	4.6
23 day	24	19	12	8.6	2.3
15 days	23	16	8.4	5.9	1.5
5 days	19	8.1	3.0	2.0	0.49
5.5 hours	2.1	0.39	0.14	0.09	0.022
20 minutes	0.13	0.023	0.0083	0.0055	0.0013

Table 6. Time for antimycin to get below LOC concentration (0.004 ppb) when applied at 25 ppb.

Assumed half-life	Time required to reach 0.004 ppb
47 days	592 days
15 days	189 days
5 days	63 days
5.5 hours	2.9 days
20 minutes	4 hours

5 ECOLOGICAL EFFECT CHARACTERIZATION

Toxicity testing reported in this section does not represent all species of bird, mammal, or aquatic organism. Only a few surrogate species for both freshwater fish and birds are used to represent all freshwater fish (2000+) and bird (680+) species in the United States. For mammals, acute studies are usually limited to Norway rat or the house mouse. Estuarine/marine testing is usually limited to a crustacean, a mollusk, and a fish. Also, neither reptiles nor amphibians are tested. The assessment of risk or hazard assumes that avian toxicity is similar to that of terrestrial-phase amphibians and reptiles. The same assumption is made for fish and aquatic-phase amphibians.

5.1 Categories of Acute Toxicity

In general, categories of acute toxicity ranging from "practically nontoxic" to "very highly toxic" have been established for aquatic organisms based on LC_{50} values (**Table 7**), terrestrial organisms based on LD_{50} values (**Table 8**), and avian species based on LD_{50} values (**Table 9**). Subacute dietary toxicity for avian species is based on the LC_{50} values (**Table 10**).

[40] *Ibid* Tiffan and Bergersen 1996.

Table 7. Categories for aquatic animal acute toxicity based on median lethal concentration in mg per liter (parts per million).	
LC_{50} (ppm)	**Toxicity Category**
<0.1	Very highly toxic
0.1–1	Highly toxic
>1–10	Moderately toxic
>10–100	Slightly toxic
>100	Practically non-toxic

Table 8. Categories for mammalian acute toxicity based on median lethal dose in mg per kilogram body weight (parts per million).	
LD_{50} (mg a.i./kg)	**Toxicity Category**
<10	Very highly toxic
10–50	Highly toxic
51–500	Moderately toxic
501–2000	Slightly toxic
>2000	Practically non-toxic

Table 9. Categories of avian acute oral toxicity based on median lethal dose in milligrams per kilogram body weight (parts per million).	
LD_{50} (ppm)	**Toxicity Category**
<10	Very highly toxic
10-50	Highly toxic
51-500	Moderately toxic
501-2000	Slightly toxic
>2000	Practically non-toxic

Table 10. Categories of avian subacute dietary toxicity based on median lethal concentration in milligrams per kilogram diet per day (parts per million).	
LC_{50} (ppm)	**Toxicity Category**
<50	Very highly toxic
50–500	Highly toxic
501–1000	Moderately toxic
1001–5000	Slightly toxic
>5000	Practically non-toxic

5.2 Toxicity to Aquatic Animals

5.2.1 Freshwater Fish, Acute

A total of 15 acute toxicity studies of technical grade (>95% active ingredient) on freshwater fish are contained in the EFED ecotoxicity database for antimycin A (**Table 11**). Paddlefish (*Polyodon spathula*) are the most sensitive (LC_{50}=0.001 µg/L) freshwater fish species tested. To ensure the risk assessment is as protective as possible of non-target species, the lowest scientifically defensible toxicity value available is used to evaluate acute risks to freshwater fish. Although paddlefish are the most sensitive species reported, the raw data used to support this endpoint could not be evaluated and therefore this value cannot be used to quantitatively assess risk. The most sensitive species for which raw data could be reviewed is coho salmon (*Oncorhynchus kisutch*); therefore, the freshwater fish acute toxicity endpoint (96-hr LC_{50}) is 0.009 µg/L. Based on the sensitivity of freshwater fish to antimycin, the piscicide is classified as very highly toxic to fish on an acute exposure basis.

Based on the available data, fish in the family Ictaluridae (catfish), like black bullhead (*Ictalurus melas*) and channel catfish (*Ictalurus punctatus*), are the least sensitive species tested which accounts for how antimycin can be used to selectively remove scaled fish from catfish aquaculture ponds. Although paddlefish do not have scales and yet are the most sensitive species to antimycin, this phylogenic primitive fish is not related to the catfish but is instead distantly related to sturgeon.

Toxicity testing of formulated product (1 to 10% active ingredient) indicates that formulated products are less toxic than the technical grade active ingredient; the 96-hr LC_{50} for bluegill sunfish is 1.18 µg/L for the formulated product (**Table 12**) whereas the technical grade active ingredient has a 96-hr LC_{50} of 0.034 µg/L. Similarly, rainbow trout had LC_{50} values ranging from 0.63 to 185 µg/L for formulated product while technical grade antimycin had an LC_{50} averaging 0.011 µg/L.

OPP utilized the ECOTOX (Ecotoxicology Database System)[41] on-line database to conduct an open literature review in an attempt to supplement the registrant-submitted data. ECOTOX is a comprehensive computer-based system that provides single chemical toxic effect data for aquatic life, terrestrial plants and terrestrial wildlife derived predominately from peer-reviewed literature. The literature relevant to the exposure and toxic effects of antimycin A and the metabolites was collected, reviewed and evaluated for inclusion into this chapter. Citations and abstracts were obtained by searching the following commercial or publicly available databases: TOXLINE, MEDLINE, BIOSIS previews, AGRICOLA, and AQUIRE, as well as Dissertation Abstracts. For a more in-depth discussion of the ECOTOX on-line database see http://www.epa.gov/ecotox/. While a number of acute toxicity studies using various fish species are available through ECOTOX, none of the toxicity values obtained from the open literature are more sensitive than that of the coho salmon.

Additional data were submitted by the registrant and represented a compendium of unpublished studies[42]; however, information on the toxicity of antimycin was not of sufficient quality to use quantitatively in this ecological risk assessment.

[41] ECOTOX (ECOTOXicology database). 2005. Maintained by the U.S. EPA ORD and the National Health and Environmental Effects Research Laboratory's (NHEERL's) Mid-Continent Ecology Division. http://www.epa.gov/ecotox.

[42] Ayerst Laboratories. 1964. Efficacy of Antimycin. (Compilation: unpublished study received in 1994 under 8991-5l CDL: 005666-A (Accession No. l 00135924))

Table 11. Freshwater fish acute toxicity of technical grade antimycin A.					
Species	**% ai**	**96-hour LC_{50} (µg a.i./L) (95%C.I.)**	**Toxicity Category**	**MRID/ Accession No.**	**Study Classification**
Bluegill (*Lepomis macrochirus*)	95.5	0.034 (0.008 - 0.141)	very highly toxic	400980-01[43]	Supplemental
Green Sunfish (*Lepomis cyanellus*)	95.5	0.22 (0.128 - 0.416)	highly toxic	400980-01	Supplemental
Rainbow Trout (*Oncorhynchus mykiss)*	95.5	0.012 (0.0066 - 0.023)	very highly toxic	400980-01	Supplemental
Rainbow Trout (*Oncorhynchus mykiss)*	95.5	0.010 (0.0056 - 0.019)	very highly toxic	400980-01	Supplemental
Cutthroat Trout (*Oncorhynchus clarki*)	95.5	0.057 (0.019 - 0.166)	very highly toxic	400980-01	Supplemental
Coho Salmon (*Oncorhynchus kisutch*)	95.5	0.009 (0.006 - 0.014)	very highly toxic	400980-01	Supplemental
Lake Trout (*Salvelinus namaycush*)	95.5	0.053 (0.045 - 0.063)	very highly toxic	400980-01	Supplemental
Goldfish (*Carassius auratus*)	95.5	0.180 (0.099 - 0.348)	highly toxic	400980-01	Supplemental
Fathead Minnow *(Pimephales promelas*)	95.5	0.025 (0.008 - 0.074)	very highly toxic	400980-01	Supplemental
Black Bullhead (*Ictalurus melas*)	95.5	4.8 (3.4 - 6.8)	highly toxic	400980-01	Supplemental
Channel Catfish (*Ictalurus punctatus*)	95.5	1.36 (1.02 - 0.82)	highly toxic	400980-01	Supplemental
Mosquitofish (*Gambusia affinis*)	95.5	0.19 (0.114 - 0.324)	highly toxic	400980-01	Supplemental
Largemouth Bass (*Micropterus salmoides*)	95.5	0.24 (0.16 - 0.35)	highly toxic	400980-01	Supplemental
Yellow Perch (*Perca flavescens*)	95.5	0.04 (0.031 - 0.052)	very highly toxic	400980-01	Supplemental
White Crappie (*Pomoxis annularis*)	95.5	0.34 (0.27 - 0.42)	highly toxic	400980-01	Supplemental
Paddlefish (*Polyodon spathula*)	95.5	0.001 (0.0004 - 0.003)	very highly toxic	400980-01	Supplemental

[43] Mayer, F. and M. Ellersieck. 1986. Manual of Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater animals. U. S. Fish and Wildlife Service. Resource Publication 160. 579 p. (MRID 400980-01)

Table 12. Freshwater fish acute toxicity of formulated antimycin A					
Species	**% ai**	**96-hour LC_{50} (µg a.i./L) (95%C.I.)**	**Toxicity Category**	**MRID/ Accession No.**	**Study Classification**
Bluegill (*Lepomis macrochirus*)	10	1.18 (0.9 - 1.51)	very highly toxic	TN 901	supplemental
Rainbow Trout (*Oncorhynchus mykiss)*	10	185 (134 - 255)	highly toxic	TN 944	supplemental
Rainbow Trout (*Oncorhynchus mykiss)*	1	0.63 (0.58 - 0.68)	very highly toxic	TN 35	supplemental
Bluegill (*Lepomis macrochirus*)	1	48-hr LC_{50}=29.5 (26 – 33)	very highly toxic	TN 1533	supplemental
Bluegill (*Lepomis macrochirus*)	1	48-hr LC_{50}=29.5 (20.1 – 25.2)	very highly toxic	TN 153	supplemental

The toxicity of antimycin to fish and the efficacy of treatments have been related to pH, temperature[44], and the gradient (velocity/flow rate) of water being treated. Increased temperatures and more alkaline pH levels tend to increase the toxicity of antimycin while cooler water temperatures and more acidic conditions tend to decrease the toxicity/effectiveness of antimycin. **Figure 3** depicts that at 12°C, 0.8 µg/L did not kill any green sunfish after 24 hours; however, at 22°C and the same treatment concentration, all of the sunfish were killed. Similarly at 12°C no bluegill were killed after 24 hrs following treatment at 0.2 µg/L; however, at 22°C all of the bluegill were dead after 24 hours after exposure to 0.2 µg/L[45].

[44] Berger, B.L., R.E. Lennon and J.W. Hogan. Laboratory Studies of Antimycin as a Fish Toxicant. Pages 1 – 21

[45] Walker, C.R., R.E. Lennon and B.L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. Pages 1 – 18 in Investigations in Fish Control No. 2. Bureau of Sport Fisheries and Wildlife Circular 186

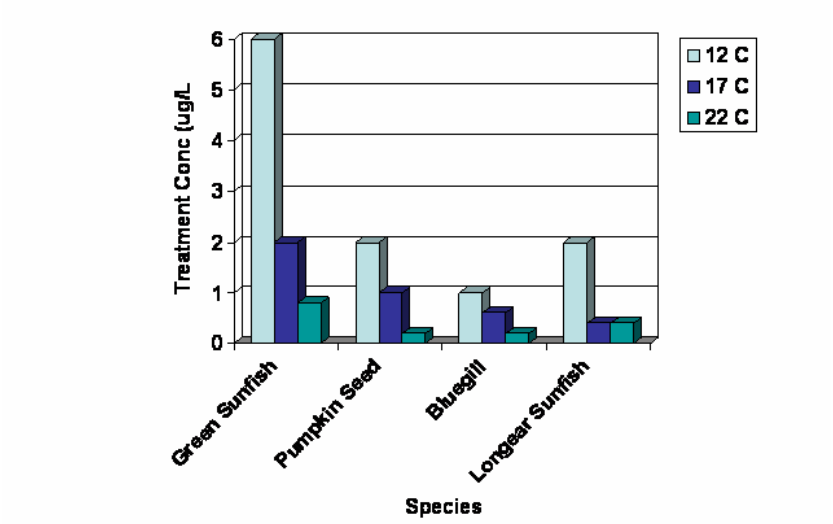


Figure 3 Complete survival of fish treated with antimycin A at 12, 17 and 22°C

5.2.2 Freshwater Fish, Chronic

No chronic toxicity data on antimycin A are available for freshwater fish. A search of the open literature did not identify any additional data on the chronic toxicity of antimycin A to fish.

5.2.3 Freshwater Invertebrate, Acute

Although an acute toxicity test measuring effects of technical grade antimycin A to waterfleas (*D. magna*) was submitted (MRID 400980-01), the study failed to establish a definitive EC_{50} value (**Table 13**) since greater than 50% mortality was observed in all of the treatment groups. However, a 96-hr EC_{50} value is available for the scud, *Gammarus fasciatus* (EC_{50}=0.008 µg/L). Based on the sensitivity of *Gammarus* to antimycin A, the compound is classified as very highly toxic to freshwater invertebrates on an acute exposure basis.

A search of the open literature did not identify other studies that would provide additional information concerning the acute toxicity of freshwater invertebrates exposed to antimycin A.

Table 13. Freshwater invertebrate acute toxicity of technical grade antimycin A					
Species	**% ai**	**96-hour EC_{50} (µg a.i./L) (95%C.I.)**	**Toxicity Category**	**MRID/ Accession No.**	**Study Classification**
Waterflea (*Daphnia magna*)	95.5	48 hr EC_{50} <10 (5 – 10)	very highly toxic	400980-01	Supplemental
Scud (*Gammarus fasciatus*)	95.5	0.008 (0.0058 - 0.011)	very highly toxic	400980-01	Supplemental
Aquatic Isopod (*Asellus brevicaudus*)	95.5	>1.0	very highly toxic	400980-01	Supplemental

5.2.4 Freshwater Invertebrate, Chronic

No chronic toxicity data on antimycin A are available for freshwater invertebrates. A search of the open literature did not identify other studies that would provide additional information concerning the chronic toxicity of antimycin A to freshwater invertebrates.

5.2.5 Estuarine/Marine Fish, Acute

A single estuarine/marine fish acute toxicity study of technical grade antimycin A with the spot (*Leiostomus xanthurus*) resulted in a 48-hr LC_{50} of 0.23 µg/L; therefore, antimycin A is classified as very highly toxic to estuarine/marine fish on an acute exposure basis.

5.2.6 Estuarine/Marine Fish, Chronic

No chronic estuarine/marine fish toxicity data were submitted and no useable data were located in the open literature for antimycin A; therefore, chronic risks associated with estuarine/marine fish exposure to antimycin A are unknown.

5.2.7 Estuarine/Marine Invertebrates, Acute

Antimycin A is very highly toxic to estuarine/marine invertebrates on an acute exposure basis (pink shrimp 96-hr LC_{50}=24 µg/L) (**Table 14**). Acute toxicity data were also available on the Eastern oyster, *Crassostrea virginica,* showing that antimycin is very highly toxic to mollusks as well.

Table 14. Estuarine/marine invertebrate acute toxicity of technical grade antimycin A					
Species	**% ai**	**96-hour EC_{50} (µg a.i./L)**	**Toxicity Category**	**MRID/ Accession No.**	**Study Classification**
Pink Shrimp *(Panaeus duorarum)*	95.5	48-hr LC_{50}=24	very highly toxic	402284-01	Supplemental
Blue Crab *(Callinectes sapidus)*	95.5	48-hr LC_{50}>100	highly toxic	402284-01	Supplemental
Eastern Oyster *(Crassostrea virginica)*	95.5	62	very highly toxic	402284-01	Supplemental

5.2.8 Estuarine/Marine Invertebrates, Chronic

No chronic estuarine/marine invertebrate toxicity data were submitted and no useable data were located in the open literature for antimycin A; therefore, chronic toxicity associated with estuarine/marine invertebrate exposure to antimycin A is unknown.

5.3 Toxicity to Terrestrial Animals

5.3.1 Birds, Acute and Subacute

No toxicity data are available for technical grade antimycin. However, based on acute oral (14-day) toxicity studies using formulated product of unspecified strength (MRID 135924), antimycin is classified as very highly toxic to water fowl (mallard duck LD_{50}=2.9 mg/kg bw) and highly toxic to upland game birds (bobwhite quail LD_{50}=39 mg/kg bw) (**Table 15**). No data are available to evaluate the subacute dietary toxicity of antimycin A to birds.

Table 15. Avian acute oral toxicity.					
Species	**% a.i.**	**Toxicity Value**	**Toxicity Category**	**MRID No. Author, Year**	**Study Classification**
Mallard duck *(Anas platyrhynchos)*	NS[a]	LD_{50}: 2.9 mg/kg	Very highly toxic	135924	Supplemental
Bobwhite quail (*Colinus virginianus*)	NS[a]	LD_{50}: 39 mg/kg	Highly toxic	135924	Supplemental

[a] Not stated.

5.3.2 Birds, Chronic

No chronic toxicity data are available for birds.

5.3.3 Mammals, Acute

No toxicity data are available on technical grade antimcyin A; however, the acute oral LD_{50} for Fintrol® Concentrate (20% solution) is 286 mg/kg for male and 361 mg/kg for female

rats[46] (MRID 455279-01). Based on these data, the formulated endproduct is classified as moderately toxic to mammals on an acute oral exposure basis.

5.3.4 Mammals, Chronic

No chronic toxicity data are available for mammals.

5.4 Aquatic Effects Characterization of Potassium Permanganate Toxicity

Potassium permanganate (CAS No. 7722-64-7) is a strong oxidizing agent[47] and is commonly used as a treatment for deactivating antimycin in water bodies treated to control or eradicate undesirable fish, quantify fish populations, or restore listed species[48]. Given that potassium permanganate is typically applied at 1 ppm to deactivate antimycin, permanganate may be present at exposure concentrations that present a secondary risk to non-target aquatic organisms. Presumably though, the permanganate reacts with antimycin and most organic matter in the aquatic environment and is therefore short-lived. A search for toxicity data on potassium permanganate in U.S. EPA's ECOTOX database[49] (ECOTOX, 2005) yielded a total of 57 toxicity values (96-hour LC_{50}s) for freshwater fish. More than 30 of the LC_{50} values in ECOTOX are reported[50] and include toxicity values for bluegill sunfish (*Lepomis macrochirus*) and rainbow trout, the recommended test species for assessing acute toxicity to warm water and cold water fish species, respectively[51] (OPPTS Guideline 850.1075). Twelve 96-hour LC_{50} values for estuarine/marine fish are described in ECOTOX; however, none of the studies included guideline-recommended surrogate estuarine/marine species. Five toxicity values for potassium permanganate toxicity to freshwater invertebrates, *i.e.*, daphnids, are available in ECOTOX.

5.4.1 Acute Toxicity of Potassium Permanganate to Aquatic Vertebrates

Table 16 summarizes examples of 96-hour LC_{50} values presented by Marking and Bills[52] and Birdsong and Avault[53]; however, the studies from which these results were obtained were not evaluated and verified. The data suggest that potassium permanganate can be categorized as highly to moderately toxic to fish on an acute exposure basis. Channel catfish appear to be more sensitive to potassium permanganate compared to other fish species. Although the LC_{50} values presented in **Table 16** vary across species by almost three orders of magnitude, intra-species

---

[46] Kuhn, J.O. 2001. Fintrol® Concentrate (Antimycin) Final Report Acute Oral Toxicity in Rats. Stillmeadow, Inc. Report No. 6025-00. Submitted to Aquabiotics Corporation, Bainbridge Island, WA. (MRID 455279-01).

[47] Marking, L.L. and T.D. Bills. 1975. Toxicity of potassium permanganate to fish and its effectiveness for detoxifying antimycin. Trans. Am. Fish. Soc. 104(3): 579-583.

[48] Finlayson, B.J., R.A. Schnick, R.L. Cailteux, L. DeMong, W.D. Horton, W. McClay, C.W. Thompson, and G.J. Tichacek. 2000. Rotenone Use in Fisheries Management: Administrative and Technical Guidelines Manual. American Fisheries Society, Bethesda, Maryland.

[49] ECOTOX (ECOTOXicology database). 2005. Maintained by the U.S. EPA ORD and the National Health and Environmental Effects Research Laboratory's (NHEERL's) Mid-Continent Ecology Division. http://www.epa.gov/ecotox.

[50] *Ibid* Marking and Bills 1975.

[51] OPPTS 850.1075. 1996. Ecological effects test guidelines: fish acute toxicity test, freshwater and marine. U. S. Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances: EPA712-C-96-118. http://www.epa.gov/offtsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-1075.pdf

[52] *Ibid* Marking and Bills 1975.

[53] Birdsong, C.L. and J.W. Avault Jr. 1971. Toxicity of certain chemicals to *Juvenile pompano*. Prog. Fish-Cult. 33(2): 76-80.

variability is relatively low for all species except striped bass (*Morone saxatilis*). This variation may partly reflect temperature and salinity effects on toxicity. Reardon and Harrell[54] demonstrated that the toxicity of potassium permanganate to striped bass is inversely related to salinity of the test waters. However, studies with Florida Pompano (*Trachinotus carolinus*) suggest that toxicity increases with increasing salinity[55]. As shown in **Figure 4**, studies with rainbow trout and channel catfish both suggest that acute toxicity of potassium permanganate is inversely related to water temperature.[56] Additionally, in a recent study by Hobbs *et al*.[57], the 96-hr LC_{50} for fathead minnows (*Pimephales promelas*) in synthetic and pond water was 2,130 and 11,280 µg/L, respectively. The difference in toxicity estimates between the synthetic and pond water is attributed by the study authors to increased organic carbon in the pond water. The toxicity estimates for potassium permanganate in synthetic water from the Hobb's study are consistent with the 96-hr LC_{50} values reported in Marking and Bills[58] for freshwater fish.

Table 16. Acute toxicity of potassium permanganate to freshwater and saltwater fish.[a]

Fish Species	96-hr LC50 (ppb)	Temperature	Salinity	References
Bluegill Sunfish[b] (*Lepomis macrochirus*)	2300 – 3600	Warm Water	Freshwater	Marking and Bills, 1975
Common Carp (*Cyprinus carpio*)	3050 – 3450	Warm Water	Freshwater	Marking and Bills, 1975
Channel Catfish (*Ictalurus punctatus*)	750	Warm Water	Freshwater	Marking and Bills, 1975
Rainbow Trout[c] (*Oncorhynchus mykiss*)	1220 – 1800	Cold Water	Freshwater	Marking and Bills, 1975
Striped Bass (*Morone saxatilis*)	960 – 4920	--	Freshwater; brackish; marine	Reardon and Harrell, 1994
Florida Pompano (*Trachinotus carolinus*)	1600 – 2900	--	Brackish; marine	Birdsong and Avault, 1971

[a] All data obtained from studies summarized in ECOTOX (2005).
[b] Preferred surrogate warm water fish species.
[c] Preferred surrogate cold water fish species.

54 Reardon, I.S. and R.M. Harrell. 1994. Effects of varying salinities on the toxicity of potassium permanganate to larval and juvenile striped bass, *Morone saxatilis* (Walbaum). Aquacult. Fish. Manag. 25(6): 571-578.

55 *Ibid* Birdsong and Avault 1971.

56 *Ibid* Marking and Bills 1975.

57 Hobbs. M. S., R. S. Grippo, J. L. Farris, B. R. Griffin and L. L. Harding. 2006. Comparative Toxicity of Potassium Permanganate to Nontarget Aquatic Organisms. Environmental Toxicology and Chemistry 25(1): 3046 – 3052.

58 *Ibid* Marking and Bills 1975.

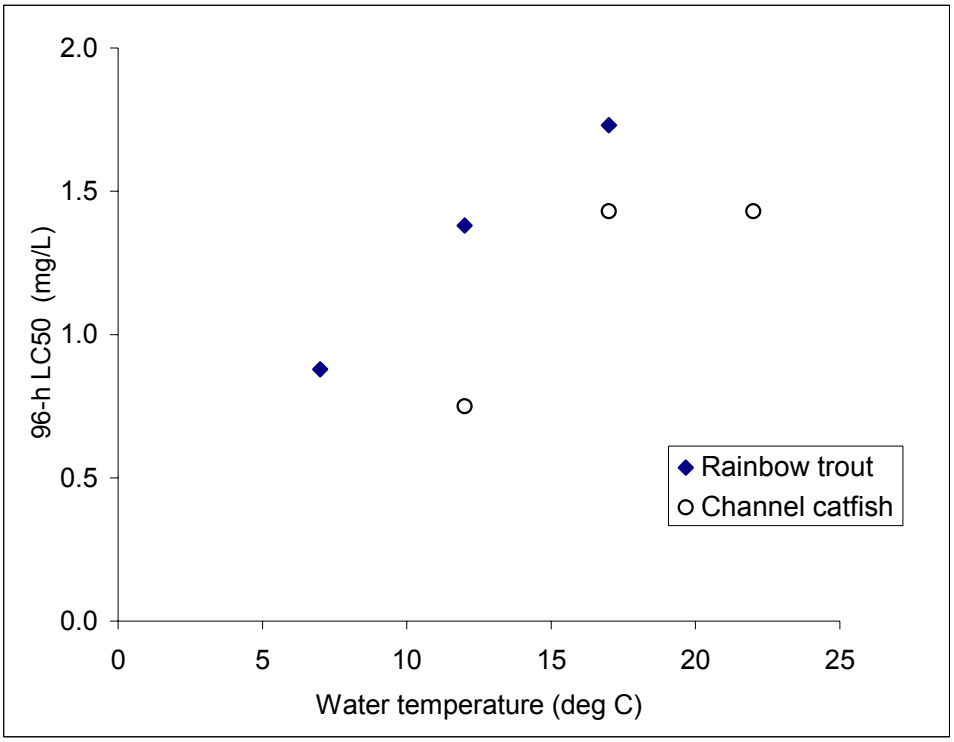


Figure 4. Effect of temperature (°C) on potassium permanganate toxicity to rainbow trout at pH 7.5 and 40-48 mg/L $CaCO_3$. Source: Marking and Bills (1975).

5.4.2 Acute Toxicity Potassium Permanganate to Aquatic Invertebrates

Only two 48-hour EC_{50} values for potassium permanganate in *D. magna* were available in ECOTOX. These values are 84 and 3500 µg/L. The studies reporting these toxicity values were not available for evaluation and verification of the results. The lower value suggests that potassium permanganate is very highly toxic to aquatic organisms on an acute exposure basis. Sreekala *et al*[59] and Anderson[60] (1944) reported 2-hour EC_{50} values for potassium permanganate of 540 and 1200 µg/L, respectively, in *Daphnia* spp. Anderson[61] also showed a dose-related increase in toxicity of potassium permanganate to *D. magna* with a NOAEL of 630 µg/L. In a recent study, Hobbs *et al*.[62] reports 96-hr LC_{50} values for *D. magna* and *Ceriodaphnia dubia* of 53 and 58 µg/L in synthetic water; however, in pond water under similar testing conditions the 96-hr LC_{50} is 1980 and 2390 µg/L, respectively. Hobbs *et al*.[63] also report that *Chironomous tentans* and *Hyallela azteca* tested with artificial substrates have 96-hr LC_{50} values of 4,430 and 4,740 µg/L, respectively, in synthetic water versus 13,550 and 12,300 µg/L, respectively, in pond

[59] Sreekala, K.G., J. Jennita, and V.R. Prakasam. 1991. Tolerance and heart rate of Daphnia sp. (crustacean) in response to disinfectants, bleaching powder and potassium permanganate. Pollut. Res. 10(1): 33-36.

[60] Anderson, B.G. 1944. The toxicity thresholds of various substances found in industrial wastes as determined by the use of *Daphnia magna*. Sewage Works J. 16(6): 1156-1165.

[61] *Ibid* Anderson 1944.

[62] *Ibid* Hobbs *et al*. 2006.

[63] *Ibid* Hobbs *et al*. 2006.

water. The decreased toxicity of potassium permanganate to aquatic animals in pond water compared to the toxicity in synthetic water is attributed to the higher organic carbon content of the pond water.

6 RISK CHARACTERIZATION

6.1 Risk Estimation

A means of integrating the results of exposure and ecotoxicity data is called the quotient method. For this method, risk quotients (RQs) are calculated by dividing exposure estimates by ecotoxicity values, both acute and chronic.

RQ = EXPOSURE/TOXICITY

RQs are then compared to OPP's levels of concern (LOCs). These LOCs are criteria used by OPP to indicate potential risk to non-target organisms and the need to consider regulatory action. The criteria indicate that a pesticide used as directed has the potential to cause adverse effects on non-target organisms. LOCs currently address the following risk presumption categories: (1) **acute high** - potential for acute risk to Federally non-listed species is high, regulatory action may be warranted in addition to restricted use classification (2) **acute restricted use** - the potential for acute risk is high, but this may be mitigated through restricted use classification (3) **acute endangered species** - the potential for acute risk to endangered (Federally listed) species is high, regulatory action may be warranted, and (4) **chronic risk** - the potential for chronic risk is high, regulatory action may be warranted. Currently, EFED does not perform assessments for chronic risk to plants, acute or chronic risks to non-target insects, or chronic risk from granular/bait formulations to mammalian or avian species.

The ecotoxicity test values (*i.e.*, measurement endpoints) used in the acute and chronic risk quotients are derived from the results of required studies. Examples of ecotoxicity values derived from the results of short-term laboratory studies that assess acute effects are: (1) median lethal concentrations (LC_{50}) (fish and birds) (2) median lethal doses (LD_{50}) (birds and mammals) (3) median effects concentrations (EC_{50}) (aquatic plants and aquatic invertebrates) and (4) first quartile effects concentration (EC_{25}) (terrestrial plants). Examples of toxicity test effect levels derived from the results of long-term laboratory studies that assess chronic effects are: (1) lowest observed adverse effect concentration (LOAEC) (birds, fish, and aquatic invertebrates) and (2) no observed adverse effect concentration (NOAEC) (birds, fish and aquatic invertebrates). For birds, mammals, and all aquatic organisms, the NOAEC is the ecotoxicity test value used in assessing chronic risk. Other values may be used when justified. Risk presumptions, along with the corresponding RQs and LOCs, are summarized in **Tables 17** through **19.**

Table 17. Risk presumptions for terrestrial animals (birds and wild mammals)

Risk Presumption	RQ	LOC
Acute High (Non-listed) Risk	EEC[1]/LC_{50} or LD_{50}/ft^2 or LD_{50}/day[3]	0.5
Acute Restricted Use	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day (or LD_{50} < 50 mg/kg)	0.2
Acute Endangered (Listed) Species	EEC/LC_{50} or LD_{50}/ft^2 or LD_{50}/day	0.1
Chronic Risk	EEC/NOAEC	1

[1] abbreviation for Estimated Environmental Concentration (ppm) on avian/mammalian food items

[2] $\frac{mg/ft^2}{LD_{50} * wt. of bird}$ [3] $\frac{mg of toxicant consumed/day}{LD_{50} * wt. of bird}$

Table 18. Risk presumptions for aquatic animals		
Risk Presumption	**RQ**	**LOC**
Acute High (Non-listed) Risk	EEC[1]/LC_{50} or EC_{50}	0.5
Acute Restricted Use	EEC/LC_{50} or EC_{50}	0.1
Acute Endangered (Listed) Species	EEC/LC_{50} or EC_{50}	0.05
Chronic Risk	EEC/NOAEC	1

[1] EEC = (ppm or ppb) in water

Table 19. Risk presumptions for plants		
Risk Presumption	**RQ**	**LOC**
Terrestrial and Semi-Aquatic Plants		
Acute High (Non-listed) Risk	EEC[1]/EC_{25}	1
Acute Endangered (Listed) Species	EEC/EC_{05} or NOAEC	1
Aquatic Plants		
Acute (Non-listed) Risk	EEC[2]/EC_{50}	1
Acute Endangered (Listed) Species	EEC/EC_{05} or NOAEC	1

[1] EEC = lbs ai/A
[2] EEC = (ppb/ppm) in water

For the assessment of antimycin risks, the deterministic risk quotient (RQ) method is used to compare exposure and toxicity values. A summary of toxicity values used to calculate RQs is provided in **Table 20**; a more detailed discussion of antimycin toxicity can be found in **Section 5**.

Table 20. Summary of toxicity data for most sensitive test species used to calculate risk quotients to evaluate ecological risks of antimycin A use			
Taxonomic Group	**Exposure Category**	**Most Sensitive Species**	**Toxicity Value**
Birds[a]	Acute (LD_{50}/LC_{50})	Mallard duck (*Anas platyrhynchos*)	LD_{50} = 2.9 mg/kg
	Chronic (NOAEC)	Not available	Not available
Mammals	Acute LD_{50}	Laboratory rat (*Rattus norvegicus*)	LD_{50} = 286 mg/kg bw
	Chronic NOAEL (NOAEC)	Not available	Not available
Terrestrial insects	Acute LD_{50}	Honey bees (*Apis mellifera*)	Not available

Table 20. Summary of toxicity data for most sensitive test species used to calculate risk quotients to evaluate ecological risks of antimycin A use			
Taxonomic Group	**Exposure Category**	**Most Sensitive Species**	**Toxicity Value**
Freshwater fish[b]	Acute LC_{50}	Coho Salmon (*Oncorhynchus mykiss*)	96-h LC_{50} = 0.009 µg/L
	Chronic NOAEC	Rainbow trout	Not available
Freshwater invertebrates	Acute EC_{50}	Water flea (*Daphnia magna*)	48-h EC_{50} = 0.008 µg/L
	Chronic NOAEC	Water flea	Not available
Estuarine/marine fish	Acute LC_{50}	Spot *(Leiostomus xanthurus)*	96-h LC_{50}=0.23 µg/L
	Chronic NOAEC	Not available	Not available
Estuarine/marine invertebrates	Acute EC_{50}	Pink Shrimp *(Panaeus duorarum)*	48-hr LC_{50}=24 µg/L
	Chronic NOAEC	Not available	Not available
Terrestrial plants	Acute	Not available	Not available
Aquatic plants and algae	Acute EC_{50}	Not available	Not available

[a]Birds are used as surrogates for terrestrial phase amphibians and reptiles (US EPA, 2004).
[b]Freshwater fish are used as surrogates for aquatic phase amphibians (US EPA, 2004).

6.1.1 Freshwater Fish and Invertebrates

At a treatment rate of 25 µg/L, the acute estimated environmental concentration (EEC) of antimycin is expected to be equivalent to the application rate. At this exposure concentration, RQ values (RQ=EEC/LC_{50}) for freshwater fish and invertebrates are 2,778 (25/0.009) and 3,125 (25/0.008), respectively. Acute high risk levels of concern (RQ≥0.5) for fish and invertebrates are exceeded by factors of 5,556 and 6,250X for fish and invertebrates, respectively (**Table 21**). Even if RQ values were based on the least sensitive fish, *i.e.*, black bullhead 96-hr LC_{50}=4.8 µg/L, the RQ (5.6) would exceed the acute high risk LOC by a factor of 11X. It should be noted, however, that RQ values in this screening-level assessment compare peak concentrations to toxicity values based on a 96-hr exposure; it is likely that that shorter-duration toxicity studies would have lower LC_{50} values and in turn, RQ values would be lower. Additionally, at the lower treatment concentration (5 µg/L)[64] recommended to remove scaled fish from aquaculture ponds, the RQ is roughly equal to unity (5 µg/L ÷ 4.8 µg/L=1.04*)* and exceed the acute high risk LOC.

[64] Avery, J.L. 2006. Use of Fintrol® to remove scaled fish in catfish ponds. *The Catfish Journal.* April 2006

Table 21. Acute risk quotients for freshwater fish and invertebrates based on a targeted antimycin A treatment rate of 25 µg/L.			
Species	**Estimated Exposure Concentration (µg/L)**	**Toxicity LC_{50} (µg/L)**	**Acute RQ Value**
Coho Salmon (*Oncorhynchus kisutch*)	25	0.009	2,778[a]
Scud (*Gammarus fasciatus*)	25	0.008	3125[a]

[a] Exceeds acute risk (RQ≥0.5), restricted use (RQ≥0.1), and endangered species (RQ≥0.05) levels of concern.

No acceptable toxicity data are available with which to evaluate the chronic risk to aquatic animals associated with the use of antimycin; however, given that the treatment concentrations exceed acute median lethal concentrations by several orders of magnitude, it is reasonable to believe that most aquatic animals will be killed by current treatment concentrations of antimycin A. In situations where antimycin is applied to flowing water, it is typically deactivated with potassium permanganate; however, deactivation is not required by the label. The combination of flow through, dilution with untreated water and deactivation with potassium permanganate makes chronic exposure to antimycin unlikely. Additionally, the acute toxicity of antimycin makes it likely that few biological receptors would be present to exhibit effects. However, to the extent that treated water is not deactivated using permanganate nor diluted by untreated water, the potential for chronic risks is uncertain.

6.1.2 Estuarine/Marine Fish and Invertebrates

At the maximum application rate, the acute RQ values for estuarine/marine fish and invertebrates are 109 and 1.0, respectively (**Table 22**). Both fish and invertebrate RQ values exceed the acute high risk LOC by factors of 218X and 2X, respectively. Although the toxicity value for estuarine/marine mollusks (62 µg/L) is less sensitive than that of pink shrimp, an RQ value for mollusks (RQ=0.40) exceeds the acute restricted use LOC (RQ≥0.1).

Table 22. Acute risk quotients for estuarine/marine fish and invertebrates based on a targeted antimycin A treatment rate of 25 µg/L.			
Species	**Estimated Exposure Concentration (µg/L)**	**Toxicity LC_{50} (µg/L)**	**Acute RQ Value**
Spot (*Leiostomus xanthurus*)	25	0.23	109[a]
Pink Shrimp *(Panaeus duorarum)*	25	24	1.0[a]

[a] Exceeds acute high risk (RQ≥0.5), restricted use (RQ≥0.1), and endangered species (RQ≥0.05) levels of concern.

6.1.3 Birds

The use of antimycin to achieve fishery management objectives, where the compound is applied directly to water, is not likely to represent a means of exposure to birds relative to

consumption of antimycin residues on terrestrial forage items since the compound is applied directly to water. On the chance that birds forage on dead or dying fish, it is possible to estimate the potential amount of antimycin in their diet. For example, the weight of food ingested (FI) by a great blue heron *Ardea herodias* (body weight=2.576 kg) is given by taking the antilog of the value estimate using formula (**EQ1)** recommended by the Wildlife Exposure Factors Handbook[65].

$$\text{Log}_{10}\,(\text{FI}) = 0.966\ \log_{10}\,(\text{body weight in grams}) - 0.640 \quad \textbf{(EQ 1)}$$

Based on this relationship, a blue heron will consume 452 g of fish per day. Piscivorous birds are likely to consume small to medium-sized fish and therefore rainbow trout are used as a surrogate. Fish tissue residue data for fish killed with antimycin indicate that rainbow trout contained as much as 172 µg antimycin/kg[66]. Therefore, a heron eating 0.45 kg would consume 77 µg (0.45 kg/day x 172 µg/kg) of antimycin.

Extrapolations from one bird to another need to consider differences in the scaling of toxicity for differences in body weight. The LD_{50} can be adjusted for body weight based on the formula (**EQ 2**) recommended by Mineau *et al*[67]:

$$\text{Adjusted } LD_{50} = LD_{50}\,(AW/TW)^{(a-1)} \quad \textbf{(EQ 2)}$$

where adjusted LD_{50} is the median 50% lethal dose for the species being assessed, LD_{50} is the median lethal dose in the test organism, AW is the body weight of the assessed organism, TW is the body weight for the test organism, and *a* is the slope of the regression line for estimating the assessed species LD_{50} from the test species LD_{50} (EFED default value of 1.15). In the case of assessing a blue heron, 2576 g is a suitable value (adult bird) for AW. The test organism is a mallard duck which weighs about 1230 g. With a test LD_{50} of 2.9 mg kg^{-1}, the adjusted LD_{50} is 3.2 mg kg^{-1}. The actual dose is then the LD_{50} times the weight of the heron (2.576 kg) which equals 8.246 mg. Therefore, the amount of antimycin potentially consumed by herons is several orders of magnitude less than the acute median lethal dose; the dose-based RQ value is less than 0.01 and is below acute risk levels of concern for non-listed and listed species (**Table 23**).

According to the Wildlife Exposure Factors Handbook[68] the mean food ingestion rate for a bald eagle (*Haliaeetus leucocephalus*) is 0.12 kg/kg day. A bald eagle weighing 5.089-kg bird would consume 610 g of fish per day representing a potential antimycin exposure of 105 µg (0.610 kg/day x 172 µg/kg). The adjusted LD_{50} for bald eagles is 3.6 mg kg^{-1} and the equivalent dose for a 5.089-kg bird is 18.32 mg; therefore, the dose-based RQ value is less than acute risk levels of concern (**Table 23**).

---

[65] ORD 1993. Wildlife Exposure Factors Handbook. Office of Health and Environmental Assessment, Office of Research and Development. EPA/600/R-93/187. http://www.epa.gov/ncea/pdfs/birds.pdf

[66] Ritter, P.O. and F.M. Strong. 1966. Residues in tissues of fish killed by antimycin. Journal of Agricultural Food Chemistry 14(4): 403 – 407 (MRID 461534-01)

[67] Mineau, P., B.T. Collins, and A. Baril. 1996. On the use of scaling factors to improve interspecies extrapolation of acute toxicity in birds. *Regulatory Toxicology and Pharmacology.* 24: 24-29.

[68] *Ibid* ORD 1993.

Similarly, the mean for food ingestion rate for a belted kingfisher (*Ceryle alcyon*) is 0.11 g/g-day[69]. Based on this rate, an adult kingfisher weighing 148 g would consume 16 g of fish per day representing a potential antimycin exposure of 2.8 µg (0.016 kg/day x 172 µg/kg). The adjusted LD_{50} for kingfishers is 2.1 mg kg^{-1} and once again, the estimated exposure is well below the effects threshold.

Table 23. Acute dose-based risk quotients for piscivorous birds based on potential residues in dead or dying fish.

Species	Estimated Daily Dose (µg)	Adjusted LD_{50} (µg)	Acute RQ Value
Blue Heron (*Ardea Herodias)*	77	8246	<0.01
Bald Eagle *(Haliaeetus leucocephalus)*	105	18,320	<0.01
Belted Kingfisher (Ceryle alcyon)	2.8	311	<0.01

Although ecological risk assessments do not typically take drinking water exposure into account, birds could potentially drink water from treated areas. The Wildlife Exposure Factors Handbook[70] estimates that bald eagles ingest 0.037 g water/g bw day. Therefore, a 5-kg bird would ingest 188 g water/day. At a treatment rate to water of 25 µg/L (25 µg/kg), an eagle could consume 4.7 µg/day. The potential contribution from drinking water (4.7 µg) and diet (105 µg) is still two orders of magnitude below the adjusted LD_{50}.

6.1.4 Mammals

Similarly, the use of antimycin as a piscicide is not likely to represent a means of exposure to wild mammals relative to consumption of antimycin residues on terrestrial forage items (plants, seeds, insects) since the compound is applied directly to water. Based on the chance that different-sized mammals forage on dead or dying fish, it is possible to estimate the potential amount of antimycin in their diet. Using daily food intake as estimated by Nagy[71] (**EQ3**), a 350-g mammal will consume about 68 g of food based on the allometric equation:

$$F=0.621*BW^{0.564}/(1-W) \qquad \textbf{(EQ3)}$$

where F is the food intake in grams of fresh weight, BW is the body mass of the organism in grams, and W is the mass fraction of water in the food. For this assessment W is assumed to be 0.75[72]. Piscivorous mammals are not confined to eating small fish and may feed opportunistically on any sized fish that may succumb to antimycin; therefore, common carp (*Cyprinus carpio*) are used as surrogates. Based on the data on antimycin tissue residues in common carp with a body weight of 99 grams, a small mammal would only consume 68%

[69] *Ibid* ORD 1993

[70] *Ibid* ORD 1993

[71] Nagy, K.A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. *Ecological Monographs* **57:** 111-128.

[72] *Ibid* Nagy 1987.

(68/99) of the total carp body mass. According to the data for common carp, maximum total body residues of antimycin in carp tissue amounted to 261 µg/kg[73]. A 350-g mammal consuming 68 grams represents an equivalent dose of 18 mg of antimycin; this value is below the median lethal dose of antimycin (286 mg/kg*0.350 kg=100 mg) for similarly sized mammals.

Similar to birds, extrapolations from one size of mammal to another must consider differences in the scaling of toxicity for difference in body weight. The LD_{50} can be adjusted for body weight based on the formula recommended by Mineau *et al.*[74] (**EQ4**):

$$\text{Adjusted } LD_{50} = LD_{50}(TW/AW)^{(0.25)} \qquad \textbf{(EQ4)}$$

where adjusted LD_{50} is the median 50% lethal dose for the species being assessed, LD_{50} is the median lethal dose in the test organism, AW is the body weight of the assessed organism and TW is the body weight for the test organism. Since we are assessing a large mammal, 1000 g is a suitable value for AW. The test organism is a rat weighing about 350 g. With a test LD_{50} of 286 mg/kg, the adjusted LD_{50} is 220 mg/kg. Using the daily intake equation, a 1000-g mammal will consume about 122 g of food. If the animal fed exclusively on carp killed by antimycin, the equivalent dose would be 0.122 kg *261 µg/kg or 32 µg of antimycin. Once again, this value is well below the estimated median lethal equivalent dose adjusted for body weight (220 mg/kg * 1 kg=220 mg) and the RQ value is less than 0.01 (**Table 24**). Therefore, even if fish are available for consumption by mammals scavenging along the shoreline for dead or dying fish, it is not likely that the mammals would consume sufficient quantities of antimycin to result in acute toxicity.

Table 24. Acute dose-based risk quotients for piscivorous mammals based on potential residues in dead or dying fish.

Species	Estimated Daily Dose (µg)	Adjusted LD50 (µg)	Acute RQ Value
Large Mammal (1000 g)	8.9	220,000	<0.01

6.2 Risk Discussion

Antimycin A (Fintrol®) is applied directly to water and as a piscicide; it is intended to kill fish. Niclosamide (CAS No. 50-65-7), 3-trifluoromethyl-4-nitrophenol (TFM; CAS No. 99-30-2) and rotenone (CAS No. 83-79-4) are other piscicides that have recently been evaluated by OPP for ecological effects; however, of these piscicides, antimycin is one of the most toxic to fish (**Table 25**). Antimycin's high toxicity to fish accounts for the low treatment concentrations (typically 25 µg/L or less) needed and may account for why antimycin does not evoke an avoidance response in most fish. Based on the available information it is reasonable to believe that one component of the initial risk hypothesis discussed in the problem formulation cannot be rejected, *i.e.*, the use of antimycin A in accordance with the label will likely result in adverse effects, such as acute mortality, to aquatic animals when the chemical is used at typical rates of

[73] *Ibid* Ritter and Strong. 1966.

[74] *Ibid* Mineau *et al.* 1996.

25 µg/L as identified in the use closure memo (dated October 26, 2005). The extent to which non-target mortality will occur is uncertain; field studies suggest that while aquatic invertebrate populations are immediately affected in terms of decreased numbers, invertebrate communities recover to pretreatment levels in terms of both abundance and diversity within several months[75]. While the initial risk hypothesis included potential risk to terrestrial animals feeding on dead or dying fish or through the ingestion of antimycin-treated water, the likelihood of adverse effects on these taxa is considered low based on available data.

Table 25. Acute toxicity values for freshwater fish (LC_{50}) and invertebrates (LC_{50}) for selected piscicides based on most sensitive endpoints used in OPP Ecological Risk Assessment chapters in support of the Reregistration Eligibility Decisions.

Chemical	Freshwater Fish 96-hr LC_{50} (µg/L)	Freshwater Invertebrate 48-hr EC_{50} (µg/L)
Antimycin A	0.009	0.008
Rotenone[a]	1.94	3.7
Niclosamide[b]	30	34
TFM[c]	600	3800

[a] Rotenone RED Chapter DP Barcode D307382
[b] Niclosamide RED Chapter DP Barcode D255595
[c] TFM 3-trifluoromethyl-4-nitrophenol RED Chapter DP Barcode D219182

Although there are data indicating that antimycin A is subject to abiotic degradation and, under alkaline conditions, may degrade rapidly by hydrolysis, the susceptibility of antimycin to biotic degradation is unknown. Preliminary *in vitro* tests with rat hepatocytes[76] indicate that antimycin *in vitro* Phase 1 metabolism leads to hydrolysis of the acyloxy group along with deformylation of the N-formylaniline (**Figure 5**; see **Appendix D** for further discussion). Deformylation of antimycin has been hypothesized as resulting in its deactivation as a fish toxicant.[77] There are data to support that the toxicity of antimycin is related to both pH and temperature and label application rates are adjusted accordingly; at higher pH levels, the degradation rate increases and thus larger amounts of antimycin are required for treatment. At lower water temperatures, *i.e.*, below 15.5°C (60°F), the label recommends higher treatment concentrations and likely reflects the decreased toxicity of antimycin at lower water temperatures due to decreased fish metabolic rates.

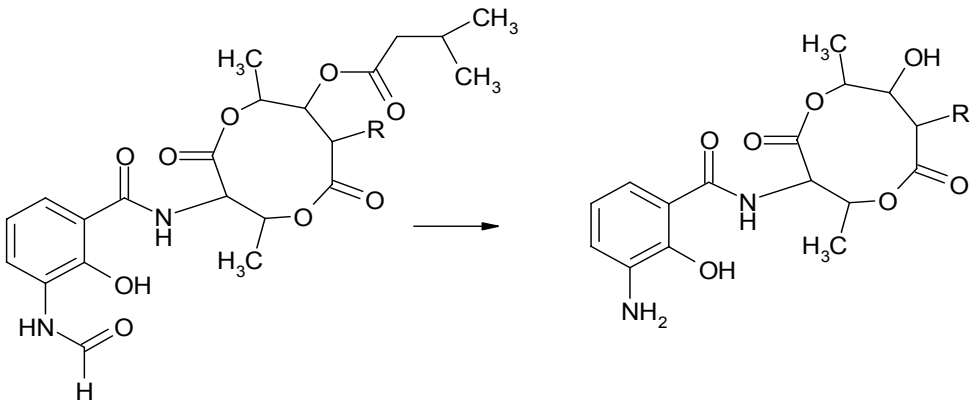
[75] Moore, S.E., M.A. Kulp, J. Hammonds, and B. Rosenlund. 2005. Restoration of Sams Creek and an Assessment of Brook Trout Restoration Methods, Great Smoky Mountain National Park. National Park Service, U. S. Department of the Interior. Technical Report NPS/NRWRD/NRTR-2005.

[76] personal communication John Kenneke, Chemist, U.S. EPA ORD 2006; these data are considered preliminary and have not undergone extensive peer review or quality assurance that would permit their use in a more quantitative fashion.

[77] Ritter, P.O. and F.M. Strong. 1966. Residues in tissues of fish killed by antimycin. Journal of Agricultural Food Chemistry 14(4): 403 – 407.

6.3 Risks to Aquatic Animals

Antimycin A is very highly toxic to fish on an acute exposure basis. With fish LC_{50} values as low as 1 nanogram (n) per liter (part per trillion (ppt*r*)) and treatment rates 25 hundred times higher than this LC_{50} value, the direct application of antimycin to flowing and static water to eliminate undesirable fish species is likely to kill many of the fish species present. **Figure 6** depicts the distribution of 96-hr LC_{50} values in parts per trillion across fish genera and shows that roughly 80% of the genera have LC_{50} values of less than or equal to 20 ng/L; 95% of the genera are at or below 79 ng/L. Therefore, at treatment rates of 25 µg/L, the concentration of antimycin exceeds the 96-hr LC_{50} for 95% of the species reported. Since fish serve as surrogates for aquatic phase amphibians, risk is presumed for aquatic-phase amphibians as well.



R = n-butyl; n-hexyl

Figure 5. Metabolites tentatively identified from the phase 1 *in vitro* metabolism of antimycin A at pH 7.4, 37°C, with rat hepatic microsomes.

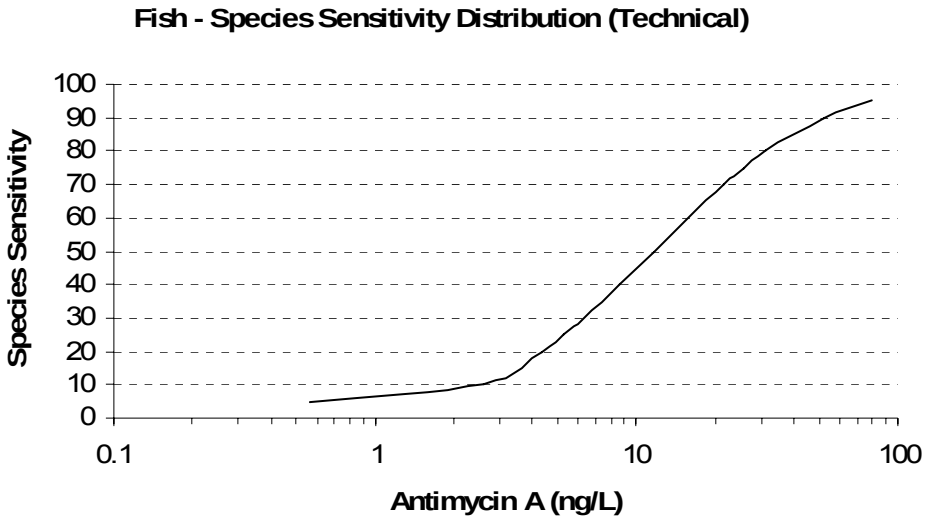


Figure 6. Distribution of acute 96-hr median lethal concentrations (LC_{50}) for fish genera exposed to antimycin A.

Although typical treatment rates are likely to kill most fish species, the chemical is used to selectively remove scaled fish from catfish (fingerling and food-fish) aquaculture production ponds by taking advantage of the differential toxicity of antimycin to scaled and non-scaled fish. In studies of channel catfish conducted by the U.S. Fish and Wildlife Service[78] (see **Appendix F** for further study details), exposure of catfish fry to 0.75 µg/L resulted in 100% mortality; however, fingerling catfish in the same study were roughly an order of magnitude less sensitive with a 96-hr LC_{50} value of 8.4 µg/L. Additionally, study results are consistent with data reported in **Section 5** indicating that catfish are several orders of magnitude less sensitive to antimycin than scaled species (**Table 26**).

Antimycin's use in aquaculture to remove scaled fish from catfish production ponds does not typically involve subsequent deactivation with potassium permanganate; rather, the antimycin is allowed to degrade naturally in the production pond water. Fingerling catfish would not be harvested as food fish for roughly 1 – 1.5 years post-treatment.

The use of antimycin to remove scaled fish from food-fish production ponds is conducted relatively close to the time of harvest; however, again the antimycin is not typically deactivated. Water from harvested aquaculture ponds is frequently reused for fish production. To the extent

[78] Hogan, J. 1966. Antimycin as a Fish Toxicant in Catfish Culture. Presented at the 20th Annual Meeting of the Southeastern Association of Game and Fish Commissioners, Oct 24 - 26, 1966. Bureau of Sport Fisheries and Wildlife, U.S. Fish and Wildlife Service. (Accession No. 00045801; MRID 2400798-13)

that recently treated water is released to adjoining waterbodies, nontarget mortality of both fish and invertebrates could occur.

Table 26. Fingerling fish bioassay of antimycin 96-hr LC_{50} values (nominal concentrations).	
Species	**96-hr LC_{50} µg/L**
Goldfish	0.137
Common Carp	0.200
Fathead Minnow	0.074
Green Sunfish	0.060
Bluegill Sunfish	0.051
Largemouth Bass	0.104
Channel Catfish	8.4

Although no data were submitted with which to evaluate the chronic toxicity of antimycin to fish, quantitative structure-activity relationship models can be used to estimate the potential toxicity of antimycin. The Ecological Structure Activity Relationships (ECOSAR)[79] is a component of the Estimation Program Interface (EPI) Suite™ [80] developed by EPA's Office of Pollution Prevention and Toxics and Syracuse Research Corporation. ECOSAR is a model which predicts acute and chronic aquatic animal/plant toxicity. Based on this model (see **Appendix B** for model output), the 90-day chronic toxicity value (ChV) for freshwater fish is 67 µg/L; however, this value is higher than all of the 96-hr median lethal dose estimates reported in this assessment and therefore is not considered a realistic estimate of a chronic toxicity endpoint.

The available laboratory data indicate that freshwater invertebrates have roughly similar sensitivities as fish to antimycin on an acute exposure basis and many of these species may die as well at treatment rates of 25 µg/L. Although there are anecdotal reports[81] suggesting that plants and invertebrates are relatively unaffected by antimycin treatments, no laboratory data have been reviewed by EPA that can substantiate these claims. To the extent that application rates are less than 25 µg/L and less sensitive animals are in the treatment areas, aquatic invertebrate mortality could be substantially reduced. Although invertebrates are less conspicuous members of the aquatic community, they are a major component of aquatic ecosystems and food webs. Any significant effects on invertebrates would most likely influence other components of the ecosystem. Effects may not be limited to merely a change in total biomass as a result of widespread mortality but any changes associated with differential sensitivity could bring about significant changes in the community structure, which could alter system function.

There are no data on freshwater mollusks; however, data on estuarine/marine mollusks indicate that they are less sensitive to antimycin than other invertebrates although risk quotients

[79] ECOSAR. Ecological Structure Activity Relationships http://www.epa.gov/opptintr/newchems/tools/21ecosar.htm

[80] EPI Suite http://www.epa.gov/opptintr/exposure/docs/episuite.htm

[81] Wisconsin Alumni Research Foundation. 1965. Antimycin as a Fish Toxicant: a resume of information and data pertaining to the use of antimycin in fish management procedures in ponds and lakes. (Accession No. 00135924)

based on mollusk toxicity data exceed acute restricted use and endangered species levels of concern.

Based on PBT Profiler[82] modeling (**Appendix B**), which generated the estimated bioconcentration factor for antimycin (350X), the compound does not appear likely to bioconcentrate in fish. In general, chemicals that have the potential to bioconcentrate also have the potential to bioaccumulate. Since a bioconcentration factor (BCF) in fish can be readily measured in the laboratory and bioaccumulation is much more complicated to determine, the BCF is frequently used to predict the importance of bioaccumulation. Because of antimycin's relatively low BCF, the chemical is not considered likely to bioaccumulate in aquatic food chains.

In addition to acute risks, there is a potential for chronic effects to freshwater invertebrates when antimycin is applied directly to water. The limited data on freshwater invertebrates suggest that they are as sensitive to antimycin as fish, and it is possible that many invertebrates will not survive antimycin treatment concentrations in the targeted area at the typical application rate; therefore, chronic effects will be limited by a lack of receptors. Additionally, at the maximum recommended treatment rate, a single application of antimycin directly to water is intended to kill all fish in the treated area; therefore, it is unlikely that repeated applications of antimycin will occur within the same year. Also, antimycin degrades rapidly in the environment, particularly at warmer temperatures in alkaline waters and can be deactivated with potassium permanganate to prevent its movement to non-target areas, thus further reducing the likelihood of chronic exposure.

A field study conducted by the National Park Service[83] as part of Southern Appalachian brook trout (*Salvelinus fontinalis*) restoration effort in the Great Smoky Mountain National Park evaluated the impacts of antimycin A (Fintrol®) treatment at 8 µg/L for 8 hours in high gradient streams. Pre- and post-treatment monitoring of freshwater invertebrates indicated an 18 – 25% decline in total taxa in two of the three sites treated with antimycin roughly two weeks post-treatment; mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddis flies (Trichoptera), collectively referred to as EPT, being the most sensitive families. Sampling conducted approximately one year after treatment indicated no statistical difference (ANOVA) between pre- and post-treatment for total taxa and EPT taxa. Furthermore, crayfish were observed feeding on dead fish in the antimycin treatment area and there was no difference in the numbers of crayfish observed pre- and post-treatment. In this study, potassium permanganate (1 ppm) was used to inactivate the antimycin A and some of the effects noted on macroinvertebrates soon after treatment were believed to result from the permanganate.

---

[82] PBT Profiler Model http://www.pbtprofiler.net/

[83] Moore, S.E., M.A. Kulp, J. Hammonds, and B. Rosenlund. 2005. Restoration of Sams Creek and an Assessment of Brook Trout Restoration Methods, Great Smoky Mountain National Park. National Park Service, U.S. Department of the Interior. Technical Report NPS/NRWRD/NRTR-2005.

Similarly, in a study of Bear Creek in Great Smokey Mountain National Park[84] benthic macroinvertebrate communities were sampled before and after treatment with antimycin A with subsequent deactivation with potassium permanganate to determine whether aquatic invertebrates were impacted and if so, how. Based on Jaccard and Morisita-Horn similarity indices[85] the report concluded that benthic [invertebrate] communities were at pre-treatment conditions within 4 months post-treatment. Where decreased numbers of invertebrates were noted 2 weeks post-treatment, it was uncertain whether the reductions were due to antimycin or potassium permanganate. It is also uncertain though how representative these study results are for the recovery of aquatic invertebrates in other areas/conditions in which antimycin may be used in both lotic and lentic environments.

In Great Basin National Park, Nevada, efforts to restore the native Bonneville cutthroat trout (*Oncorhynchus clarki utah*) also relied on antimycin A[86]. Macroinvertebrates were surveyed in Snake Creek following the EPA Rapid Bioassessment of Creeks and Small Rivers protocols[87] three years prior to treatment and following treatment at one week, one month, nine months and one year. Roughly 7.6 kilometers of Snake Creek were treated over a 6 day period. Segments of the creek were maintained at 8 µg/L for 8 hours and were later deactivated with permanganate (4 mg/L). The efficacy of treatment was monitored by using brook trout (*Salvelinus fontinalis*) in live cars maintained throughout and downstream of the treatment areas. Overall numbers of aquatic invertebrates declined by 61% and 54% for EPT taxa one month after treatment; macroinvertebrate diversity declined by 29% one month post-treatment. However, by 9 months post-treatment, invertebrate populations had returned to pretreatment conditions and in some cases exceeded pre-treatment abundance by over 300% and diversity was within 95% of pre-treatment levels. The deactivation of antimycin with potassium permanganate was considered effective; however, there was a gradient of effect. Brook trout in live cars at 100, 200 and 500 m downstream of the deactivation station experienced 90%, 25% and 0% mortality, respectively. The downstream mortality was attributed to the permanganate treatment (personal communication N. Darby, Biologist, Great Basin National Park, NV, 2006).

Although antimycin is likely to partition to sediments containing organic matter, given K_{oc} estimates ranging from 2500 – 10000 mg/g, there are no data available to quantify the extent to which antimycin may be available to benthic invertebrates. Additionally, no laboratory data are available to assess the toxicity of antimycin to benthic invertebrates; however, the National Park Service studies discussed above only show transient effects in invertebrate communities and many of these taxa are associated with the benthic environment.

---

[84] Etnier, D. a. and C. D. Hulsey. 2005. Effects of Antimycin A treatment on benthic macroinvertebrates in Bear Creek tributaries to Forney Creek, Great Smokey Mounthain National Park, Swain Couty, North Carolina. University of Tennessee Final report prepared under cooperative agreement CA-5460-A1-005.

[85] Krebs, D. J. 1999. Ecological Methodology. Addison-Welsey Educational Publishers, Inc., Menlo Park, CA, 620p.

[86] Baker, G. M., N. W. Darby and T. B. Williams. 2004. Balancing Bonneville cutthroat trout with non-native salmonids in Great Basin National Park. Wild Trout III Symposium: Working Together to Ensure the Future of Wild Trout: P141 – 150.

[87] Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition. EPA 841-B-99-002. U. S. Environmental Protection Agency; Office of Water, Washington D.C. http://www.epa.gov/owow/monitoring/rbp/

Studies conducted by Cerreto[88] in conjunction with the Wyoming Game and Fish Department indicated that benthic invertebrate communities were not significantly ($p>0.05$) affected in terms of invertebrate biomass, richness or diversity by 8-hr treatments of first-order streams with antimycin A at 10 µg/L relative to reference streams. *In situ* bioassays with mayflies (*Cinygmula* spp.) and caddisflies showed no effect on survival (*Brachycentrus* spp.). Although there was high variability in invertebrate drift estimates the study had a low a ability to detect treatment effects (poor statistical power), the results indicate that invertebrate communities were not eliminated during and immediately after a 8-hour treatment with antimycin A at 10 µg/L.

As mentioned earlier, potassium permanganate is sometimes used to detoxify antimycin following stream treatments. Permanganate is typically applied at a treatment concentration of 1 mg/L. Based on the most sensitive fish (channel catfish 96-hr LC_{50}=750 µg/L; **Table 16**) and invertebrate (waterflea EC_{50}=84 µg/L) species reported, RQ values for fish and invertebrates would exceed the acute high risk level of concern. In the National Park Service Sams Creek survey[89], total taxa declined by 34% following treatment with 1 mg/L $KMnO_4$ for 9 days; EPT taxa declined by as much as 11%[90]. However, similar to what was observed following antimycin treatment, the number and diversity of aquatic insects in the permanganate-treated stream had returned to pretreatment levels 4 months after treatment.[91] In the same study, higher permanganate concentrations, *i.e.*, 9 days at 4 mg/L, were lethal to 40% of the trout; however, fish were not affected at lower concentrations. Also, as mentioned earlier, deactivation with permanganate is not required by the label.

6.4 Risks to Terrestrial Animals

Only acute oral toxicity data are available for birds and these indicate that antimcyin is highly to very highly toxic to birds on an acute oral exposure basis. Risk quotients are calculated using the dose-based acute toxicity values and result in values below acute risk levels of concern. The dose-based approach considers the uptake and absorption kinetics of a gavage toxicity study to approximate exposure associated with uptake from a dietary matrix. Toxic response is a function of duration and intensity of exposure. For many compounds, a gavage dose represents a very short-term, high intensity exposure. Although the dose-based estimates may not reflect reality in that animals do not receive a gavage while feeding, it is possible that a short-duration, high-intensity exposure could occur associated with feeding along side treated surface waters since many birds may gorge themselves when food items are available; this would be particular true as birds opportunistically feed on dead and dying fish.

No chronic avian toxicity data were submitted or located in the open literature for antimycin; therefore, chronic risks associated with avian exposure to antimycin are unknown. However, the likelihood of chronic exposure is considered low since dead fish would soon sink

---

[88] Cerreto, K. M. 2004. Antimycin and rotenone: short-term effects on invertebrates in first order, high-elevation streams. A master's thesis submitted to the Department of Zoology and Physiology and the Graduate School of the University of Wyoming.

[89] *Ibid* Moore *et al*. 2005.

[90] Personal communication: S. Moore, Supervisory Fishery Biologist, Great Smoky Mountains National Park, June 2006

[91] *Ibid* Moore *et al*. 2005

to the bottom of treated water. While exposure is possible through ingestion of antimycin A residues in drinking water, the likelihood is considered low due to flow-through.

Ecological risk assessments typically evaluate the potential acute and chronic effects of pesticides to terrestrial and aquatic organisms. As indicated by **Table 20**, there are a number of toxicity studies (avian sub-acute dietary, avian chronic, mammalian chronic, aquatic vertebrate/invertebrate chronic and terrestrial/aquatic plant) that are not available for antimycin A. In determining whether any of these taxa would be at risk from the use of antimycin, it must first be determined whether exposure is likely. As indicated by acute risk quotients, acute exposure estimates based on recommended treatment rates are more than sufficient to exceed acute high risk level of concern for aquatic animals. However, given the uncertainty regarding the degradation of antimycin, chronic exposure estimates vary widely. The extent to which non-target animals will be affected will depend on the extent to which antimycin moves outside of the targeted treatment area.

Although two studies were submitted by the registrant examining the potential effects on mammals from the ingestion of antimycin-treated water[92] and fish[93], there were insufficient details included in the studies to substantiate their conclusions regarding no effect (see **Appendix F** for additional study details).

6.5 Ecological Incident Reports

There are no incidents related to the use of antimycin A reported in the Ecological Incident Information System database. However, the fact that incidents have not been formally reported does not mean that incidents have not occurred. It is noteworthy that there are incidents reported for the other piscicides mentioned in **Table 25**. The primary use of antimycin in high gradient streams and the low overall amount of antimycin used nationally may limit opportunities to observe adverse effects on non-target organisms.

6.6 Uncertainties

Although the ecological risks assessed in this chapter are based on a treatment rate of 25 µg/L maintained for roughly 8 hours, the label does not specify a maximum application rate. At this treatment rate, though, the available toxicity data suggest that a substantial number of aquatic species will be subject to acute mortality. Higher application rates can be reasonably expected to cause acute mortality on a larger proportion of the aquatic community.

Resource management agencies have developed guidance, such as that depicted in **Appendix C,** for the use of piscicides in New Mexico. Additionally, professional fishery organizations such as the Fish Management Chemicals Subcommittee of the American Fisheries

---

[92] Arslaneglau, L. and V. Korths. 1967. Antimycin Toxicity Studies: Administration of Water Treated with Antimycin to Rats and Dogs. Ayerst Research Laboratories. (MRID 2400798-10)

[93] Arslaneglau, L. and V. Korths. 1967. Antimycin Toxicity Studies: Administration of Fish Killed with Antimycin to Rats and Dogs. Ayerst Research Laboratories. (MRID 2400798-08)

Society have completed monographs for the use of antimycin in streams[94] and in lakes/reservoirs[95]; however, the label does not currently require that these application methods be followed. Mr. Stephen Moore and Mr. Matt Kulp with the U.S. National Park Service in collaboration with Mr. James Brooks and Mr. Bruce Rosenlund of the U.S. Fish and Wildlife Service and Dr. David Probst with the New Mexico Department of Game and Fish are currently developing a standard operating procedure (SOP) for the use of antimycin. While this manual is intended to serve National Park Service biologists in the proper use of antimycin on National Park Service lands, it is also being developed on behalf of the registrant[96] and will serve as a SOP to accompany the label for the use of antimycin in streams, lakes and reservoirs. Mississippi State University (MSU) Agricultural Research Extension Service has completed a SOP for the use of antimycin in catfish aquaculture (**Appendix E**). These documents are intended to assure the consistent use of antimycin in targeted treatment areas and minimize non-target species mortality.

6.6.1 Analytical Method Development

The available data on both the environmental fate and ecological effects of antimycin have been limited by the lack of an analytical method for detecting antimycin. The low application rates, typically less than 25 µg/L, coupled with the fact that antimycin is composed of 4 subunits that may readily dissociate from one another, would require fairly sensitive methods of detection. During the problem formulation phase of this risk assessment, there were relatively frequent discussions with the registrant and stakeholders; EPA emphasized the need to develop an analytical method that could be applied in the field. Through these discussions and with funding provided by the U.S. Fish and Wildlife Service, methodology is currently under development by the U.S. Geological Survey in La Crosse, Wisconsin. Research thus far has resulted in levels of quantification (LOQ) at 50 ng/L. The methodology requires that a sample size of 50 mL is first concentrated on a solid phase extraction (SPE) column then eluted with and stored in an organic solvent to stabilize the antimycin A. Increased sensitivity can be achieved through higher sample sizes (a 10 ppt*r* quantification limit has been achieved by extracting 250-mL water samples); however, 50 mL is considered a reasonable sample size to process through an SPE column under field conditions[97]. The extracted sample is then eluted off the SPE column with acetone and is minimally stable for 2 weeks and may be stable for several months.

In the lab, the extracted sample is then analyzed using reversed-phase high pressure liquid chromatography (HPLC) with a mass spectroscopy (MS) detector. Using a gradient elution method, antimycin A resolves into four quantifiable peaks (A_1, A_2, A_3 and A_4). The limit of detection (LOD) is approximately 15 ppt*r* when using a sample size of 50 mL.

---

[94] American Fisheries Society. 2006. Fintrol® Stream and River Use Monograph for Complete Removal of Fish. Use in Streams and Rivers. http://www.fisheries.org/html/Antimycin_monographs.shtml

[95] American Fisheries Society. 2006. Fintrol® Pond, Lake and Reservoir Use Monograph for Complete Removal of Fish: Use in Ponds, Lakes and Reservoirs. http://www.fisheries.org/html/Antimycin_monographs.shtml

[96] Memo from Mary Romeo; President, Aquabiotics Corporation; dated February 13, 2006

[97] Personal communication J. Bernardy and T. Hubert, Biological Resources Division of the U.S. Geological Survey, May 2006

6.6.2 Assumptions and Limitations Related to Effects Assessment

6.6.2.1 Age Class and Sensitivity of Effects Thresholds

Test organism age may have a significant impact on the observed sensitivity to a toxicant. The screening risk assessment acute toxicity data for fish are collected on juvenile fish weighing between 0.1 and 5 grams. Aquatic invertebrate acute testing is performed on recommended immature age classes (*e.g.*, first instar for daphnids, second instar for amphipods, stoneflies and mayflies, and third instar for midges). Similarly, acute dietary testing with birds is also performed on juveniles, with mallard being 5-10 days old and quail 10-14 days old. The screening risk assessment has no current provisions for a generally applied method that accounts for uncertainty associated with study organism age. In so far as the available toxicity data may provide ranges of sensitivity information with respect to age class, the risk assessment uses the most sensitive life-stage information as the screening endpoint.

Additionally, this assessment does not evaluate whether antimycin A could be more toxic for organisms that have lower metabolic activity. This may occur in more sensitive life stages and may render these organisms more vulnerable to chronic effects.

6.6.2.2 Lack of Effects Data for Amphibians and Reptiles

Currently, toxicity studies on amphibians and reptiles are not required for pesticide registration. Since these data are lacking, the Agency uses fish as surrogates for aquatic-phase amphibians and birds as surrogates for terrestrial-phase amphibians and reptiles. These surrogates are thought to be reflective of or protective (more sensitive) of herpetofauna. Amphibians are characterized by a permeable skin. The most important route of exposure for aquatic amphibians would likely be the dermal route. Freshwater fish may be suitable surrogates since exposure would likely be surface area-dependent and the gill surface area of many fish is fairly large. Also, both fish and amphibians are ectothermic so metabolic rates and demands would likely be similar. For terrestrial species, however, the difference between amphibians and birds and reptiles and birds is quite large. Terrestrial amphibians and reptiles are both ectothermic while birds are endothermic; birds have a higher basal metabolic rate required to maintain constant body temperature. The higher metabolic demands of birds may predispose birds to higher relative exposures. However, this does not address any potential differences in toxicity. To date, there are few controlled studies on reptile species that could be used to compare to similar studies on birds. *A priori*, there is no strong reason to think that one taxon is more or less sensitive than another. Further research is required to determine whether, in general, reptiles and terrestrial-phase amphibians are suitably represented by bird species in assessing risks.

6.6.2.3 Use of the Most Sensitive Species Tested

Although the screening risk assessment relies on a selected toxicity endpoint from the most sensitive species tested, it does not necessarily mean that the selected toxicity endpoints reflect sensitivity of the most sensitive species existing in a given environment. The relative

position of the most sensitive species tested in the distribution of all possible species is a function of the overall variability among species to a particular chemical. The relationship between the sensitivity of the most tested species versus wild species (including listed species) is unknown and a source of significant uncertainty. The use of laboratory species has historically been driven by availability and ease of maintenance. A widespread comparison of species is lacking; however, even variation within a species can be quite high.

6.6.2.4 Effectiveness of Deactivation with Potassium Permanganate

There are no data to evaluate the effect of potassium permanganate on antimycin A. Although information from the National Park Service[98] suggests that permanganate is an effective means of deactivating antimycin and there is similar information for other piscicides such as rotenone[99], no data have been submitted to identify the degradation products from mixing antimycin A with potassium permanganate.

6.6.3 Assumptions Related to Exposure Assessment

In this exposure assessment, a wide range of possible EECs were derived, due primarily to the lack of fate data and the lack of use limits on the antimycin label. Chronic exposure concentrations are especially affected by these uncertainties because these values are influenced by both the uncertainty in application rates as well as the uncertainty in the degradation rate. Acute concentrations uncertainties are due primarily to the label rate ambiguities only.

Uncertainties due to application rates were not directly addressed here, but it should be noted that any change in application rate would result in directly proportional changes in EECs. The label reports a concentration of "roughly 25 ppb" as the highest in a list of recommended starting concentrations. It is unknown if there would be an application substantially higher than 25 ppb.

Chronic concentrations in a stream would likely be affected by dissipation due to hydrodynamic dispersion, degradation, mixing, and sorption as the antimycin flows downstream. Thus, antimycin chronic concentrations in streams are likely to be lower than calculated here. However, the dissipation mechanisms of antimycin in a stream are not well understood and will likely be site-specific, depending on such highly localized factors as stream velocity and turbulence; thus, no further attempts at refining stream concentrations were made in this assessment.

The label describes a method of detoxifying antimycin with 1 ppm of potassium permanganate, but the label does not require that this detoxification be performed. Furthermore, EFED has no information regarding the effectiveness of potassium permanganate at removing

---

[98] *Ibid* Moore et al. 2006.

[99] EPA. 2006. Environmental Fate and Ecological Risk Assessment for the Reregistration of Rotenone. (DP Barcode 307380); Docket Number OPP-EPA-HQ-2005-0494 http://www.regulations.gov/

antimycin and thus EFED cannot assume that potassium permanganate actually removes antimycin from a water body. EFED did not attempt to adjust recommended estimated environmental concentrations with consideration for potassium permanganate use.

6.7 Federally Threatened and Endangered (Listed) Species Concerns

Section 7 of the Endangered Species Act, 16 U.S.C. Section 1536(a)(2), requires 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, if they are proposing an "action" that may affect listed species or their designated habitat. Each federal agency is required under the Act to insure 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. 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."[100]

To facilitate compliance with the requirements of the Endangered Species Act subsection (a)(2), the Environmental Protection Agency Office of Pesticide Programs has established procedures to evaluate whether a proposed registration action may directly or indirectly 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 any listed species (U.S. EPA 2004). After the Agency's screening-level risk assessment is conducted, if any of the Agency's listed species LOCs are exceeded for either direct or indirect effects, an analysis is conducted to determine if any listed or candidate species may co-occur in the area of the proposed pesticide use or areas downstream or downwind that could be contaminated from drift or runoff/erosion. If determined that listed or candidate species may be present in the proposed action areas, further biological assessment is undertaken. The extent to which listed species may be at risk then determines the need for the development of a more comprehensive consultation package as required by the Endangered Species Act.

The federal action addressed herein is the proposed re-registration of pesticide product that contains the active ingredient antimycin.

6.7.1 Action Area

For listed species assessment purposes, the action area is considered to be the area affected directly or indirectly by the Federal action and not merely the immediate area involved in the action. At the initial screening level, the risk assessment considers broadly described taxonomic groups and so conservatively assumes that listed species within those broad groups

[100] 50 C.F.R. § 402.02

are collocated with the pesticide treatment area. This means that terrestrial plants and wildlife are assumed to be located adjacent to the treated site and aquatic organisms are assumed to be located in a surface water that is the treated site. The assessment also assumes that the listed species are located within an assumed area which has the relatively highest potential exposure to the pesticide, and that exposures are likely to decrease with distance from the treatment area. The use characterization section of this risk assessment presents the pesticide use sites that are used to establish initial collocation of species with treatment areas.

6.7.2 Taxonomic Groups Potentially at Risk

If the assumptions associated with the screening-level action area result in RQs that are below the listed species LOCs, a "no effect" determination conclusion is made with respect to listed species in that taxa, and no further refinement of the action area is necessary. Furthermore, RQs below the listed species LOCs for a given taxonomic group indicate no concern for indirect effects upon listed species that depend upon the taxonomic group covered by the RQ as a resource. 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 could 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.

Assessment endpoints, exposure pathways, the conceptual model addressing proposed antimycin re-registration uses, and the associated exposure and effects analyses conducted for the antimycin screening-level risk assessment are in **Sections 4** and **5.** The assessment endpoints used in the screening-level risk assessment include those defined operationally as reduced survival, reproduction, and growth for both aquatic and terrestrial animal species from direct acute and direct chronic exposures. These assessment endpoints address the standard set forth in the Endangered Species Act requiring federal agencies to ensure that any action they authorize does not 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. Risk estimates (i.e., RQs integrating exposure and effects) are calculated for broad-based taxa groups for the screening-level risk assessment and presented in **Section 6**.

Both acute endangered species and chronic risk LOCs are considered in the screening-level risk assessment to identify direct and indirect effects to taxa of listed species. This section identifies direct effect concerns, by taxa, triggered by exceeding listed species LOCs in the screening-level risk assessment with an evaluation of the potential probability of individual effects for exposures that may occur at the established listed species LOC. Data on exposure and effects collected under field conditions are evaluated to make determinations on the predictive utility of the direct effect screening assessment findings to listed species. Additionally, the

results of a screen for indirect effects to listed species, using direct effect acute and chronic LOCs for each taxonomic group, are presented and evaluated.

6.7.2.1 Listed Species Risk Quotients

A description of the potential direct effects associated with exposure to antimycin is discussed for each of the taxonomic groups below. **Table 27** provides a summary of the direct effects for Federally listed threatened/endangered species, including the range of RQ values and the acute dose-response slopes used in evaluating the probability of individual effects on listed species.

Table 27. Summary of direct effects for Federally listed species from pisicidal uses.

Listed Species Taxonomic Group of Concern	Direct Effects	Slope[a]	RQ
Freshwater Fish	Acute: mortality	15.5	2,778
Freshwater Invertebrates	Acute: mortality/immobilization	4.5	3,125
Saltwater Mollusc	Acute mortality	4.5	0.40
Aquatic Plants: Vascular Non-vascular	 no data no data	 — —	 — —
Birds	Acute: mortality/sublethal	4.5	<0.01* ---
Mammals	Acute: mortality	6.5	<0.01* --
Terrestrial Plants: Monocots Dicots	 Acute: no data Acute: no data	 — —	 — —

[a]Raw data were not provided so the default value of 4.5 is used.
*Dose-based value.

6.7.2.1.1 Freshwater Fish and Amphibians

Listed species acute risk LOCs for direct effects on freshwater fish and amphibians are exceeded (RQ = 2,778) for antimycin when used at the typical label rate for piscicidal use in warm waters where degradation would be fastest.

It is noteworthy that piscicides such as antimycin are used in efforts to restore listed species. For example, the Gila trout (*Oncorhynchus gilaes)* was first listed in 1967. It is currently designated as endangered in the entire range; the published range of this species

includes Arizona and New Mexico.[101] Stocking and naturalization of non-native trout with the range of Gila trout and ensuing hybridization, predation and competition are considered by the U.S. Fish and Wildlife Service to be major causes of the imperiled status of the species. The revised recovery plan for this species focuses on chemically treating non-native salmonids to re-establish viable populations of Gila trout in its historic range[102]. A vital component of the recovery and long-term survival of Gila trout is removal of non-native trout and hybrids; to that end, antimycin A has been used to eradicate the non-native trout.

6.7.2.1.2 Freshwater Invertebrates

Listed species acute risk LOCs for direct effects on freshwater invertebrates are exceeded (RQ =3,125) for antimycin when used at the typical treatment rate for piscicidal use in flowing and static waters. No chronic freshwater invertebrate toxicity data were submitted and no useable data were located in the open literature; therefore, chronic effects associated with freshwater invertebrate exposure to antimycin are unknown.

6.7.2.1.3 Estuarine/Marine Fish and Invertebrates

Listed species acute risk LOCs for direct effects on estuarine/marine fish and invertebrates are exceeded with RQ values of 109 and 1.0, respectively. No chronic estuarine/marine fish or invertebrate toxicity data were submitted and no useable data were located in the open literature for antimycin; therefore, acute and chronic effects associated with estuarine/marine fish and invertebrate exposure to antimycin are unknown.

6.7.2.1.4 Aquatic Plants

No aquatic plant toxicity data were submitted and no useable data were located in the open literature for antimycin; therefore, risks associated with aquatic plant exposure to antimycin are unknown.

6.7.2.1.5 Birds

No acute risk LOCs are exceeded for birds. No chronic bird toxicity data were submitted and no useable data were located in the open literature for antimycin; therefore, chronic risks associated with avian exposure to antimycin are unknown.

6.7.2.1.6 Mammals

No acute risk LOCs are exceeded for mammals. No chronic mammalian toxicity data were submitted and no useable data were located in the open literature for antimycin; therefore, chronic risks associated with mammalian exposure to antimycin are unknown.

---

[101] U. S. Fish and Wildlife Service Endangered Species Status. http://ecos.fws.gov/species_profile/servlet/gov.doi.species_profile.servlets.SpeciesProfile?spcode=E00E#status

[102] Pittenger, J. 1993. Gila Trout (Oncorhynchus gilae) (Third Revision) Recovery Plan. U. S. Fish and Wildlife Service, Albuquerque, NM http://ecos.fws.gov/docs/recovery_plans/2003/030910.pdf

6.7.2.2 Probit Dose Response Relationship

6.7.2.2.1 Aquatic Listed Species Probability of Effects on Individuals

The probability of individual effects at estimated acute RQs above the listed species acute risk LOC was calculated. The probit slopes used in this analysis were obtained from dose-response relationships used in calculating RQs. For freshwater fish, the probit dose-response slope is 15.5 based on the coho salmon acute toxicity test. Should exposure to listed freshwater fish occur at the typical treatment rate of 25 µg/L, the probability of one individual being affected is 1 in 1.00 (i.e., 100%).

The probability of individual effects to listed freshwater invertebrates should exposure occur at the typical treatment rate is again 100%. The probit dose-response slope used for freshwater invertebrates was 4.5 (the default value used in OPP assessments).

7 CONCLUSIONS

There are a number of uncertainties associated with the environmental fate and potential ecological effects of antimycin A. Although there are data indicating that the chemical is short-lived in warm, alkaline waters, the persistence of antimycin under cold, acidic conditions is unknown. Furthermore, the extent to which antimycin partitions to sediments is unknown. In spite of these uncertainties, available literature suggests that through a combination of environmental factors (pH, temperature, flow velocity, dilution), antimycin does not appear to persist in the environment; however, the exact mechanism underlying its limited persistence is unclear.

Relative to other piscicides and other pesticides in general, antimycin is extremely toxic to some species of fish; however, field studies indicate that through the use of rigorous application procedures, the extent of acute mortality can be limited to the targeted treatment area. Although the current label does not require that standard operating procedures be followed, the registrant has indicated that it is committed to developing such procedures and linking them to the labeled uses of antimycin.

Available data indicate that aquatic invertebrates can be as sensitive to antimycin as fish; however, field studies have indicated that effects on aquatic macroinvertebrates are transitory. Aquatic invertebrate populations appear to recover to pretreatment levels in terms of both abundance and diversity; this likely occurs through immigration from upstream, untreated areas.

Although terrestrial animals may consume dead or dying fish or ingest antimycin-treated drinking water, current data indicate that antimycin residues would not be sufficient from either source to constitute a risk of acute mortality to these animals.

There is uncertainty regarding the chronic effects of antimycin particularly for animals with low metabolic rates such as may exist in certain life stages; however, the potential for

chronic exposure appears limited. In lotic environments, antimycin is expected to move out of the treatment area by wash through and dilution by untreated tributaries. Additionally, although the current label does not require deactivation using potassium permanganate, the oxidizing agent is commonly used in lotic environments to prevent the movement of antimycin outside of the targeted treatment area. The potential for chronic effects in lentic environments, though, continues to be uncertain; however, antimycin use in aquaculture does not appear to have any reported adverse effects on fingerling catfish that are stocked afterwards. Additionally, in alpine lakes, aquatic communities apparently recover enough to sustain restocking efforts.

While there are potential risks to Federally-listed threatened/endangered aquatic species, antimycin is used in the recovery of some species. It is incumbent of the users to conduct the necessary monitoring to establish whether listed species are present; however, given the treatment rates and the vulnerability of aquatic animals to antimycin, acute mortality may result from exposure.

Finally, the lack of a quantitative method for measuring antimycin in water has been a limiting factor in being able to conduct detailed environmental fate and ecological effect studies. The current methodologies being developed by the U.S. Geological Survey are expected to vastly improve the analytical detection limits for antimycin A.

8 REFERENCES

American Fisheries Society. 2006. Fintrol® Stream and River Use Monograph for Complete Removal of Fish. Use in Streams and Rivers. http://www.fisheries.org/html/Antimycin_monographs.shtml

American Fisheries Society. 2006. Fintrol® Pond, Lake and Reservoir Use Monograph for Complete Removal of Fish: Use in Ponds, Lakes and Reservoirs http://www.fisheries.org/html/Antimycin_monographs.shtml

Anderson, B.G. 1944. The toxicity thresholds of various substances found in industrial wastes as determined by the use of *Daphnia magna*. Sewage Works J. 16(6): 1156-1165.

Arslaneglau, L. and V. Korths. 1967. Antimycin Toxicity Studies: Administration of Water Treated with Antimycin to Rats and Dogs. Ayerst Research Laboratories. (MRID 2400798-10).

Arslaneglau, L. and V. Korths. 1967. Antimycin Toxicity Studies: Administration of Fish Killed with Antimycin to Rats and Dogs. Ayerst Research Laboratories. (MRID 2400798-08)

Ayerst Laboratories. 1964. Efficacy of Antimycin. (Compilation: unpublished study received in 1994 under 8991-5l CDL: 005666-A (Accession No. l 00135924)

Bettermann, A.D., J.M. Lazorchak and J.C. Dorofi. 1996. Profile of toxic response to sediments using whole-animal and *in vitro* submitochondrial particle (SMP) assays. Journal of Environmental Toxicology and Chemistry 15(3): 319 – 324.

Birdsong, C.L. and J.W. Avault Jr. 1971. Toxicity of certain chemicals to *Juvenile pompano*. Prog. Fish-Cult. 33(2): 76-80.

Cerreto, K. M. 2004. Antimycin and rotenone: short-term effects on invertebrates in first order, high-elevation streams. A master's thesis submitted to the Department of Zoology and Physiology and the Graduate School of the University of Wyoming

ECOSAR. Ecological Structure Activity Relationships http://www.epa.gov/opptintr/newchems/tools/21ecosar.htm

ECOTOX (ECOTOXicology database). 2005. Maintained by the U.S. EPA ORD and the National Health and Environmental Effects Research Laboratory's (NHEERL's) Mid-Continent Ecology Division. http://www.epa.gov/ecotox.

EPA. 2006. Environmental Fate and Ecological Risk Assessment for the Reregistration of Rotenone. (DP Barcode 307380); Docket Number OPP-EPA-HQ-2005-0494 http://www.regulations.gov/

EPI Suite http://www.epa.gov/opptintr/exposure/docs/episuite.htm

Finlayson, B.J., R.A. Schnick, R.L. Cailteux, L. DeMong, W.D. Horton, W. McClay, and C.W. Thompson. 2002. Assessment of Antimycin A Use in Fisheries and its Potential for Reregistration. Fisheries 27(6): 10 -18.

Finlayson, B.J., R.A. Schnick., R.L. Cailteux., L. DeMong., W.D. Horton , W. McClay , C.W. Thompson, and G.J. Tichacek.. 2000. Rotenone Use in Fisheries Management: Administrative and Technical Guidelines Manual. American Fisheries Society, Bethesda, Maryland.

Heim, D. 2003. Determination of Aerobic Aquatic Metabolism for Antimycin A Complex: Amended Final Report. Lab Project No. 47313. Unpublished study prepared by ABC Laboratories, Inc. 79 p (MRID 458959-01).

Heim, D. 2003. Hydrolysis Determination for Antimycin A Complex. Project Number 47132. Unpublished study prepared by Analytical Bio-Chemistry Labs, Inc. 63 p. (MRID 460231-01).

Hobbs. M. S., R. S. Grippo, J. L. Farris, B. R. Griffin and L. L. Harding. 2006. Comparative Toxicity of Potassium Permanganate to Nontarget Aquatic Organisms. Environmental Toxicology and Chemistry 25(1): 3046 – 3052.

Hogan, J. 1966. Antimycin as a Fish Toxicant in Catfish Culture. Presented at the 20th Annual Meeting of the Southeastern Association of Game and Fish Commissioners, Oct 24 - 26, 1966. Bureau of Sport Fisheries and Wildlife, U.S. Fish and Wildlife Service. (Accession No. 00045801; MRID 2400798-13).

Hussain, A. 1969. Kinetics and mechanisms of hydrolysis of antimycin A in solution. Journal of Pharmaceutical Sciences 58: 316-320.

Kenneke, J.F. Summary of Antimycin Hydrolysis Research, US EPA, unpublished report (see Appendix G)

Kuhn, J.O. 2001. Fintrol® Concentrate (Antimycin) Final Report Acute Oral Toxicity in Rats. Stillmeadow, Inc. Report No. 6025-00. Submitted to Aquabiotics Corporation, Bainbridge Island, WA. (MRID 455279-01).

Lee, T.H., Derse, P.H., and Morton, S. 1971. Effects of Physical and Chemical Conditions on the Detoxification of Antimycin. Transactions of the American Fisheries Society 1971; 100: 13–17

Liu, W.C., E.E. van Tamelan, F.M. Strong. 1960. The chemistry of antimycen A. VIII Degradation of Antimycen A. Journal of the American Chemical Society 82: 1652-1654

Marking, L.L. and T.D. Bills. 1975. Toxicity of potassium permanganate to fish and its effectiveness for detoxifying antimycin. Trans. Am. Fish. Soc. 104(3): 579-583.

Mayer, F. and M. Ellersieck. 1986. Manual of Acute Toxicity: Interpretation and Data Base for 410 Chemicals and 66 Species of Freshwater Animals. U.S. Fish and Wildlife Service. Resource Publication 160. 579 p. (MRID 400980-01)

Mineau, P, B.T. Collins, and A. Baril. 1996. On the use of scaling factors to improve interspecies extrapolation of acute toxicity in birds. Regulatory Toxicology and Pharmacology. 24**:** 24-29.

Moore, S.E., M.A. Kulp, J. Hammonds, and B. Rosenlund. 2005. Restoration of Sams Creek and an Assessment of Brook Trout Restoration Methods, Great Smoky Mountain National Park. National Park Service, U.S. Department of the Interior. Technical Report NPS/NRWRD/NRTR-2005.

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

OPPTS 850.1075. 1996. Ecological effects test guidelines: fish acute toxicity test, freshwater and marine. U.S. Environmental Protection Agency Office of Prevention, Pesticides and Toxic Substances: EPA712-C-96-118. http://www.epa.gov/offtsfrs/publications/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Drafts/850-1075.pdf

ORD. 1993. Wildlife Exposure Factors Handbook. Office of Health and Environmental Assessment, Office of Research and Development. EPA/600/R-93/187. http://www.epa.gov/ncea/pdfs/birds.pdf

PBT Profiler http://www.pbtprofiler.net/

Pittenger, J. 1993. Gila Trout (*Oncorhynchus gilae*) (Third Revision) Recovery Plan. U.S. Fish and Wildlife Service, Albuquerque, NM http://ecos.fws.gov/docs/recovery_plans/2003/030910.pdf

Reardon, I.S. and R.M. Harrell. 1994. Effects of varying salinities on the toxicity of potassium permanganate to larval and juvenile striped bass, *Morone saxatilis* (Walbaum). Aquacult. Fish. Manag. 25(6): 571-578.

Ritter, P.O. and F.M. Strong. 1966. Residues in tissues of fish killed by antimycin. Journal of Agricultural Food Chemistry, 14(4): 403 – 407. (MRID 461534-01)

Sreekala, K.G., J. Jennita, and V.R. Prakasam. 1991. Tolerance and heart rate of *Daphnia sp*. (crustacean) in response to disinfectants, bleaching powder and potassium permanganate. Pollut. Res. 10(1): 33-36.

Tiffan, K.F. and E.P. Bergersen. 1996. Performance of Antimycen in high-gradient Streams. North American Journal of Fisheries Management 16: 465-468.

U.S. Fish and Wildlife Service Endangered Species Status. 2006. http://ecos.fws.gov/species_profile/servlet/gov.doi.species_profile.servlets.SpeciesProfile?spcode=E00E#status

Walker, C.R., R.E. Lennon, and B.L. Berger. 1964. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals. U.S. Bureau of Sport Fisheries and Wildlife, Investigations in Fish Control No. 2 (Circular No. 186). 18 pp.

Wisconsin Alumni Research Foundation. 1965. Antimycin as a Fish Toxicant: a resume of information and data pertaining to the use of antimycin in fish management procedures in ponds and lakes. (Accession No. 00135924)

9 APPENDIX A. ASTER Model Output

20-Sep-05

ASTER ECOTOXICITY PROFILE

U.S. Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Mid-Continent Ecology Division
(formerly the Environmental Research Laboratory-Duluth)

Contact: Scientific Outreach Program
218-529-5225 or FAX 218-529-5003
E-mail: ecotox.support@epa.gov

I. CHEMICAL IDENTIFICATION

Name Antimycin A

CAS number 1397-94-0
SMILES O1C(=O)C(NC(=O)C-c2c(O)c(NC=O)ccc2)C(C)OC(=O)C(CCCCCC)C(OC(=O)CC(C)C)C1
Formula C28 H40 N2 O9

II. ENVIRONMENTAL EXPOSURE ASSESSMENT

Parameter	Value	Source	Reference
Molecular Weight (g/mole)	548.6	Calc.	
Melting Point (C)	not available for this chemical		
Boiling Point (C)	550.	Calc.	
Vapor Pressure (mm of Hg)	2.31E-15	Calc.	
Ht Vaporization (cal/mole)	1.68E+04	Calc.	
Solubility in Water (mg/L)	69.0	Calc.	
Log P	3.81		
CLogP	17934		
pKa	9.92	Calc.	
Adsorption Coef (log Koc)	3.41	Calc.	
Henry's Constant (atm-m**3/mole)	2.42E-17	Calc.	
Log10(Henry's Constant) (atm-m**3/mole)	-16.6	Calc.	
Hydrolysis Half-life (days)	190.	Calc.	
BOD Half-life:		Calc.	

HALF-LIFE > 100 DAYS [E.G., SEE ALEXANDER (1965,1973), AND MCKENNA

AND HEATH (1976) FOR REVIEWS ON THE RECALCITRANCE OF POLYAROMATIC COMPOUNDS]

Mackay Level 1 Environmental Partitioning @25 C Fugacity = 1.753E-17 Pa

0.00	% into air
25.81	% into soil
50.04	% into water
0.04	% into suspended solids
0.02	% into aquatic biota
24.09	% into sediment

III. ECOTOXICOLOGICAL HAZARD ASSESSMENT

Aquatic Hazard Identification

Researchers and managers using AQUIRE data for analysis or summary projects should consult with the original scientific paper to ensure an understanding of the content of the data retrieved from AQUIRE.

BIOCONCENTRATION DATA

Species Common Name / Species Latin Name	Ex Ty	Duration (days)	Endpoint Effect	Conc Type	BCF	Source	Ref No.
FRESH WATER							
184207: Fathead minnow / Pimephales promelas	F	2.00-304	BCF	RSD calculated	410	QSAR	7

Human Health Hazard Identification

There is no information in the QSAR SYSTEM which would suggest that this chemical is a potential carcinogen or mutagen.

IV. ECOLOGICAL RISK CHARACTERIZATION

A. Environmental Exposure Assessment

Henry's Constant = 2.42E-17 atm-m**3/mole

Log10 (Henry's Constant) = -16.6 atm-m**3/mole

Lyman et al. 1982. would conclude that a chemical with these properties is non-volatile. See page 15-15.

The hydrolysis half-life of the amide moeity is expected to be greater than six months.
Hydrolysis Half-Life = 190 days

B. Ecotoxicological Hazard Assessment

Genetic/Mutagenic Assessment

There is no information in the QSAR SYSTEM which would suggest that this chemical is a potential carcinogen or mutagen.

NEUROTOXICANT: PYRETHROIDS The acute mode of toxic action for pyrethroid insecticides is generally attributed to an interaction with sodium channels in nerve membranes that ultimately results in tremors and seizures [9403].

When sufficient data is available from fathead minnow early life stage (ELS) tests (32-d exposures) completed at ERL-Duluth, QSAR models have been developed to predict chronic values for either survival or growth, which ever is the most sensitive endpoint. A chronic value is defined as the geometric mean of the LOEC(lowest observable effect concentration) and the NOEC (no observable effect concentration). These models have been developed for groups of xenobiotics that have been classified based on their acute modes of toxic action. Empirical observations suggest that when a statistically robust ELS QSAR can be established and when 96-h LC50/32-d ELS chronic value ratios are within a factor of 20 it is reasonable to assume that adverse effects are elicited through the same mode of toxic action in both 4-d and 32-d exposures. If during a chronic exposure a different mode of action is involved, or if metabolic activation is significant, the ratios between acute and chronic endpoint values for a group of xenobiotics are generally quite variable and typically exceed two orders of magnitude. In addition, the statistical strength of ELS QSARs in these instances are poor.

A chronic value cannot be calculated for the fathead minnow for pyrethroid and organochlorine type insecticides. 96-h LC50/32-d ELS chronic value ratios for neurotoxicants tested at ERL-Duluth using the fathead minnow range from 3.98 to 22.2 (log P range of 6.06 to 6.50).

C. Other Information

V. CITATION INFORMATION

REFERENCE NUMBER: 7
Veith,G.D. and P.Kosian
1983
Estimating Bioconcentration Potential from Octanol/Water
Partition Coefficients In: D.Mackay, et al., (Eds.), Physical Behavior of PCBs in the Great Lakes, Ann Arbor Sci. Publ., Ann Arbor, MI:269-282

REFERENCE NUMBER: 9403
Coats,J.R. 1990 Mechanisms of Toxic Action and Structure-Activity Relationships for Organochlorine and Synthetic Pyrethroid Insecticides Environ. Health Perspect. 87:255-262

REFERENCE NUMBER: 17934
Leo,A. and D.Weininger 1997. Daylight Software CLogP Version 3.15+ for Unix Pomona Medical Chemistry Project, Pomona College, Claremont, CA. Distributed by Daylight Chemical Information Systems, Inc., 3952 Claremont St., Irving, CA 92714

10 APPENDIX B. PBT Profiler Model Output

Persistence Summary

Partitioning

The PBT Profiler uses three environmental compartments (water, soil, and sediment) to determine the persistence of a chemical in the environment. If released to the environment, antimycin A is expected to be found predominantly in soil. It is also expected to be found in water and sediment.

The PBT Profiler does not explicitly consider a chemical's fate in the atmosphere in its persistence estimate. It also does not consider a chemical's potential to enter groundwater. Important P2 considerations in these media may be discussed on a chemical by chemical basis in the sections that follow.

Transformation and Persistence

The PBT Profiler has estimated that antimycin A is expected to be found predominantly in soil and its persistence estimate is based on its transformation in this medium. Its half-life in soil, 75 days, exceeds the EPA criteria of ≥ 2 months (and ≤ 6 months). Therefore, antimycin A is estimated to be persistent in the environment.

The PBT Profiler calculates a chemical's atmospheric half-life from the estimated gas-phase reaction rate with hydroxyl radicals and ozone. The vapor pressure of antimycin A, 0.000000000001 mm Hg, suggests that it will exist as a gas/particulate mixture in the atmosphere. Since particulates react slower with hydroxyl radicals and ozone (relative to a gas-phase reaction), the atmospheric lifetime of antimycin A is expected to be longer than that predicted by the PBT Profiler. As a result, the distribution of antimycin A in the various environment compartments may be different than that predicted by the PBT Profiler. This should be considered when identifying P2 opportunities.

Overall Persistence

The overall persistence is a calculated term that allows the persistence of different chemicals to be compared using a single value. Even though the units of the overall persistence are the same as those used for a chemical's half-life (hrs), these two terms are not inter-convertible. The overall persistence takes into account both a chemical's media-specific half-life *as well as* its rate of transport into (and out of) that compartment. Because the overall persistence takes into account transport, its value will likely be different than any of the media-specific half-lives.

The overall persistence can only be calculated in a mass-balance multimedia model. These models calculate the overall persistence by determining the weighted average of the residence time in each compartment.

The overall persistence for antimycin A is 96 days using the default emission scenario of the level III multimedia model. The overall persistence using different release scenarios is provided in the following section.

Release Scenarios

The PBT Profiler estimates persistence based on a standard release scenario emitting equal amounts to soil, water, and air. A more in-depth P2 assessment may utilize a release scenario that is more representative of an individual chemical's life cycle. This section of the PBT Profiler provides seven different release scenarios to help identify P2 opportunities for antimycin A. The seven release scenarios are based on a more realistic total release of 300 kg/hr to the environment and not the 1,000 kg/hr shown on the PBT Profiler results page. Since the fugacity model is linear, the percent in each compartment does not change based on the total release to the environment, but only on the relative amount released to air, water, and soil.

The following table provides the percent estimated in each environmental compartment using different release scenarios. The media (water, soil, and sediment) the chemical is expected to be found in predominantly (the predominant compartment) is underlined. The color of each estimate indicates if the EPA criteria have been exceeded in that specific medium. Therefore, by determining the color of the underlined value in each row, the persistence ranking for each different scenario can be compared directly to the default persistence value, **P**, estimated by the PBT Profiler.

The overall persistence, P_o (days), calculated for each release scenario is also provided.

Release to each medium (Kg/hr)			Percent in each medium				P_o
Air	Water	Soil	Air	Water	Soil	Sed	
100	100	100	0	11	84	4	96
150	0	150	0	1	98	0	110
300	0	0	0	2	97	1	100
150	150	0	0	19	73	7	90
0	150	150	0	17	77	6	92
0	300	0	0	72	0	28	76
0	0	300	0	0	100	0	110

Bioaccumulation Summary

Bioconcentration Bioaccumulation is the process by which the chemical concentration in an aquatic organism achieves a level that exceeds that in the water, as a result of chemical uptake through all possible routes of exposure. Biomagnification refers to the concentration of a chemical to a level that exceeds that resulting from its diet. Bioaccumulation includes both biomagnification and bioconcentration.

In general, chemicals that have the potential to bioconcentrate also have the potential to bioaccumulate. Since a bioconcentration factor (BCF) in fish can be readily measured in the laboratory and bioaccumulation is much more complicated to determine, the BCF is frequently used to predict the importance of bioaccumulation. The estimated bioconcentration factor (BCF) of 350 for antimycin A does not exceed the EPA bioconcentration criteria.

Bioaccumulation Estimate

The PBT Profiler estimates that antimycin A is not expected to bioaccumulate in the food chain because it does not exceed the BCF criteria.

Toxicity Summary Fish

Chronic

Toxic PBT chemicals are those that persist in the environment, bioconcentrate in aquatic organisms, and may bioaccumulate in humans, birds, and wild mammals. Exposure to PBT chemicals will result in chronic exposures which, in turn, can lead to chronic toxicity. The PBT Profiler uses an estimated fish chronic toxicity value (ChV) to allow organic chemicals lacking experimental data to be screened for P2 opportunities. A more in-depth P2 assessment requires that the potential toxicity of antimycin A to other aquatic organisms (and at other durations of exposure) be determined.

The PBT Profiler estimates that antimycin A is chronically toxic to fish. It is important to note that antimycin A may also be toxic to other aquatic organisms. Some aquatic organisms, such as daphnids, may be more sensitive to both acute and chronic exposures to antimycin A. To help assess the toxicity of antimycin A to other aquatic organisms, the PBT Profiler provides the complete ECOSAR™ estimates for this compound, as discussed below.

Other Toxicity Information

Unlike persistence and bioaccumulation, there is a wide range of different aquatic toxicity endpoints that may be of concern when assessing a chemical for P2 opportunities. The PBT Profiler determines the fish chronic aquatic toxicity for its toxicity ranking. Endpoints specific to humans, avian and terrestrial species, benthic organisms, and other aquatic animals are not included in the PBT Profiler toxicity ranking that appears on the initial results page. Other endpoints associated with the acute, sub chronic, and chronic toxicity of antimycin A should be considered for the above organisms in light of its persistence, potential for bioaccumulation, release to the environment, and life cycle when performing an in-depth P2 Assessment.

To help address some of these toxicity issues, the following information may be useful when identifying P2 opportunities for antimycin A:

ECOSAR™ is a personal computer program developed by EPA's Office of Pollution Prevention and Toxics to estimate the acute and chronic toxicity of chemicals discharged into water. ECOSAR™ predicts a chemical's effects on aquatic organisms, although its results should be evaluated together with information on persistence and biodegradation.

The ECOSAR™ program, as well as more information on its use, is available from EPA's web site. The following is the complete ECOSAR™ output for antimycin A:

SMILES : O1C(=O)C(NC(=O)Cc2c(O)c(NC(=O))ccc2)C(C)OC(=O)C(CCCCCC)C(OC(=O)CC(C)C
)C1
MOL FOR: C28 H40 N2 O9
MOL WT : 548.64
Log Kow: 4.21 (KowWin estimate)
Melt Pt:
Wat Sol: 13.59 mg/L (calculated)

ECOSAR v0.99g Class(es) Found

Esters
Phenols

ECOSAR Class	Organism	Predicted Duration	End Pt	mg/L (ppm)
Neutral Organic SAR (Baseline Toxicity)	Fish	14-day	LC50	8.757
Esters	: Fish	96-hr	LC50	5.457
Esters	: Daphnid	48-hr	LC50	4.248
Esters	: Green Algae	96-hr	EC50	0.471
Esters	: Green Algae		ChV	0.382
Esters	: Fish		ChV	0.473
Phenols	: Fish	96-hr	LC50	3.507
Phenols	: Daphnid	48-hr	LC50	3.685
Phenols	: Green Algae	96-hr	EC50	3.016
Phenols	: Fish	30-day	ChV	0.509
Phenols	: Fish	90-day	ChV	0.067
Phenols	: Daphnid	21-day	ChV	0.381
Phenols	: Green Algae	96-hr	ChV	1.082

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect.
Esters:
Fish and daphnid acute toxicity log Kow cutoff: 5.0
Green algal EC50 toxicity log Kow cutoff: 6.4
Chronic toxicity log Kow cutoff: 8.0
MW cutoff: 1000

Phenols:
Fish and daphnid acute toxicity log Kow cutoff: 7.0
Green algal EC50 toxicity log Kow cutoff: 7.0
Chronic toxicity log Kow cutoff: 9.0
MW cutoff: 1000

Close Window

Developed by the Environmental Science Center under contract to the *Office of Pollution Prevention and Toxics , U.S. Environmental Protection Agency*
Computer Resources Donated by *Syracuse Research Corporation* Ver 1.203 Last Updated August 27, 2004

11 APPENDIX C. New Mexico Standard Operating Procedure for the Use of Piscicides.

PROCEDURE FOR DEPLOYMENT OF

PISCICIDE DURING RESTORATION

OF LENTIC WATERS

Introduction:

New Mexico Department of Game and Fish (NMDGF) manages fisheries in waterbodies (lakes, reservoirs, and ponds) to support the recreational fishing and conservation of native fishes. Natural lakes and anthropogenic impoundsments are important components to the state's fisheries program. They provide biological diversity and geographically dispersed opportunities for recreation. In addition, they are complex systems that may be inhabited by nuisance species or unwanted normative species. To effectively manage waterbodies for public's recreational interest and/or species conservation, the deployment of a piscicide into lentic waters is a valuable tool.

To meet management plan objectives, NMDGF will consider piscicide use in appropriate waterbodies. Deployment of a piscicide is a complex task requiring guidance by specific objectives, public scoping, interagency coordination, and identified benefits to the public and the resource.

Pre-treatment information gathering will include input from appropriate agencies, diverse public representatives, private landowners, and other interested parties. This investigation process will address public concerns for the deployment of a piscicide into surface waters of the state.

Purpose:

Because of the complexities involved with piscicide deployment, a standardized procedure is essential to provide guidance during the treatment of any waterbody in the state. This document provides general methods that will facilitate successful use of piscicides hi lentic systems for fishery management activities in New Mexico.

Project Development and Preliminary Assessments

1. The use of a piscicide in waterbodies is an important fishery management tool that will support the programs administrated by NMDGF. Use of a piscicide hi a lentic system hi New Mexico must be supported by objectives listed in a fishery management plan. A particular management plan should provide objective criteria for prioritizing and selecting waterbodies for chemical reclamation.

2. Identify Issues and Potential Conflicts

- Identify public opinions and attitudes hi the community
- Identify land status
- Identify recreational and commercial interests

- Identify water development and user groups

3. Description of waterbody proposed for treatment.
    - Physical character- surface acres, waterbody volume, depth profiles, thermal stratification, water retention time, springs, inflow and outflow channels, water quality, waterbody longevity, barrier presence, barrier function and stability.
    - Biological character- identify fishery, natural reproduction, conduct genetics testing if appropriate, characterize macroinvertebrate community.
    - Determine access to waterbody and potential tributaries.

4. Complete all state and federal permit requirements.
    - *e.g.* NEPA, SHPO, ESA, CWA 404

5. After a comprehensive evaluation of the proposed project by all planning entities, a decision on whether to continue with the proposed project will be made.

6. Staff will develop a treatment plan to include piscicide application plan, staff needs, safety protocol, and emergency response protocol. State certified pesticide applicators will supervise chemical restoration projects in New Mexico. In addition, personnel supervising restoration projects in New Mexico will have successfully completed the National Conservation Training Center (USFWS) course, Rotenone and Antimycin Use in Fisheries Management.

Pretreatment Activities

1. Determine waterbody boundaries, basin profile, inflows and outflows and other pertinent information (e.g. fish movements).

2. Determine surface acres and volume of waterbody. If appropriate, calculate stream discharge of tributaries. Calculate flow through time if appropriate. Conduct water quality monitoring.

3. Determine fish species present (target and non-target species).

4. Determine best method for treating lake and, if necessary, lake tributaries in accordance with product label. Methods selected should consider pH, temperature, organic load, plant material, and basin morphology. Logistical considerations include cost and accessibility.

5. Determine concentration of piscicide to remove target species.

6. Calculate amount of piscicide needed to treat waterbody including tributaries if required. If treatment of tributaries is required, follow the appropriate provisions in the Protocol for Deployment of Piscicides during Restoration of Native Fisheries in Lotic Waters.

7. If required, locate a neutralization site on the outflow from the waterbody.

8. Supervisory personnel will review deployment schedule, personnel responsibilities, methods of communication, and outline procedures for handling unexpected issues with field staff.

9. Personnel will post notices of the application at points of entry into the treatment areas as well as in appropriate offices and websites. Such notices will be posted at least 48 hours before deployment.

Deployment of Piscicide

Boat deployment of liquid rotenone or antimycin

1. Determine amount of piscicide to be deployed in the waterbody using directions on the label, water volume and inflow from tributaries. If necessary, tributaries can be treated in accordance with the Protocol for Deployment of Piscicides during Restoration of Native Fisheries in Lotic Waters.

2. Safety equipment will be worn according to label recommendations.

3. Set up live-cars with target fish to assess treatment effectiveness at varying points and depths in the waterbody.

4. Determine if it is necessary to divide lake into sections for deployment. Marker buoys should be used for guidance to ensure total coverage. Deploy the piscicide in a manner to effectively cover the treatment area, e.g. in a grid-like pattern. Backpack sprayers should be used to treat the shoreline and small inlets where boat access is limited. Drip stations can be deployed on larger inlets concurrently with lake application.

5. Shallow waters can be treated using venturi apparatus that pulls diluted piscicides into the prop wash of the boat motor. Deeper sections of the waterbody should be treated with bilge pumps and hoses. To ensure toxicity overlap between the sections, the entire volume of piscicide should be deployed within 48 hours.

6. Suspected problem areas may be treated with several of these methods. Sand mix formulas may be useful for underwater spring areas.

7. Treatment times will depend on turnover time in the lake and the type of piscicide chosen.

8. Where feasible, dead fish will be collected and buried in appropriate locations.

9. All deployment equipment will be emptied, rinsed with water to remove residue, disassembled, collected and stored in accordance with the product label.

Boat deployment of powdered rotenone product

1. Determine amount of piscicide to be deployed in the water body using directions on the label, water volume, and inflow from tributaries. If necessary, tributaries can be treated in accordance with the Protocol for Deployment of Piscicides during Restoration of Native Fisheries hi Lotic Waters.

2. Safety equipment will be worn according to the labeling requirements.

3. Powdered rotenone should be applied using a closed system to limit drift. An example of this type of system is an aspirator system that suctions the powdered rotenone from the containers into water that is pumped through the system.

4. Set up live-cars with target fish to assess treatment effectiveness at varying points and depths in the waterbody.

5. Determine if it is necessary to divide lake into sections for deployment. Marker buoys should be used for guidance to ensure total coverage. Deploy the piscicide in a manner to effectively cover the treatment area, e.g. in a grid-like pattern. Backpack sprayers should be used to treat shoreline and small inlets where boat access is limited. Drip stations can be deployed on larger inlets concurrently with lake application using a liquid rotenone formulation.

6. To ensure toxicity overlap between the sections, the entire volume of piscicide should be deployed within 48 hours.

7. Where feasible, dead fish will be collected and buried in appropriate locations.

8. All deployment equipment will be emptied, rinsed with water to remove residue, disassembled, collected and stored in accordance with the product label.

Application of sand mix rotenone

May be effective in shallow impoundments.

1. Determine amount of piscicide to be deployed hi the water body using directions on the label, water volume and results from bioassays. Inflow rates need to be factored into treatment concentration and duration.

2. Staff mixing and applying sand formulation will wear safety equipment according to label requirements.

3. Sand mixture can be applied using burlap sacks or other types of pesticide application equipment that would be appropriate for dry materials. Equipment should be chosen to assure even application.

Arial application of liquid rotenone

Aerial application is desirable in situations where access to lakes is limited. Generally, it works for lakes <20 feet deep where piscicide distribution at greater depths is not a concern.

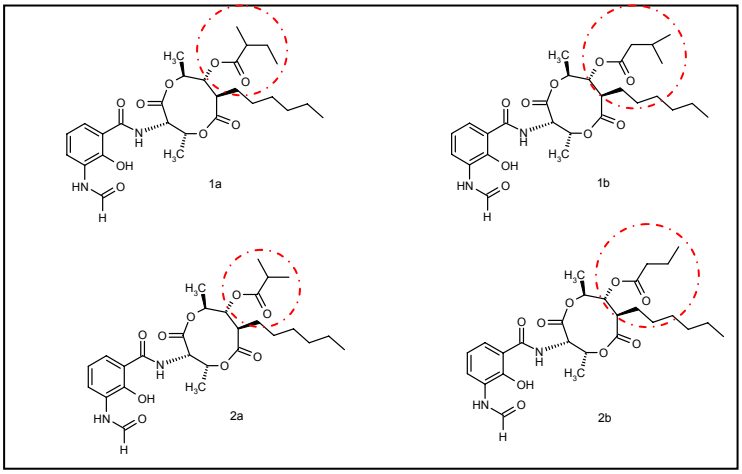
12 APPENDIX D. *In vitro* Mammalian Metabolism.

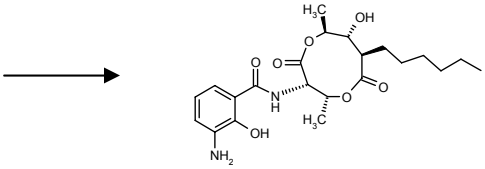
Dr. John Kenneke with the Processes and Modeling Branch in the U.S. EPA National Exposure Research Laboratory (NERL) Ecosystems Research Division has generated preliminary information on an *in vitro* metabolic pathway for antimycin A. As preliminary information, these data have not undergone rigorous internal review nor have they been subject to quality assurance measures that may permit a more quantitative use of the data.

Based on preliminary analyses using liquid chromatography/mass spectroscopy of an antimycin sample from the OPP Biological and Economic Assessment Division repository, Antimycin is composed of 8 components that group into two major categories: ones that have a butyl chain and the others that have a hexyl chain. Within each of these groups, four different structures exist based upon differences in the acyloxy side chain as indicated with the red circles (butyl and propyl structural isomers).

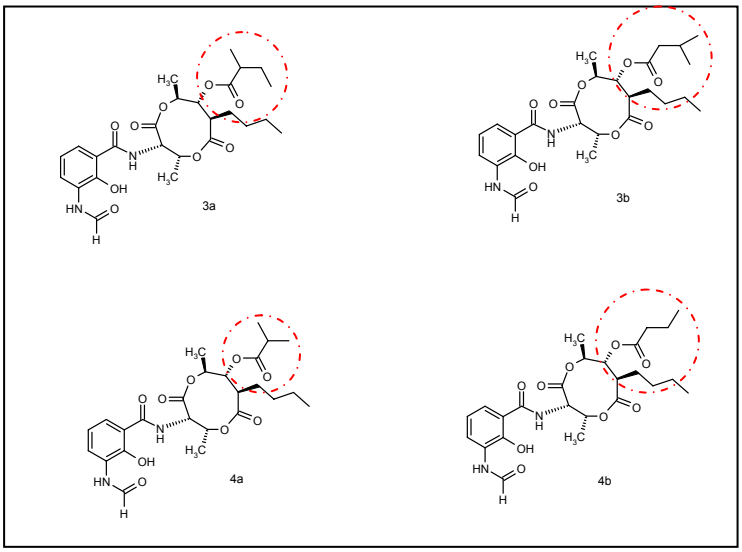
The theoretical masses of 1a/b, 2a/b, 3a/b, 4a/b are: 549, 535, 521, and 507 g/mole respectively. The mass spectral data we collected from our antimycin a standard shows 5 peaks with the same masses. *In vitro* phase 1 metabolism leads to hydrolysis of the acyloxy group (along with deformylation of the N-formylaniline)--all four of the hexyl compounds (1a, 1b, 2a, 2b) lead to the same metabolite--and all four of the butyl compounds (3a, 3b, 4a, 4b) lead to a second metabolite.

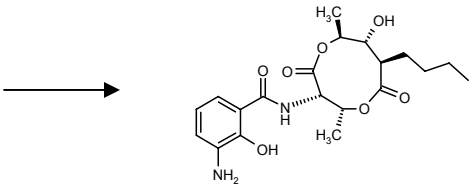
Hexyl Components





In vitro Phase 1 Metabolism





Butyl Components

13 APPENDIX E. Standard Operating Procedure for the Use of Fintrol® to Remove Scaled Fish in Catfish Ponds[103]

Fish other than catfish are sometimes intentionally stocked into catfish ponds for a specific purpose. For example, fathead minnows might be added to brood ponds to provide forage for broodfish. However, most catfish ponds are operated as monocultures, and fish other than catfish are a nuisance. In fingerlings ponds, wild fish may eat fry or compete for food. In foodfish ponds, wild fish may compete for food, interfere with harvest, or cause economic losses as "weigh-backs" at the processing plant. Accordingly, there are times when it may be necessary to remove wild fish from catfish ponds.

Two fish toxicants are registered by the Environmental Protection Agency for use in commercial catfish production. Antimycin is a non-selective toxicant and is typically used to eliminate the few remaining fingerlings from nursery ponds prior to stocking fry. Fintrol® (active ingredient antimycin A) is effective in removing scaled fish from a pond without harming catfish.

Fintrol® has been in use since the late 1960s. The chemical is absorbed through the fish's gills where it then interferes with respiration leading to death. The attributes that make Fintrol® a desirable management tool are a wide difference in toxicity to catfish and scaled fish, a low toxicity to mammals, and how quickly it degrades.

Fintrol® can selectively remove scaled fish because they are much more susceptible to low concentrations of the toxicant. Problematic scaled fish (usually shad, carp, or sunfish) can be eliminated at concentrations of 5 to 10 parts per billion (ppb), while it may take in excess of 20 ppb to kill catfish under normal conditions. It should be pointed out that concentrations high enough to remove unwanted bullheads would also be fatal to catfish.

To determine an effective concentration of Fintrol®, you need to know the species of fish to be killed as well as the pH and temperature of the pond water. The toxicant is most effective when the pH of the pond water is 8.5 or lower and at water temperatures above 60° F (a rough estimate of the required concentrations are given in Table 1). In moderately buffered catfish ponds (total alkalinity above 50 ppm), the ideal application time would be early in the morning (because pH is lowest at that time) after pond temperatures have stabilized above 60° F. Avoid treating ponds when catfish may be stressed due to high water temperatures or periods of low dissolved oxygen.

To determine the amount of Fintrol® needed to treat the pond, you will need to estimate the pond volume, in acre-feet of water. First, measure the surface area of the water to be treated. Multiply the surface area in acres by the average depth of the pond in feet. The result is the number of acre-feet to be treated.

[103] Avery, J.L. 2006. Use of Fintrol® to remove scaled fish in catfish ponds. *The Catfish Journal.* April 2006 (reproduced with permission of author).

Fintrol® is sold in a metal can containing two bottles. The bottle with the green label contains 240 cubic centimeters (cc) of Fintrol®-concentrate (20% solution of active ingredient) while the blue label bottle contains 240 cc of diluent (inactive solution). These two liquids are mixed together in equal amounts to obtain a solution used to treat the pond. Treatment rates are therefore expressed as the amount of final mixture needed to treat a certain pond volume. To get a correct volume of concentrate and diluent, use Table 2 to determine the amount of final solution needed to achieve a desired concentration per acre-foot. Multiply that number by the number of acre feet to be treated. Then thoroughly mix together half that amount of Fintrol®-concentrate and half that amount of diluent. For example, if you need 80 ounces of mixture to treat a pond, mix together 40 ounces of Fintrol®-concentrate and 40 ounces of diluent to obtain the 80 ounces you need. The final mixture retains potency for seven days. However, once this solution is mixed with water, it must be applied within eight hours.

As an example, let's say a catfish farmer wants to remove shad from a 10-acre catfish pond that is 4 feet deep with a water temperature of 75° F and a pH of 7.9. From Table 1, we see that a concentration of 5 ppb should selectively remove the shad without harming catfish. Table 2 indicates that we need to apply 2 ounces of Fintrol® solution per acre-foot to achieve a 5 ppb concentration. Therefore, 10 acres × 4 feet × 2 ounces per acre-foot = 80 ounces of Fintrol® solution needed to treat the pond. You would then mix 40 ounces of Fintrol®-concentrate and 40 ounces of diluent to obtain the 80 ounces you need.

After dilution with water, the Fintrol® solution can be applied by drip tubes, spray equipment, or mixing tanks on a chemical boat. During mixing, handling, or applying Fintrol®, workers should wear protective goggles and protective gloves. If any contact occurs with eyes or skin, flush immediately with water.

Treat ponds with Fintrol® only when ponds are not overflowing. Do not treat during rainy weather or when water is being added to ponds. This will prevent the chemical from being diluted or flushed out of the pond, thereby assuring a more effective treatment. This also helps protect the environment by preventing the chemical from being discharged into other water bodies.

If the chemical is fresh and treatment rates are calculated correctly, Fintrol® is one of the most dependable pond-management tools available to the catfish farmer. Fintrol® is, however, a relatively slow-acting chemical so be patient when waiting to see results. Depending on water temperature, it may be a day or two before you start to see signs of an effective treatment. As with all pesticides, read and follow all label recommendations.

Table 1. Rough estimation of concentrations of Fintrol ® needed for eradication of different fish species under various combinations of pH and water temperature.

	Effective Concentration of Fintrol (in ppb of active ingredient)			
	pH is 8.5 or less		pH is 8.5 or higher	
Target Species	Water temp. above 60° F	Water temp. below 60° F	Water temp. above 60° F	Water temp. below 60° F
Gizzard shad, carp, minnows, sunfish	5	7.5	7.5	10
Catfish, bullheads	15	20	20	25

Table 2. Amount of Fintrol® solution to achieve desired concentration.

Desired Concentration (ppb active ingredient)	Amount of Fintrol® solution (Fintrol®-concentrate + Diluent) per acre-foot (ml)[1]	(ounces)
1 ppb	12.3	0.50
2 ppb	24.6	0.75
3 ppb	36.9	1.25
4 ppb	49.2	1.50
5 ppb	61.5	2.00
6 ppb	73.8	2.50
7 ppb	86.1	2.75
8 ppb	98.4	3.25
9 ppb	110.7	3.75
10 ppb	123.0	4.00

[1] 1 cc = 1 ml

14 APPENDIX F. Review of Selected Papers

Ritter, P.O. and F.M. Strong. 1966. Residues in Tissues of Fish Killed by Antimycin. (Magnitude of Residue Study). Aquabiotics Corporation. Case No. 4121 (MRID461534-01)

Deformylantimycin A3 hydrochloride was mixed with H^3-formic acid to yield H^3-antimyicin. While the resulting antimycin was as active as unlabeled (cold) antimycin in inhibiting reduced coenzyme Q-cytochrome c reductase, the deformylated starting material only showed trace activity. The tritium-labeled antimycin did not show a significant exchange of its tritium label.

Small (40 - 115 g) rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) and common carp (*Cyprinus carpio*) were placed individually in 19-liter glass jars containing 15 liters of water treated with antimycin A. After the fish died, the fish were removed and the water was discarded. Larger (230 - 320 g) brook trout and common carp (100 g) were placed individually in metal tanks containing 43 liters of water treated with antimycin A. After a trout was exposed and died, it was followed by a carp and then a trout; after 3 fish had been exposed successively using the same water, the water was discarded. Fish were then frozen. Afterward small trout and carp were divided into 3 factions: head plus viscera, gills, and the remainder of the body. All tissue samples were homogenized except the gills. The gills were divided as equally as possible into left and right halves. Samples were then lyophilized and the radioactivity in each freeze-dried sample was determined by combusting the sample in oxygen and collecting and counting the water sample. Spiked (H^3-antimycin) samples showed recoveries of 96%.

Heart, liver, kidney, muscle, gills and skin from each of the large trout were sampled separately. The remainder of the carcass was divided into two fractions: head plus remaining viscera and the balance of the carcass.

Small carp (90 - 200 g) were collected from West Salem, Wisconsin and poisoned with 10 ppb of non-radioactive antimycin (presumably in the lab). Tissues were homogenized and then extracted with chloroform six times, the extracts combined and dried and then redissolved in ethanol. The antimycin content of the extracts was then estimated by determining the toxicity to goldfish. Glass beakers (3 L) containing 2 goldfish (1 - 3 g) in 1.5 L of distilled water. The volume of ethanol introduced with the test solution was not over 2 mL. Controls were run with 2.5 mL ethanol/1.5 L; responses were gauged relative to a standard bioassay of goldfish with antimycin at known concentrations. Spiked homogenates were also extracted to determine the extraction efficiency.

Results:

Radioactive residues in tissues were assumed to be intact antimycin A. **Table 1** depicts estimated antimycin tissue concentrations of fish killed by antimycin at two different treatment concentrations (5 and 10 ppb). As might be expected, the time required to kill fish with antimycin tended to be shorter at higher antimycin A concentrations. Tissue residues in fish also tended to be higher at higher treatment concentrations. Overall, of the fish studied (16), tissue residues averaged 203 µg/kg fish weight. Tissue residues in larger trout killed with antimycin at 10 ppb were similar to residues in small trout killed with antimycin at 5 ppb; the lowest tissue concentrations were identified in larger trout killed with antimycin at 5 ppb. Of the fish studied, carp appeared to be the least sensitive to antimycin A and required the longest time to kill; carp required roughly 5 times longer to die from antimycin than the trout.

Table 1. Whole body H^3-antimycin levels in fish killed with antimycin.

Number Used	Species	Survival Time (hrs)	Antimycin Dosage (ppb)	Tissue Residue[1] µg/kg fish weight
4	Carp	15 - 22	5	219, 238, 247, 286
4	Carp	12 - 16	10	323, 334, 355, 450
3	Small Trout	2.5 - 16	5	109, 135, 137
3	Small Trout	2 - 4	10	152, 160, 172
2	Larger Trout	2 - 5	5	59.2, 92.3
2	Larger Trout	2 - 3	10	109, 79.3

[1] Tissue residues based on radioactivity and presume radioactivity reflects 100% antimycin.

Fish			Antimycin Residues µg/kg fresh weight			
Number	Body Weight (g)	Survival Time (hrs)	Antimycin Concentration (ppb)	Gills	Head and Viscera	Remainder
			Brook Trout			
1	70.0	2.5 - 4.3	5	158, 166	217	75.2,74.8
2	43.5	6.2	5	179, 203	239, 258	98.7, 101
3	52.0	5.2	5	176, 177	232, 228	103, 105
4	47.4	2.2 - 4.0	10	224, 236	204, 190	132, 141
5	66.2	2.2 - 4.0	10	282	244	138, 123
6	44.3	2.2 - 4.0	10	223, 222	178, 263	133, 130
			Carp			
1	106	22.2	5	299	460, 480	139, 146
2	112	20.3	5	279	473	167, 164
3	107	20.8	5	238, 242	362, 417	218, 203
4	98.5	15.3	10	331	700, 780	261, 248
5	100	14.3	10	386, 406	413	290
6	90.2	16.7	10	298, 275	751, 811	190, 181

Brook trout and carp treated with 5 ppb antimycin tended to take longer to die than when fish were treated at 10 ppb. Although trout treated with 5 ppb showed that the highest tissue residues (µg/kg fish weight) based on radioactivity were associated with the head and viscera, trout treated with 10 ppb tended to show the highest residues in the gills (**Table 2**). Once again, carp treated with antimycin took roughly 5 times longer to die than trout; however, the carp were roughly 2 times as large as the trout. For carp, the highest tissue residues of antimycin were associated with the head and viscera.

Table 3 summarizes the tissue distribution of H^3 antimycin [equivalents in µg/kg] and indicates that residues appear to be predominately located in the liver and kidney. In this case, survival time was not clearly correlated with dose, as fish treated with 5 and 10 µg/L took roughly equivalent periods of time to die. The authors conclude from Tables 2 and 3 that the lowest antimycin concentrations occurred in the body. Average residues in the muscle were 79 ± 14.4 µg/kg (mean ± std error). The authors conclude that the ability of the fish to absorb and accumulate antimycin against a steep concentration gradient, e.g., 5 ppb in the water versus up to 900 ppb in certain tissues, points to a firm binding of the toxicity with some body constituents which may be the antimycin-sensitive site of the electron transport system.

Table 3. Tissue distribution of antimycin in rainbow trout killed with H^3-antimycin.

Fish No. Body Wt. Survival Time Dose	Antimycin Equivalent to Radioactivity (µg/kg Fresh Weight)						
	Liver	**Kidney**	**Gills**	**Muscle**	**Skin**	**Head plus Viscera**	**Remainder of Body**
1 319 g 2.7 hrs 5 ppb	470 475 563 502	334 300 446	153 143 168	66.6 74.4 22.6	95.3 38.1	80.6 74.6	41.6 40.8
2 263 g 2.3 hrs 10 ppb	954 790 762 855	304 598 461	159 163 151	170 96.7 48.5	100 68.4	127 127	83.3 82.9
3 230 g 5.0 hrs 5 ppb	702 795 797	496 406 210	134 178 132	150 40.0 44.2	30.4	101 42.4	69.4 65.7
4 271 g 2.7 hrs 10 ppb	670 538	397 319	121 149 148	110 46.6	145	77.7 87.9	145

The authors attempted to quantify the tissue concentrations of intact antimycin A by extracting the tissues with solvent and then exposing carp to the concentrated extract in a mixture of acetone and ethanol and compare the results to survival times at known concentrations of antimycin. Fish survival times after treatment at 2, 5 and 10 µg/L were 9.5 hrs, 2.7 to 6.8 hrs, and 4.6 hrs, respectively. Based on this response and the survival time of carp treated with the tissue extracts, estimates of antimycin ranged from 30 µg/kg to less than 15 µg/Kg (**Table 4**). Recovery of antimycin from spiked tissues ranged between 8.5 and 21%. While these data suggest that fish killed with antimycin have sufficient antimycin in their tissues to be toxic to other fish, the poor recoveries from spiked samples make it difficult to even qualitatively estimate tissue residues. The authors conclude, though, that given the oral LD50 values reported for mammals (10 - 55 mg/kg) and birds (2.9 mg/kg), coupled with the results from feeding studies where rats were fed 50% of their diet composed of fish killed with 5 to 10 ppb of antimycin, no ill effects are likely from the consumption of fish killed with antimycin.

Table 4. Goldfish assay of antimycin extracted from homogenized carp tissue.

Sample Tested	Survival Time (hours)	Qualitative Estimate of Activity
100-g Carp	8.1, 9.7	30 µg/Kg
200–g Carp	13.7, 14.7	<15 µg/Kg
93-g control carp with 35.5 µg	8.8, 10.1	3.5 µg/Kg
95-g control carp with 35.5 µg	6.5, 8.5	7.5 µg/kg

Uncertainties: It is unclear whether the 96% recovery of labeled material following combustion and counting also included the lyophilization procedure. It's not clear from the study to what concentration of antimycin (labeled and cold combined) the fish were actually exposed.

The goldfish bioassay for estimating antimycin A residues in tissues contained roughly 1.3 mL/L (2 mL/1.5 L) and exceeds the EPA recommended maximum of 0.1 mL/L.

Water quality conditions are not reported for the bioassays. According to the methods section, distilled water was used. Distilled water can be very damaging to fish and is contrary to EPA's recommended use of reconstituted water or natural waters that have been characterized.

The assumption that radioactive residues in tissues are representative of parent antimycin A was not adequately substantiated. The authors acknowledge the questionable validity of their assumption since antimycin was labeled in its formyl group and this group is relatively labile.

The authors conclusions that the ability of the fish to accumulate antimycin against a steep concentration gradient points to a firm binding of the toxicant with some body constituent that may be the antimycin sensitive site of the electron transport system is not supported by the data. Rather, these data suggest that antimycin is lipophillic and that it is partitioning to tissues where lipids are more likely.

Thompson, C.M., J. Griffen, P. Boudreau, W. Cranor, and K. Lawsin. 1980. Acute Toxicity of BOLERO 10G (SX-1252) to Rainbow Trout (*Oncorhynchus mykiss* formerly *Salmo gairdneri*). ABC Analytical Bio Chemistry Laboratories, Inc., P.O. Box 1097, Columbia, MO. ABC Report #26078. (Accession No. 00050664; MRID 2401255-02).

Although the study focused on Bolero® 10G (Thiobencarb), the positive control for this static study was antimycin A (Sigma Chemical Co.; Type III, crystalline, Lot 125c-0152). Report cites following Standard Methods for Examination of Water and Wastewater and Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Trout (mean wt = 1.1 g; mean length 41 mm) obtained from Mt. Lassen Trout Hatchery (Red Bluff, CA); 14-day acclimation period. Animals fasted 48 hrs prior to study. Study conducted in 19-liter glass vessels with 15 liters of soft, reconstituted water (DO 8.9 mg/L, pH 7.6, hardness 45 mg/L at $CaCO_3$; 12°C; 16-hr light: 8-hr dark cycle). Each treatment consisted of 10 fish. Study conducted in compliance with Good Laboratory Practice Regulations. DO dropped as low as 5.5 mg/L.

Uncertainties: Procedure for measuring concentrations of thiobencarb are reported; however, it is not stated whether the concentration of antimycin was measured. Purity of antimycin A is not specified. Test concentrations used to determine the LC_{50} of antimycin A are not reported and no raw data on mortality resulting from exposure to antimycin A are provided. At normal atmospheric pressure (760 mm Hg) and at 12°C, DO saturation is 10.8 mg/L; however, with readings of 5.5 mg/L at 96 hrs, the DO is at roughly 51% saturation and is below the recommended limit of 60% saturation.

48 hr LC_{50}= 0.00014 mg/L (95% CI: 0.000075 - 0.00024 mg/L)
96 hr LC_{50}= 0.000062 mg/L (95% CI: 0.000042 - 0.00014 mg/L)

Hogan, J. 1966. Antimycin as a Fish Toxicant in Catfish Culture. Presented at the 20th Annual Meeting of the Southeastern Association of Game and Fish Commissioners, Oct 24 - 26, 1966. Bureau of Sport Fisheries and Wildlife, U.S. Fish and Wildlife Service. (Accession No. 00045801; MRID 2400798-13)

Liquid formulation: crystalline antimycin dissolved in acetone diluted to obtain antimycin concentrations ranging from 0.01 ppb to 500 ppb.

Sand formulation: Fintrol 5 (antimycin incorporated in Carbowax and coated on 40-mesh sand; sand to carbowax weight ratio 1:99)

Twelve species of fish utilized in the study: paddlefish (*Polyodon spathula*), gizzard shad (*Dorosoma cepedianus*), goldfish (*Carassius auratus*), carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), buffalo hybrid (*Ictiobus* spp.), white catfish (*Ictalurus furcatus*), channel catfish (*I. punctatus*), green sunfish (*Lepomis cyanellus*), bluegill (*L. macrochirus*), and largemouth bass (*Micropterus salmoides*). Eggs of carp and channel catfish obtained from Marion National Fish Hatchery, AL; goldfish eggs obtained from Southeastern Fish Control Laboratory. Channel catfish eggs treated with acriflavine to inhibit fungal growth. All eggs less than 48-hrs old when exposed to antimycin. Prolonged exposure consisted of exposing eggs until all had visibly hatched; 2-hr exposures and prolonged exposures conducted according to Berger, B.L., R.E. Lennon, and J. Hogan. Investigations in Fish Control: Laboratory studies on antimycin as a fish toxicant. Bureau of Sport Fisheries and Wildlife, Resource Publication. Fry obtained by hatching channel catfish eggs at laboratory.

Adult fish treated in 0.25-acre pond at the Fish Farming Experimental Station, Stuttgart, Arkansas.

Four vinyl wading pools containing 3,785 liters of water stocked with 80 channel catfish fingerlings from the same lot. A bioassay cage containing 10 bluegills was placed in each pool. Two weeks later, Fintrol 5 (10 ppb) was added to two pools while the other two pools served as controls. Cage containing dead bluegills was emptied and an additional 10 bluegills were added to cage. Catfish in each pool fed daily at a rate of 3% of body weight; however, because of decreased water temperatures and ice formation, feeding was reduced to once or twice a week. Four months after stocking, the pools were drained and the surviving channel catfish were counted, weighed and measured.

Field Trial: 0.25-acre pond treated in mid-December (water temp=11^{o}C); by Day 8 water temperatures had declined to 4.4^{o}C. Seining indicated the presence of paddlefish, shad, carp, buffalo, white catfish, blue catfish, and channel catfish. Pond treated 10 ppb antimycin (400 g Fintrol 5). Stability of the antimycin tested by placing cages containing 10 fingerling rainbow trout into treated pond.

Results

Egg Studies

Control mortality in goldfish study was 37%. Among antimycin-treated goldfish, there was 83% mortality at 1 ppb and 100% mortality at 2.5, 5.0 and 10 ppb. Control mortality in the carp study was 81.3%; average mortality in antimycin-treated carp was 73% at 1.0 ppb and 100% at 5 and 10 ppb. For channel catfish, mortality was 4%; 100% of the eggs treated with antimycin at 27.5 ppb or higher died (failed to hatch); at 25 ppb, there was 74% mortality; antimycin concentrations of 20 ppb or less did not appear to affect the hatchability of the eggs. Treatment of channel catfish eggs with antimycin for 2 hours at 500 ppb resulted in 100% mortality while treatment with 250 ppb resulted in 81% mortality. Concentrations at or less than 100 ppb for two hours did not affect total hatch; control fish exhibited 95% hatching success.

Fry Studies

Channel catfish control mortality was 11%; for fingerlings treated with 0.75 ppb there was 100% mortality within 96 hours.

Fingerling Bioassay

96-hr LC_{50} Values
goldfish: 0.137 ppb
carp: 0.200 ppb
fathead minnow: 0.074 ppb
green sunfish: 0.060 ppb
bluegill: 0.051 ppb
largemouth bass: 0.104 ppb
channel catfish: 8.4 ppb

Based on these data, the authors conclude that on average channel catfish are 42 to 165 times less sensitive to antimycin than fingerlings of 6 other species.

Tests in Vinyl Pools

After 4 months of acclimatization, control survival averaged 88% while survival in treated pools averaged 97%.

Uncertainties: Purity of the antimycin liquid formulation is not specified; purity of the sand formulation is not specified. Presumably, all 7 species of adult fish tested (paddlefish, shad, carp, buffalo, white catfish, blue catfish and channel catfish) were tested concurrently in the same pond. Static bioassays were conducted in indoor and outdoor facilities. Indoor tests with eggs, fry and fingerling fish conducted in accord with Berger *et al.* Water temperatures in catfish study presumably fluctuated considerably and there was ice formation in the water. Feeding rates varied because of fluctuating water temperatures.

Presumably, mortality in the egg study is referring to hatching success. Control mortality in the 0.25-acre ponds (37% for goldfish and 81% for carp) was above the EPA-recommended maximum of 20%.

Kuhn, J.O. 2001. Acute Oral Toxicity in Rats. Stillmeadow, Inc. 12852 Park One Dr., Sugar Land, TX. Laboratory Study No. 6025-00. Sponsor: Aquabiotics Corporation, 10750 Arrow Point Dr., Bainbridge Island, WA. (MRID 455279-01)

Study is GLP-compliant. Study conducted according to OPPTS 870.1100.
Fintrol Concentrate (20%); certificate of analysis not provided.
Dark brown liquid maintained at room temperature.

Sprague-Dawley rats (approximately 2 months old) from Texas Animal Specialists, Humble, TX, acclimated for 5 days. Twenty males (234 - 350 g) and twenty females (158 - 210 g) housed individually in suspended, wire bottom stainless steel cages. Animals fed Formulab #5008 (PMI Feeds Inc) *ad libitum* except 16 hrs before dosing. Drinking water was from municipal water supply.

Test substance mixed with acetone to produce 10% v/v concentration. Treatment levels consisted of 50, 200, 350 and 500 mg/kg. Presumably, the highest treatment level received 5 mL/kg of the test mixture. Body weights measured just prior to dosing and on Days 7 and 14 post-dosing. Observations for mortality and clinical/behavioral signs of toxicity were made 3 times the day of dosing and at least once daily thereafter for 14 days. On Day 14, animals were euthanized by CO_2 and necropsied.

Table 1. Summary of 14-day male, female and combined rat mortality following oral administration of antimycin A.

Dose Level		Number Dead/Number Treated		
mg/kg	**mL/kg**	**Males**	**Females**	**Combined**
50	0.5	0/5	0/5	0/10
200	2.0	1/5	0/5	1/10
350	3.5	3/5	2/5	5/10
500	5.0	5/5	5/5	10/10

The authors calculated the 14-day LD50 as follows: mean (95% confidence interval)
male 286 (258 - 312 mg/kg)
female 361 (344 – 381 mg/kg)

combined 316 (291 – 343 mg/kg)
Clinical signs included decreased activity, ataxia, circling, diarrhea, hunched posture, ocular discharge, piloerection, respiratory chirp/gurgle, soft feces, splayed legs and walking on tiptoe. No clinical signs were apparent in surviving animals after 12 days. Gasping, lateral recumbency, muscle tremors, ptosis, salivation and swollen/red penile area was observed only in animals that died on test.

No effect on body weight was reported except in one animal treated with 50 mg/kg.

Reviewer comments: No solvent control was run. Given the increasing amount of acetone administered to the animals with increasing concentration of antimycin, it would be helpful to know whether the highest dose of acetone alone had any effect on the animals.

Probit Analysis: using the Probit procedure of the Statistical Analysis System (Release 8.2; SAS Institute, Inc., Cary, NC), the following LD50 values were calculated:

Males: 285 mg/kg (95% CI: 152 - 419 mg/kg)
Slope: 6.506

Females: 354 mg/kg (95% CI: –)
Slope: 46.92

Combined: 317 mg/kg (95% CI: 253 - 386 mg/kg)
Slope: 7.759

Probit Procedure

Iteration History for Parameter Estimates

Iter	Ridge	Loglikelihood	Intercept	Log10(DOSE)
0	0	-13.862944	0	0
1	0	-8.1502514	-5.385105992	2.2762102896
2	0	-7.002163	-8.8714038	3.6774671031
3	0	-6.4405464	-13.17710389	5.389266402
4	0	-6.3505756	-15.76829862	6.4228097624
5	0	-6.350107	-15.97344454	6.5056129262
6	0	-6.350107	-15.97469006	6.5061219203
7	0	-6.350107	-15.97469006	6.5061219203

Model Information

Data Set	WORK.MALES
Events Variable	RESPONSE
Trials Variable	N
Number of Observations	4
Number of Events	9
Number of Trials	20
Name of Distribution	Normal
Log Likelihood	-6.350107015

Parameter Information

Parameter	Effect
Intercept	Intercept
DOSE	DOSE

Last Evaluation of the Negative of the Gradient

Intercept	Log10(DOSE)
-8.139419E-9	-2.305914E-8

Last Evaluation of the Negative of the Hessian

	Intercept	Log10(DOSE)
Intercept	6.2699709446	15.556556047
Log10(DOSE)	15.556556047	38.732077222

Probit Procedure

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	0.7113	2	0.7007
L.R. Chi-Square	0.9661	2	0.6169

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	4

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of 1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(DOSE)	1	5.6883	0.0171

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	-15.9747	6.7801	-29.2634	-2.6860	5.55	0.0185
Log10(DOSE)	1	6.5061	2.7279	1.1595	11.8527	5.69	0.0171

Estimated Covariance Matrix

	Intercept	Log10(DOSE)
Intercept	45.969367	-18.463379
Log10(DOSE)	-18.463379	7.441548

Probit Procedure

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
2.45533211	0.15370139

Estimated Covariance Matrix for Tolerance Parameters

	MU	SIGMA
MU	0.003885	-0.000697
SIGMA	-0.000697	0.004153

Probit Procedure

Probit Analysis on Log10(DOSE)

Probability	Log10(DOSE)	95% Fiducial Limits	
0.01	2.09777	0.30737	2.29340
0.02	2.13967	0.53976	2.31911
0.03	2.16625	0.68684	2.33578
0.04	2.18625	0.79726	2.34855
0.05	2.20252	0.88689	2.35912
0.06	2.21636	0.96304	2.36827
0.07	2.22850	1.02967	2.37641
0.08	2.23937	1.08922	2.38382
0.09	2.24926	1.14327	2.39067
0.10	2.25836	1.19291	2.39708
0.15	2.29603	1.39717	2.42490

0.20	2.32597	1.55740	2.44912
0.25	2.35166	1.69257	2.47219
0.30	2.37473	1.81124	2.49563
0.35	2.39611	1.91781	2.52074
0.40	2.41639	2.01450	2.54900
0.45	2.43602	2.10217	2.58223
0.50	2.45533	2.18069	2.62269
0.55	2.47465	2.24940	2.67295
0.60	2.49427	2.30788	2.73537
0.65	2.51456	2.35663	2.81157
0.70	2.53593	2.39730	2.90259
0.75	2.55900	2.43214	3.00986
0.80	2.58469	2.46352	3.13672
0.85	2.61463	2.49387	3.29082
0.90	2.65231	2.52638	3.49039
0.91	2.66141	2.53360	3.53922
0.92	2.67129	2.54124	3.59248
0.93	2.68216	2.54941	3.65127
0.94	2.69430	2.55830	3.71716
0.95	2.70815	2.56818	3.79257
0.96	2.72441	2.57948	3.88147
0.97	2.74441	2.59300	3.99114
0.98	2.77100	2.61046	4.13743
0.99	2.81290	2.63710	4.36889

Probit Procedure

Probit Analysis on DOSE

Probability	DOSE	95% Fiducial Limits	
0.01	125.24754	2.02940	196.51730
0.02	137.93296	3.46543	208.50207
0.03	146.63968	4.86233	216.66029
0.04	153.54979	6.26987	223.12724
0.05	159.41009	7.70713	228.62391
0.06	164.57401	9.18411	233.48878
0.07	169.23920	10.70710	237.91027
0.08	173.52844	12.28056	242.00518
0.09	177.52364	13.90803	245.85151
0.10	181.28249	15.59247	249.50439
0.15	197.71101	24.95572	266.01352
0.20	211.82331	36.09130	281.26891
0.25	224.73054	49.26892	296.61507
0.30	236.99055	64.75072	313.06020
0.35	248.94753	82.75820	331.69889
0.40	260.85088	103.39622	354.00085
0.45	272.90895	126.52444	382.14562
0.50	285.31993	151.59697	419.45988
0.55	298.29532	177.58408	470.92802
0.60	312.08429	203.17883	543.71895
0.65	327.00651	227.31810	647.99672
0.70	343.50511	249.63332	799.07375
0.75	362.24477	270.48260	1022.95451
0.80	384.31777	290.74757	1370.00320
0.85	411.74977	311.79589	1953.51927
0.90	449.06413	336.03323	3093.04746
0.91	458.57252	341.66721	3461.14703
0.92	469.13039	347.72631	3912.74395
0.93	481.02012	354.33209	4479.88125
0.94	494.65565	361.66008	5213.84388
0.95	510.67947	369.97876	6202.55507
0.96	530.16981	379.73188	7611.56116
0.97	555.15302	391.73958	9798.07301
0.98	590.19588	407.81322	13722.4898
0.99	649.97255	433.60996	23382.7088

Probit Procedure

Iteration History for Parameter Estimates

Iter	Ridge	Loglikelihood	Intercept	Log10(DOSE)
0	0	-13.862944	0	0
1	0	-7.9715155	-5.188842121	2.0827991207
2	0	-6.1158799	-9.615657623	3.8243117826
3	0	-4.3503851	-18.68775826	7.3794044299
4	0	-3.7445168	-26.96984034	10.594420783
5	0	-3.4882962	-36.01561997	14.093313455
6	0	-3.3979154	-45.06079648	17.615148405
7	0	-3.3749799	-51.72867808	20.233517291
8	0	-3.3681842	-57.4172856	22.469502454
9	0	-3.3660707	-62.50282289	24.4684792
10	0	-3.3653926	-67.14537026	26.293330996
11	0	-3.3651703	-71.444983	27.983385137
12	0	-3.3650962	-75.46899564	29.565108803
13	0	-3.3650713	-79.26517807	31.057278969
14	0	-3.3650628	-82.86885014	32.473778844
15	0	-3.3650599	-86.30700008	33.825216732
16	0	-3.3650589	-89.60080622	35.119917219
17	0	-3.3650585	-92.76725537	36.364557337
18	0	-3.3650584	-95.8202236	37.564591368
19	0	-3.3650584	-98.77122247	38.724544175
20	0	-3.3650583	-101.6299294	39.848219658
21	0	-3.3650583	-104.4045736	40.938852547
22	0	-3.3650583	-107.102224	41.999221331
23	0	-3.3650583	-109.7290061	43.031733838
24	0	-3.3650583	-112.2902697	44.038492926
25	0	-3.3650583	-114.7907254	45.021350158
26	0	-3.3650583	-117.2345325	45.9819405
27	0	-3.3650583	-119.6254092	46.921725371
28	0	-3.3650583	-119.6254092	46.921725371

Model Information

Data Set	WORK.FEMALES
Events Variable	RESPONSE
Trials Variable	N
Number of Observations	4
Number of Events	7
Number of Trials	20
Name of Distribution	Normal
Log Likelihood	-3.365058335

Probit Procedure

Parameter Information

Parameter	Effect
Intercept	Intercept
DOSE	DOSE

Last Evaluation of the Negative of the Gradient

Intercept	Log10(DOSE)
-4.11444E-11	-1.11047E-10

Last Evaluation of the Negative of the Hessian

	Intercept	Log10(DOSE)
Intercept	3.1095948584	7.9110209101
Log10(DOSE)	7.9110209101	20.126175496

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	0.0000	2	1.0000
L.R. Chi-Square	0.0000	2	1.0000

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	4

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of 1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(DOSE)	1	0.0000	0.9999

Probit Procedure

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	-119.625	966847.0	-1895105	1894866	0.00	0.9999
Log10(DOSE)	1	46.9217	380039.8	-744817	744911.2	0.00	0.9999

Estimated Covariance Matrix

	Intercept	Log10(DOSE)
Intercept	934793186240	-3.674403E11
Log10(DOSE)	-3.674403E11	144430226507

Probit Model in Terms of Tolerance Distribution

MU	SIGMA
2.5494674	0.02131209

Estimated Covariance Matrix for Tolerance Parameters

	MU	SIGMA
MU	1912.467729	7548.803851
SIGMA	7548.803851	29796.290457

Probit Procedure

Probit Analysis on Log10(DOSE)

Probability	Log10(DOSE)	95% Fiducial Limits	
0.01	2.49989	.	.
0.02	2.50570	.	.
0.03	2.50938	.	.
0.04	2.51216	.	.
0.05	2.51441	.	.
0.06	2.51633	.	.
0.07	2.51802	.	.
0.08	2.51952	.	.
0.09	2.52089	.	.
0.10	2.52215	.	.
0.15	2.52738	.	.
0.20	2.53153	.	.
0.25	2.53509	.	.
0.30	2.53829	.	.
0.35	2.54126	.	.
0.40	2.54407	.	.
0.45	2.54679	.	.
0.50	2.54947	.	.
0.55	2.55215	.	.
0.60	2.55487	.	.
0.65	2.55768	.	.
0.70	2.56064	.	.
0.75	2.56384	.	.
0.80	2.56740	.	.
0.85	2.57156	.	.
0.90	2.57678	.	.
0.91	2.57804	.	.
0.92	2.57941	.	.
0.93	2.58092	.	.
0.94	2.58260	.	.
0.95	2.58452	.	.
0.96	2.58678	.	.
0.97	2.58955	.	.
0.98	2.59324	.	.
0.99	2.59905	.	.

PROBIT ANALYSIS (LOG10) OF Female Rat MORTALITY AFTER 14-Days 10

Probit Procedure

Probit Analysis on DOSE

Probability	DOSE	95% Fiducial Limits	
0.01	316.14627	.	.
0.02	320.40385	.	.
0.03	323.13482	.	.
0.04	325.20456	.	.
0.05	326.89790	.	.
0.06	328.34615	.	.
0.07	329.62126	.	.
0.08	330.76716	.	.
0.09	331.81278	.	.
0.10	332.77819	.	.
0.15	336.80524	.	.
0.20	340.04054	.	.
0.25	342.84089	.	.
0.30	345.37534	.	.
0.35	347.74061	.	.
0.40	350.00000	.	.
0.45	352.19995	.	.
0.50	354.37853	.	.
0.55	356.57058	.	.
0.60	358.81183	.	.

0.65	361.14315	.	.
0.70	363.61641	.	.
0.75	366.30444	.	.
0.80	369.32109	.	.
0.85	372.86873	.	.
0.90	377.38092	.	.
0.91	378.47892	.	.
0.92	379.67536	.	.
0.93	380.99528	.	.
0.94	382.47484	.	.
0.95	384.16931	.	.
0.96	386.16969	.	.
0.97	388.64317	.	.
0.98	391.95579	.	.
0.99	397.23429	.	.

Probit Procedure

Iteration History for Parameter Estimates

Iter	Ridge	Loglikelihood	Intercept	Log10(DOSE)
0	0	-27.725887	0	0
1	0	-16.377206	-5.286974057	2.1795047052
2	0	-13.514288	-9.168833802	3.7208641131
3	0	-11.610453	-15.25789857	6.1242487778
4	0	-11.301432	-18.87651954	7.5516228007
5	0	-11.296755	-19.39400601	7.7553925872
6	0	-11.296753	-19.40426205	7.7594259804
7	0	-11.296753	-19.40426205	7.7594259804

Model Information

Data Set	WORK.COMBINED
Events Variable	RESPONSE
Trials Variable	N
Number of Observations	4
Number of Events	16
Number of Trials	40
Name of Distribution	Normal
Log Likelihood	-11.29675287

Parameter Information

Parameter	Effect
Intercept	Intercept
DOSE	DOSE

Last Evaluation of the Negative of the Gradient

Intercept	Log10(DOSE)
5.5390553E-7	1.0686071E-6

Last Evaluation of the Negative of the Hessian

	Intercept	Log10(DOSE)
Intercept	11.260491197	28.307489577
Log10(DOSE)	28.307489577	71.367913469

Probit Procedure

Algorithm converged.

Goodness-of-Fit Tests

Statistic	Value	DF	Pr > ChiSq
Pearson Chi-Square	1.6783	2	0.4321
L.R. Chi-Square	2.2289	2	0.3281

Response-Covariate Profile

Response Levels	2
Number of Covariate Values	4

Since the chi-square is small (p > 0.1000), fiducial limits will be calculated using a t value of 1.96.

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Log10(DOSE)	1	12.4251	0.0004

Analysis of Parameter Estimates

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	-19.4043	5.5418	-30.2660	-8.5425	12.26	0.0005
Log10(DOSE)	1	7.7594	2.2013	3.4450	12.0739	12.43	0.0004

Estimated Covariance Matrix

	Intercept	Log10(DOSE)
Intercept	30.711791	-12.181577
Log10(DOSE)	-12.181577	4.845733

Probit Procedure
Probit Model in Terms of Tolerance Distribution

MU	SIGMA
2.50073422	0.12887551

Estimated Covariance Matrix
for Tolerance Parameters

	MU	SIGMA
MU	0.001489	-0.000136
SIGMA	-0.000136	0.001337

Probit Procedure

Probit Analysis on Log10(DOSE)

Probability	Log10(DOSE)	95% Fiducial Limits	
0.01	2.20092	1.79320	2.32854
0.02	2.23606	1.87047	2.35298
0.03	2.25835	1.91926	2.36871
0.04	2.27511	1.95581	2.38070
0.05	2.28875	1.98543	2.39057
0.06	2.30036	2.01055	2.39906
0.07	2.31054	2.03250	2.40659
0.08	2.31965	2.05207	2.41340
0.09	2.32794	2.06981	2.41965
0.10	2.33557	2.08608	2.42547
0.15	2.36716	2.15269	2.45032
0.20	2.39227	2.20450	2.47120
0.25	2.41381	2.24783	2.49022
0.30	2.43315	2.28556	2.50848
0.35	2.45108	2.31926	2.52669
0.40	2.46808	2.34981	2.54537
0.45	2.48454	2.37782	2.56500
0.50	2.50073	2.40367	2.58603
0.55	2.51693	2.42770	2.60889
0.60	2.53338	2.45020	2.63403
0.65	2.55039	2.47152	2.66195
0.70	2.56832	2.49207	2.69329
0.75	2.58766	2.51238	2.72898
0.80	2.60920	2.53317	2.77055
0.85	2.63431	2.55555	2.82085
0.90	2.66589	2.58170	2.88615
0.91	2.67352	2.58777	2.90218
0.92	2.68181	2.59427	2.91967
0.93	2.69093	2.60132	2.93901
0.94	2.70111	2.60908	2.96071
0.95	2.71272	2.61782	2.98559
0.96	2.72635	2.62793	3.01496
0.97	2.74312	2.64018	3.05126
0.98	2.76541	2.65620	3.09977
0.99	2.80054	2.68097	3.17670

Probit Procedure

Probit Analysis on DOSE

Probability	DOSE	95% Fiducial Limits	
0.01	158.82722	62.11498	213.07913
0.02	172.20917	74.21111	225.41137
0.03	181.27837	83.03504	233.72949
0.04	188.41421	90.32645	240.27218
0.05	194.42534	96.70178	245.79350
0.06	199.69260	102.45948	250.64663
0.07	204.42824	107.76937	255.02767
0.08	208.76366	112.73835	259.05776
0.09	212.78633	117.43856	262.81749
0.10	216.55769	121.92124	266.36344
0.15	232.89670	142.13101	282.04570
0.20	246.75721	160.13927	295.93443
0.25	259.30387	176.94083	309.18794
0.30	271.11393	193.00330	322.46652
0.35	282.53733	208.57174	336.26765
0.40	293.82178	223.77603	351.04813
0.45	305.16839	238.68147	367.28354
0.50	316.76283	253.32207	385.50656
0.55	328.79779	267.73043	406.34199
0.60	341.49508	281.96815	430.55569
0.65	355.13427	296.15646	459.14286
0.70	370.09788	310.50847	493.49958
0.75	386.95408	325.37317	535.76861
0.80	406.62922	341.32278	589.59119
0.85	430.82917	359.37410	661.99468
0.90	463.33470	381.68287	769.40136
0.91	471.54670	387.05142	798.31806
0.92	480.63294	392.88774	831.13743
0.93	490.82598	399.31695	868.97886
0.94	502.46575	406.52066	913.50931
0.95	516.07827	414.77764	967.36283
0.96	532.54312	424.55001	1035.05229
0.97	553.50616	436.69360	1125.27285
0.98	582.65591	453.10200	1258.25550
0.99	631.74744	479.69949	1502.11462

Arslaneglau, L. and V. Korths. 1967. Antimycin Toxicity Studies: Administration of Water Treated with Antimycin to Rats and Dogs. Ayerst Research Laboratories. (MRID 2400798-10)

Single nominal concentration of antimycin (R-17, 001-17, Lot P 13326) at 125 ppb

Antimycin of unknown purity (12.5 mg) was dissolved in 200 ml of 70% ethanol and added to 100 liters to yield a final concentration of 125 ppb. Since the purity of antimycin is not stated, the actual concentration of antimycin A in solution is unknown. Co-solvent concentration is 2 ml/L and exceeds EPA-recommended concentration of 0.1 ml/L. "Certified" food coloring (unspecified quantity or quality) added to distinguish treatment groups. Stock solution of drinking water stored in plastic bottles and aerated once weekly throughout the test. The conditions under which the stock solutions were stored were not specified.

Four-month-old beagles (4 males and 4 females) were exposed to either control or antimycin-treated water. Although the study reported that control animal water was treated in the same way it is unclear whether there was a solvent control. Animals provided 1 L of treated or control water per day along with 400 g of Purina dog chow. Daily food and water consumption measured along with body weight. Prior to start of study and afterwards at 4-wk intervals, hematology (blood cell counts, white blood cell differential, hemoglobin, prothrombin time, sedimentation rate and hematocrit), blood chemistry (alkaline phosphatase, serum glutamic-oxaloacetic transaminase, blood urea nitrogen, lactic dehydrogenase and glucose) and urinalysis (albumin, ketone bodies, sugar; appearance) measured. Afterwards, dogs were sacrificed and the following organs measured; thymus, thyroid, adrenals, pituitary gland, spleen, heart, liver, brain, kidneys, testes, prostate, uterus, ovaries, lung, pancreas and submaxillary salivary glands. Tissue samples collected for histology; additional tissues for histology included: eye, trachea, lymph node, esophagus, skin, stomach, small intestine, large intestine, sternal bone, urinary bladder, aorta, gall bladder, muscle and mammary gland.

Additionally 50 - 60 g Charles River strain rats (20 males and 20 females) received treated or control water for 3 months. Animals were housed 6 - 7 rats/cage at 74 - 76^{o}C and 46 - 48% humidity. Prior to and at regular intervals during treatment, the following data were collected: weekly individual body weights; daily food and water intake [for each group housed together], hematology (red and white blood cell counts, differential white blood cell count and hemoglobin determination at 4-wk intervals), plus daily clinical observations. At the end of the study, the animals were sacrificed and the following organs measured: thymus, thyroid, adrenals, pituitary gland, spleen, heart, liver, brain, kidneys, testes, prostate, seminal vesicles, uterus, ovaries and submaxillary glands. Additional samples were taken for histology and included: eye, trachea, lymph node, esophagus, skin, stomach, small intestine, large intestine, sternal bone, urinary bladder, aorta, muscle, lung, pancreas and mammary gland.

Results

According to the authors, with one exception, there was a decrease in the intake of treated water; the treated water tasted bitter [to man] and the authors speculated that the bitter taste may have "caused" the lower water intake during the first month of the study. The authors also stated that lower than normal water intake will result in lower food intake and that this will be reflected in the body weight gain but that the growth pattern is considered normal. Only line graphs are presented to depict water and food intake and no raw data are available for analysis in the report. The graphs suggest that for male dogs, food and water intake was reduced for the first 5 weeks of the study. For female dogs, both water and feed consumption were considerably more erratic; however, there appeared to be a trend where both water and feed consumption were reduced for treated animals during the first 5 weeks of the study. Body weights

among treated male dogs were also reduced relative to controls; however, the same pattern was not apparent for females.

For rats, there was no apparent difference in either food or water intake between control and treated males or females.

The study authors did not observe any differences between control or treated male or female dogs or rats for any of the other measurement endpoints. However, it is unclear whether any statistical analysis had been conducted.

The study concludes that water treated with 125 ppb of antimycin is not toxic *per se* and does not degrade to products which are toxic when consumed by rats and dogs for three months.

Reviewer's comments: This study is classified as unacceptable since the purity of the test substance is not stated. Exposure has not been verified through measurements. The study does not contain sufficient detail to determine whether controls were negative controls (water only) or solvent controls and therefore whether the decreased water intake observed among dogs was due to a solvent effect or whether it was due to antimycin.

Rejection Factors:

Purity of the test compound is not stated and nominal concentration was not verified.
Treatment solution was aerated; however, concentration was not verified.
Co-solvent (ethanol) concentration 2 ml/L exceeded the recommended concentration of 0.1 ml/L and no solvent control was included.
Raw data are not available to test whether there are statistical differences for food and water consumption and for body weights.

Arslaneglau, L. and V. Korths. 1967. Antimycin Toxicity Studies: Administration of Fish Killed with Antimycin to Rats and Dogs. Ayerst Research Laboratories. (MRID 2400798-08)

Frozen rainbow trout (0.6 - 1 lb each), killed by antimycin at 125 ppb or by suffocation. Cooked fish mixed with standard laboratory animal diets in a preparation of 1:1.

Rejection Factors:

Treated diets were prepared by first cooking the fish. The effects of heat on antimycin are unknown.

Review discontinued since quality of the photocopy was so poor.

Hobbs. M. S., R. S. Grippo, J. L. Farris, B. R. Griffin and L. L. Harding. 2006. Comparative Toxicity of Potassium Permanganate to Nontarget Aquatic Organisms. Environmental Toxicology and Chemistry 25(1): 3046 – 3052.

In-house cultures of cladocerans (*Daphnia magna*, *Ceriodaphnia dubia*), fathead minnow (*Pimephales promelas*), a midge (*Chironomus tentans*) and an amphipod (*Hyalella azteca*) at Arkansas State University Ecotoxicology Research Facility used in 96-hr acute toxicity tests. Treatment solutions were renewed at 48 hours for the cladocerans and the fathead minnow following U.S. EPA procedures; however, amphipod studies were conducted with a substrate (spiked water) and were static 96-hr toxicity tests without renewal and reportedly conducted according to U. S. EPA and American Public Health Association water-only sediment test procedures. Cladocerans were <25 hours old, midges were third instars, amphipods were 7 – 14 days old and fathead minnows were 4 – 10 days old. Water temperature for cladoceran and fish was 25 $\pm$ 1°C while midges and amphipods were tested at 23 $\pm$ 1°C. Substrate for toxicity tests with Hyalella consisted of two 2.5-cm diameter maple leaf discs; substrate for toxicity tests with chironomids consisted of glass beads (150 – 212 μm). Testing consisted of 5 cladocerans per replicate with 4 replicates per treatment with six treatment concentrations. For the remaining test species, there were 10 organisms per replicate with 4 replicates per treatment and six treatment concentrations. Organisms were not fed during treatments. Tests were conducted with "moderately hard" synthetic water (hardness range: 89 – 100 mg/L as $CaCO_3$) and experimental pond water water (hardness range: 87 – 104 mg/L as $CaCO_3$) obtained from H.K. Dupree Stuttgart National Aquaculture Research Center in Stuttgart, AR, with historical records of previous chemical treatments. Total organic carbon in synthetic and pond water was 24.3 $\pm$ 1.04 and 34.8 $\pm$ 26.7 mg/L, respectively. Dissolved oxygen ranged between 7.5 to 8.1 mg/L. Measured concentrations of MnO_4^- ranged between 86.5 to 109% of nominal in synthetic water; however, measured concentrations ranged between 5.5 – 65% of nominal in pond water. In synthetic water, the pH ranged between 7.95 to 8.14 and in pond water pH ranged between 8.06 – 8.63.

Table 1 summarizes the 96-hr LC_{50} and no-observed effect concentrations (NOEC) for aquatic animals in synthetic water and in pond water. The study authors and the data suggest that potassium permanganate is less toxic to the aquatic species tested when studies were conducted in pond water and they conclude that pond water offers an ameliorating effect on the toxicity of potassium permanganate. The decreased toxicity of permanganate in the pond water is attributed to higher "potassium permanganate demand" (PPD) related to the higher organic carbon.

Table 1. Acute toxicity estimates (96-hr LC_{50} and NOEC) for potassium permanganate in moderately hard synthetic and pond water based on nominal treatment concentrations.

Test Organism	Synthetic Water 96-hr LC_{50} mg/L	Synthetic Water 96-hr NOEC mg/L	Pond Water 96-hr LC_{50} mg/L	Pond Water 96-hr NOEC mg/L
Daphnia magna	0.053	0.049	1.98	1.75
Ceriodaphnia dubia	0.058	0.047	2.39	2.25
Pimephales promelas	2.13	1.36	11.28	9.45
Chironomus tentans	4.43	<3.2	13.55	9.45
Hyalella azteca	4.74	3.56	12.30	7.83

This study provides useful information on the acute toxicity of potassium permanganate to aquatic organisms. Mean measured concentrations of permanganate were unreasonably low in the pond water; therefore, exposure in this portion of the study is uncertain.

Cerreto, K. M. 2004. Antimycin and rotenone: short-term effects on invertebrates in first order, high-elevation streams. A master's thesis submitted to the Department of Zoology and Physiology and the Graduate School of the University of Wyoming.

In conjunction with the Wyoming Game and Fish Department, this study examined the effect of antimycin and rotenone/antimycin treatments of two streams within the LaBarge Creek watershed in the Bridger-Teton National Forest of western Wyoming. Both streams are first-order, ground water-fed tributaries of LaBarge Creek and are located at an average elevation of 2,400 m. Cabin Creek was treated with antimycin alone in the summer of 2002 and 2003 while Indian Creek was treated with both antimycin and a combination of antimycin and rotenone in 2001, 2002 and 2003. Both streams were treated during the summer; drift and bioassays were conducted during the treatments in 2002 and 2003. An insect bioassay was also conducted in a third stream (Crystal Creek) that was treated for the first time with antimycin alone in 2003. The headwater portions of the streams had summer temperatures at or below 5^{o}C while the lower reaches had water temperatures as high as 20^{o}C during the day; pH ranged between 8.7 and 9.0 in the afternoon. Higher water temperatures in the lower reaches were considered due to decreased tree canopy compared to headwater portions.

Antimycin A in an acetone carrier was added to streams over an 8-hr period at an estimated treatment concentration of 10 µg/L using multiple drip stations. In addition to the antimycin A treatment, Indian Creek was also treated with a 1 mg/L solution of rotenone in kerosene carrier applied using a backpack sprayer to backwater areas and groundwater seeps yielding an approximated rotenone concentration in the water of 0.1 – 0.3 µg/L. Untreated headwater portions of each of the streams served as reference sites; however, these sites were smaller in terms of channel width and length relative to the treatment reaches. A potassium permanganate detoxification station was established near the confluence of each treatment stream and LaBarge Creek. Potassium permanganate was used at 2 mg/L over a 20-hr period to deactivate the piscicides. Bioassays using brook trout were used to monitor whether treatments were killing fish.

In Cabin and Indian Creeks invertebrate drift was measured over a 12 – 14 hour period starting 1 hour before treatment and ending approximately 3 hours after treatment stopped using 250-µm mesh drift nets. In 2002, 30-min drift samples were collected every 3 hrs and in 2003, drift sampling time was reduced to 20 minutes.

Benthic samples were collected from Cabin and Indian Creek before and after treatment using a 250-µm Hess net which covered an area of 0.086 m^2; benthic samples were approximately 250 m apart on riffle/run stream reaches; however, due to the narrowness of the reference site channels, benthic samples could not be collected at reference sites.

Drift and benthic samples were sieved in nested 1-mm 250-µm sieves and then subsampled; invertebrates were then identified to family or genus depending on the taxon.

Bioassays using mayflies (*Cinygmula* spp.) were conducted in Cabin and Crystal Creeks and bioassays using the caddisfly (*Brachycentrus* spp.) were conducted in Indian Creek. Nine bioassay cages (500-ml Nalgene bottles with 3-mm mesh screen covered windows on the sides, top and bottom), each containing 10 individuals, were spaced 120-m apart and tethered to cobbles in the substrate in Cabin Creek. In Indian Creek, cases were spaced 30-m apart in the antimycin-treated reach and 75-m apart in the antimycin/rotenone treated reach the evening before treatment. The morning after treatment the bioassay cages were collected for analysis.

Results

According to the study author, antimycin treatments in Cabin and Indian Creeks had no detectable effects on invertebrate drift relative to control reaches; however, invertebrate drift increased nearly 40-fold in Indian Creek after rotenone addition in the antimycin plus rotenone treatment. Antimycin treatment in Cabin Creek had no statistically significant effects on invertebrate density, biomass, or taxa richness. Mean invertebrate density was 22,000 invertebrates per m^2 less and mean invertebrate biomass was 1.2 g ash-free dry mass per m^2 than before treatment; however, there differences were not statistically significant. The author acknowledges though that the power of the study to detect statistical differences was low; a minimum reduction of 81,000 invertebrates per m^2 would have been necessary for a significant difference. Similar to Cabin Creek, antimycin treatment of Indian Creek had no statistically significant effect on invertebrate density, biomass or taxa richness. Rotenone and antimycin treatment of Indian Creek significantly ($p=0.024$) reduced invertebrate density from 84,000 to 47,000 invertebrates/m^2 and the number of taxa in the benthos was reduced from 46 pretreatment to 35 post-treatment. Invertebrate biomass was significantly ($p=0.047$) reduced from 9.4 to 5.8 g ash-free dry weight.

Antimycin treatments had no observed toxic effect on insects in *in-situ* bioassays; mean survival of mayflies was 82% + 9.8 and was not significantly different than survival at reference sites (87% +18.9). Similarly, survival of caddisflies in antimycin treated areas (96% + 5.5) was not statistically different than in reference sites (94.3% + 7.9); however, caddisfly survival was 58% lower in areas treated with both antimycin and rotenone compared to reference sites.

This study provides useful information on invertebrate responses to antimycin and antimycin plus rotenone treatments; however, the ability of the studies to detect treatment effects appears to be relatively low. Additionally, the areas selected as reference sites do not appear to be suitable controls given the reduced size and presumably lower invertebrate populations; this likely contributed to the low statistical power of the study. Also, the significant effects attributed to antimycin A in combination with rotenone would be difficult to attribute to rotenone alone since it was formulated with kerosene. In spite of the low statistical power of the study, the in-situ bioassays indicate that at least the species of mayflies and caddisflies used are not particularly sensitive to antimcyin A treatment concentrations of 10 µg/L for 8 hours. Also, although there was high variability in estimates of invertebrate biomass, richness and diversity, the results indicate that invertebrate communities were not eliminated during and immediately after a 8-hour treatment with antimycin A at 10 µg/L.

15 Appendix G: Memo from John Kenneke to Thomas Steeger and "Summary of Antimycin Hydrolysis Research by John F. Kenneke"

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL EXPOSURE RESEARCH LABORATORY
ECOSYSTEMS RESEARCH DIVISION
960 COLLEGE STATION ROAD ¦ ATHENS, GA 30605-2700

November 20, 2006

OFFICE OF
RESEARCH AND DEVELOPMENT

Subject: Antimycin Report

From: John F. Kenneke, Ph.D. /s/
Research Scientist

Thru: J. MacArthur Long, Ph.D. /s/
Chief, Processes and Modeling Branch

Eric Weber, Ph.D. /s/
Acting Director

To: Thomas M. Steeger, Ph.D.
Senior Biologist
Environmental Fate and Effects Division

Please find attached a copy of the report, "Summary of Antimycin Hydrolysis Research." The report has undergone internal review for technical merit and quality assurance and is being submitted to OPP at their request to aid in the re-registration assessment of antimycin A. Antimycin A is a bacteria fermentation product containing a nine member dilactone ring (i.e., potential for hydrolysis to occur) and exists as a mixture of at least eight structural isomers. Antimycin A is one of the most potent known inhibitors of mitochondrial respiration, and in spite of the fact that it has been used for over fifty years as a piscicide, little information exists on its environmental fate. Several reports on the hydrolysis of antimycin A were published in the late 1960s and early 1970s; however, the results are incomplete and inconsistent.

In response to OPP's request for additional hydrolysis data, laboratory studies were conducted over a seven-month period using certified buffers at eleven different pHs between pH 1 and pH 9. Both static and mixed batch reactors were used, and for the first time first-order hydrolysis half-lives were measured for individual antimycin isomers in addition to total antimycin. These results indicate that antimycin A hydrolyzes the quickest at pH 9, and more slowly with little noticeable difference between pH 1 and pH 8. Additionally, the $3_{a,b}$ isomer hyrolyzes more slowly than the $1_{a,b}$ isomer under basic conditions.

As expected, the mixed batch systems exhibited faster hydrolysis rates than the static systems (i.e., one order of magnitude); however, only at pH <8 (i.e., at pH 8 and above the rates were nearly identical). The accelerated rates of antimycin hydrolysis under acidic conditions relative to those measured under basic conditions is inconsistent with the static results and literature values. The reason for this discrepancy is uncertain, but since product formation could not be followed due to analytical constraints, it is possible that under the mixed acidic conditions antimycin is depleted via a non-hydrolytic route. Further studies are being conducted with the static and mixed systems, and therefore, the results presented in this report should be viewed as preliminary until those studies are completed.

Attachment

SUMMARY OF ANTIMYCIN HYDROLYSIS RESEARCH

John F. Kenneke, Ph.D.

U.S. EPA
Office of Research & Development
National Exposure Research Laboratory
Athens, GA

November 20, 2006

BACKGROUND

An analytical method was developed for the determination of antimycin $4_{a,b}$, $3_{a,b}$, 2_a, 2_b, and $1_{a,b}$ in aqueous samples. Antimycin hydrolysis experiments were conducted between pH 1 and pH 9 using both static and mixed batch systems.

MATERIALS AND METHODS

Chemicals. Hydrolysis studies were conducted using certified pH buffers purchased from Fisher Scientific. Buffer pHs were: 1.00, 2.00, 4.00, 4.63, 5.00, 6.00, 6.86, 7.00, 7.40, 8.00, and 9.00. The buffers were 0.05 M and comprised of different buffer mixtures; some contained additives to prevent microbial growth. Antimycin stocks were prepared in acetonitrile (ACN) using neat antimycin obtained from the EPA Repository and were assumed to be 100% pure.

Experimental Approach. Antimycin hydrolysis was measured under both static and mixed conditions. Static studies were conducted by placing 1732 µL of pH buffer and 18 µL of antimycin stock in a 2 mL HPLC autosampler vial. The contents of the vial were sealed with a screw cap and mixed. The sample was placed on an HPLC autosampler tray heated to 25^{o}C (+/-1) and sampled every 28 minutes over a period of 14 hours. The initial concentration of antimycin was 5 ppm. All hydrolysis studies had a final concentration of 1% ACN. Studies were run in triplicate.

Mixed batch studies were conducted in 40 mL vials containing 9.9 mL of buffer and 100 µL of appropriate antimycin stock (5 ppm final concentration). All buffer solutions were heated to 25 ^{o}C prior to the addition of the antimycin stock. After spiking, the vial was placed in a temperature controlled incubator at 25^{o}C (+/0.5) and shaken at 200 rpm. After 5 minutes a 0.5 mL sample was removed and transferred to a 2 mL HPLC vial.

The sample was analyzed immediately by HPLC. Additional 0.5 mL samples were periodically removed from the 40 mL vial and analyzed immediately by HPLC.

Analysis of Antimycin. Antimycin was analyzed using an HPLC equipped with a photodiode array detector. The eluent was 75-80% ACN and 20-25%, 0.02M sodium acetate (pH 5) run isocratic at 0.4 mL/min. Sample injection volume was 50 µL. All antimycin components were monitored at 230 and 350 nm.

Calibration. Initial calibration curves (ICAL) containing a minimum of 5 concentrations of antimycin were prepared using pH 5, 6, 7, and 8 buffers. ICALs were calculated using total antimycin peak area. ICALs were calculated at both 230 nm and 350 nm and covered the entire concentration range observed in the hydrolysis studies.

Quality Assurance. Quality assurance/quality control (QA/QC) samples were prepared in ACN from neat antimycin and were analyzed by HPLC-UV prior to sample analysis and after every 10th sample. Antimycin $4_{a,b}$, $3_{a,b}$, 2_a, 2_b, $1_{a,b}$ and total antimycin peak areas and retention times were followed using control charts based upon mean peak areas determined from a cumulative historic average. QA/QC samples having peak areas deviating by less than ±10% from the historical average were considered valid.

Data Analysis. All peak integrations were subjected to internal QA/QC review. Peaks that were found to be integrated incorrectly during the original HPLC analysis and data processing were re-integrated. All data was exported to an electronic data base for sorting and QA/QC sample validation. Samples which were not bracketed by a valid QA/QC sample were flagged. Hydrolysis data was imported into a custom report and analysis template developed for antimycin hydrolysis studies to minimize calculation errors. All experimental parameters and ICAL parameters were also imported into the template.

Hydrolysis rates for antimycin $4_{a,b}$, $3_{a,b}$, 2_a, 2_b, $1_{a,b}$ and total antimycin were determined using a least-squares regression analysis of $\ln[A]_t/[A]_0$ as a function of time, where $[A]_t$ is the concentration of antimycin at time, t, and $[A]_0$ is the initial concentration of antimycin. Rather than assuming $[A]_0$ based upon the amount of antimycin stock spiked, $[A]_0$ was determined experimentally for total antimycin as well as each component, from the intercept of the ordinate at time 0 minutes (determined from an iterative least-squares regression). The experimental data was considered valid if the determined value of $[A]_0$ for total antimycin was 70% to 135% the theoretical value spiked. Additionally, the component ratios of $4_{a,b}$, $3_{a,b}$, 2_a, 2_b, $1_{a,b}$ for $[A]_0$ had to be (0.033) : (0.367) : (0.058) : (0.072) : (0.471) respectively +/-10%, which corresponds to experimentally measured ratios in the ICAL, for the data set to be considered valid. Jackknife residuals were used to detect and reject outliers in the least-squares regression. Where possible, only data between 20% and 70% antimycin depletion was used in the least-squares

regression; at a minimum at least 45% total antimycin degradation had to have occurred for the data to be considered usable.

The slope from the least-square regression is the pseudo-first order rate constant (k_{obs}) and was considered valid if it was based upon at least 5 data points and had an $r^2>0.7$, in addition to meeting the previously described criteria. Half-lives ($t_{1/2}$) were determined for each component and total antimycin using: $t_{1/2} = 0.693/k_{obs}$.

RESULTS

Antimycin Analysis. An isocratic HPLC method was developed utilizing 20%, 0.02 M sodium acetate (pH 5) and 80% ACN as the eluent. It provided reproducible results, relatively good sensitivity, and relatively consistent peak retention times. Additionally, the $4_{a,b}$, $3_{a,b}$, 2_a, 2_b, $1_{a,b}$ components could be separated in less than 20 minutes (Figure 1). Although 230 nm was significantly more sensitive than 350 nm (approx. 4.5 times) the 230 nm wavelength exhibited much greater baseline variability with some pH buffers; the 350 nm was not significantly affected in any of the studies. All antimycin components yielded similar absorbance spectra from 200 to 800 nm and were assumed to have similar extinction coefficients for purposes of quantitation.

Hydrolysis. Ideally, samples would be collected from a mixed batch reactor over a period of hours to days, however, this could only be done in some cases due to the unavoidable constraints that aqueous samples were expected to be unstable and would have to be collected and analyzed immediately, and although this could be done during the day, samples could not be collected during non-working hours. The ideal solution would have been to have the HPLC automatically sample a mixed batch reactor, however, the appropriate equipment for this was not available. Consequently, a static batch reactor was employed for a majority of these studies.

Hydrolysis results are shown in Table 1. Mean values (n=3) with 95% confidence intervals are depicted in Figure 2 along with several values from mixed batch reactor studies and literature values. The static batch values should be viewed as a conservative estimate of antimycin hydrolysis half-lives. A majority of the mixed batch studies that were performed support this position as evidenced by their shorter half-lives. Further, studies that were conducted to determine the effect of sampling frequency on antimycin half-life show that for the same pH buffer, the more frequently the HPLC sampled the static batch reactor (and consequently lifted and moved it via the autosampler) the shorter the measured half-life. These observations may help explain some of the variability observed in published literature values for antimycin hydrolysis (i.e., mixing does not appear to have been used in at least one report, while in others it is not clear at all).

Although only total antimycin hydrolysis half-lives are presented (Table 1) for sake of brevity, additional sets of data were compiled for the individual components $4_{a,b}$, $3_{a,b}$,

2_a, 2_b, and $1_{a,b}$ (i.e., over 200 $t_{1/2}$ values). Figure 3 shows the average half-life as a function of pH for $3_{a,b}$ and $1_{a,b}$, which makes up 37% and 47% of the total antimycin respectively. It appears that $3_{a,b}$ hydrolyzes significantly slower than $1_{a,b}$, above pH 6 while $1_{a,b}$ hydrolyzes more slowly than $3_{a,b}$ below pH 6. The same trend in relative hydrolysis rates was also observed for mixed batch studies (not shown).

The information in this document has been funded (wholly) or (in part) by the United States Environmental Protection Agency. Although it has been subjected to the Agency=s peer and administrative review, it may not necessarily reflect official Agency policy. Mention of trade names of commercial products does not constitute endorsement or recommendation for use.

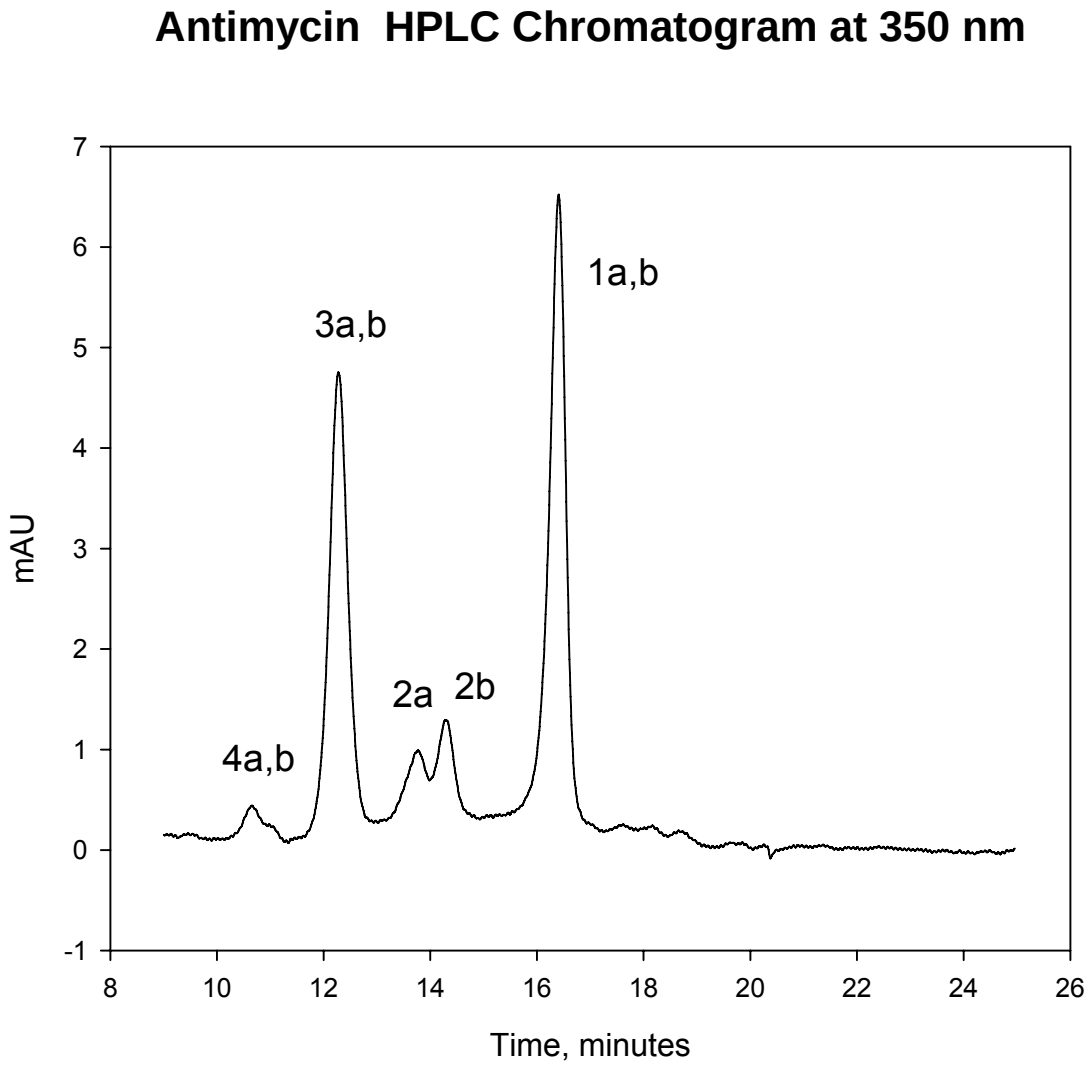


Figure 1. HPLC chromatogram of antimycin hydrolysis sample at pH 7.4. Experimental conditions are described in the text.

Table 1. Total Antimycin Hydrolysis Half-Life as a Function of pH for Static and Mixed Batch Systems

Method	Total Antimycin Half-Life[(a)] (minutes)										
	n	pH									
		1	3	4	4.63	5	6	6.86	7	7.4	8	9
Static Batch	1	672	458	497	646	619	700	542	391	517	589	268
	2	561	619	462	665	681	608	537	442	473	630	200
	3	807	543	526	851	594	678	456	444	439	586	137
	Avg	680	540	495	721	631	662	512	426	476	602	202
Mixed Batch	1	--	--	56	--	36	43	--	139	--	603	186

(a) All values have least-squares linear regression r^2>0.7, n>5, antimycin (total) recovery between 70% and 135%, >45% total antimycin degradation; data only regressed between 20-70% total antimycin degradation

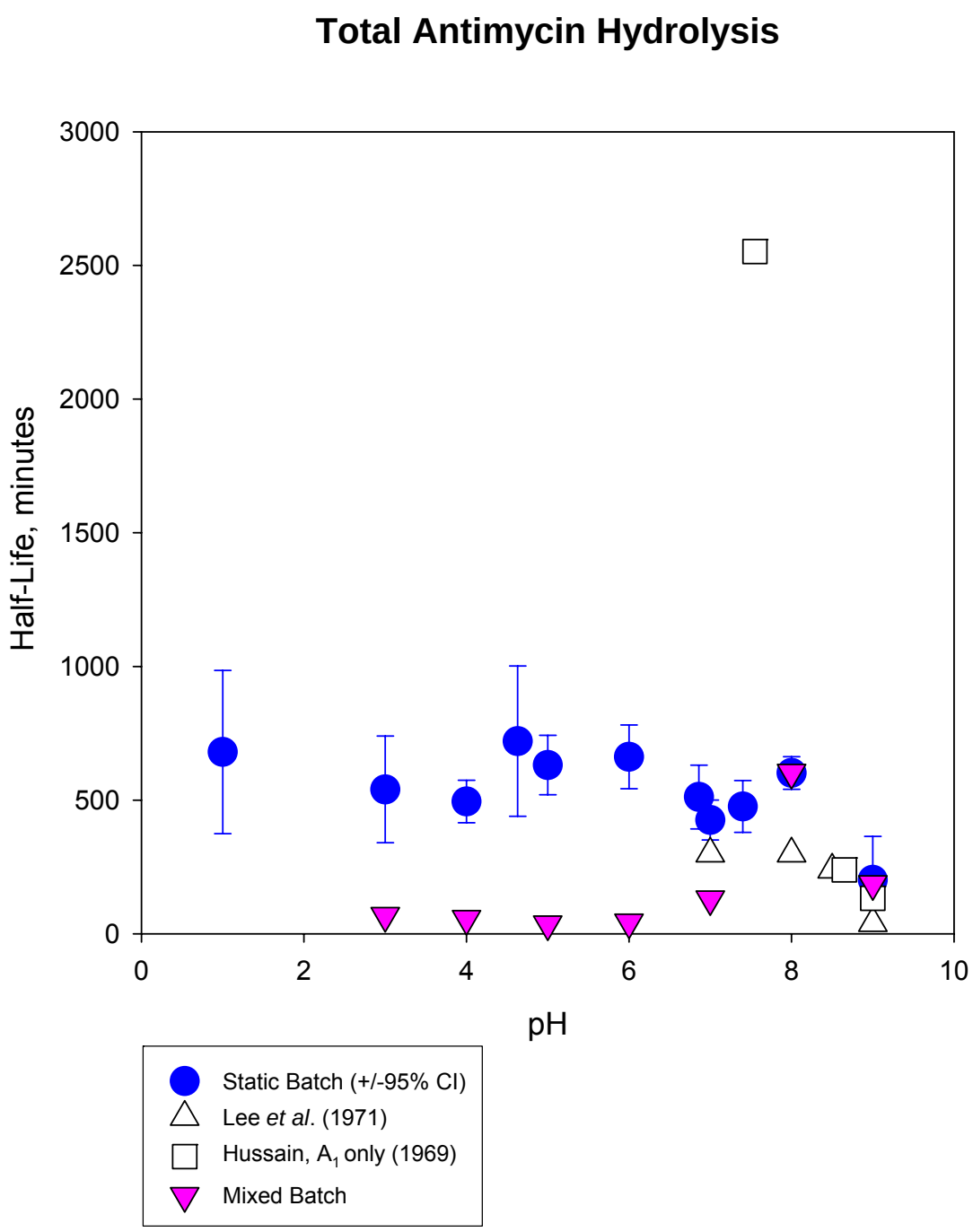


Figure 2. Half-life for total antimycin hydrolysis as a function of pH. Average values shown with 95% confidence intervals (CI) for laboratory studies conducted using static and mixed batch reactors. Literature values are shown for comparison.

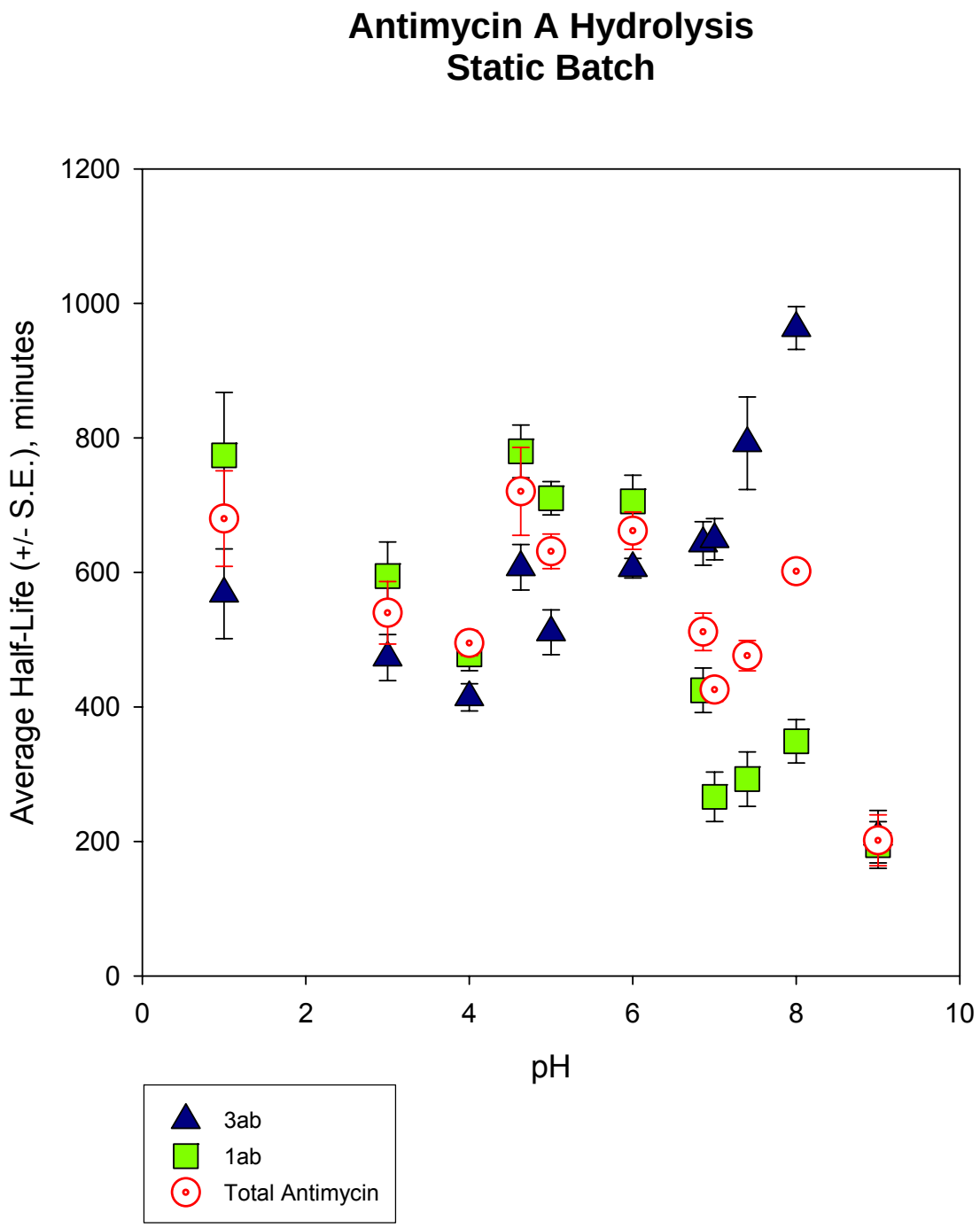


Figure 3. Average hydrolysis half-life with standard error (S.E.) for individual components of antimycin as a function of pH. Values determined using a static batch reactor.