Antimycin. A Brief Review of It's Chemistry, Environmental Fate, and Toxicology.

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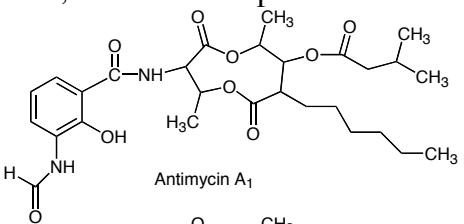
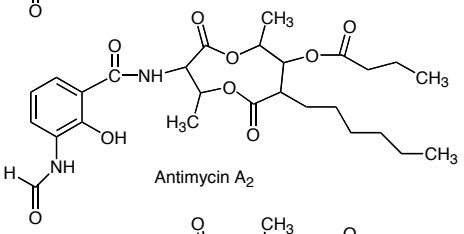
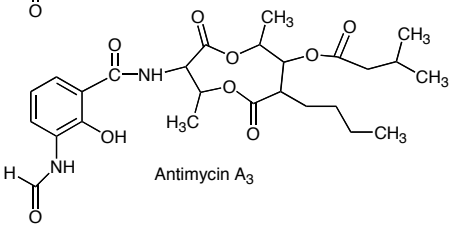
What is Antimycin? Antimycin is the active ingredient in Fintrol®, a commercial piscicide. Antimycin is a mixture of closely related molecules produced by *Streptomyces* bacteria. Antimycin A_1 was first isolated during the 1940's, and its molecular structure determined a few years later. Antimycin (used to refer to all of the antimycin variants collectively) is an antibiotic that was found to be a potent inhibitor of fungal growth (hence the name), while most bacteria are unaffected. Because of its antifungal properties, antimycin was of interest for potential commercial applications in agriculture. This interest led to a significant number of studies about its mechanism of action, and development of synthetic chemical approaches[1] to prepare the compound. It wasn't until the early '60's that antimycin was found to be highly toxic to fish, which over the last 40 years has led to antimycin being used in a large number of fisheries conservation projects across the US and in New Mexico. It is also used as a commercial fish toxicant to rid catfish farms of undesirable rough fish.







Why is Antimycin Toxic to Fish? Through mechanistic studies, molecular biologists have determined that antimycin is a highly specific inhibitor of respiration. Antimycin interrupts mitochondrial electron transport mechanism that most respiratory and photosynthetic organisms utilize in the uptake of oxygen to support metabolic function. The mitochondrial electron transport complex of proteins is very similar across all species that utilize oxygen. Antimycin interacts at a very specific site in the series of protein structures that make up the electron transport complex. Biochemists utilize this specific binding of antimycin to shunt electron flow and to study the chemical details of oxygen respiration. From these studies, much is known about the details of how antimycin binds to the enzyme site, down to the molecular level and the specifics of how side chains on antimycin influence the binding to the electron transfer protein site.[2] Antimycin binds tightly to a pocket in one of four of the main electron transport proteins. Antimycin binds at the site where ubiquinol, also called coenzyme Q, normally binds to shuttle electrons to O_2 that is bound at an adjacent iron-containing enzyme. Because the electron shuttle is blocked at this point, the bound oxygen is converted to superoxide, a very reactive form of

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[1] F. M. Strong *et al*, "The Chemistry of Antimycin A. IX. The Structure of the Antimycins", J. Am. Chem. Soc.**1960** *82*, 1513.

[2] H. Kim *et al*, "Structure of Antimycin A1, a Specific Electron Transfer Inhibitor of Ubiquinol-Cytochrome *c* Oxidoreductase", J. Am. Chem. Soc. **1999**, *121,* 4902.

oxygen. Superoxide builds up at a rate so high that the cell cannot decompose the superoxide fast enough, overwhelming the cell and leading to cell death.

Cyanide is also an electron transport inhibitor and impacts respiration. Its mechanism of toxicity is significantly different than antimycin. Cyanide binds strongly at an iron site in an adjacent protein, preventing oxygen from binding at all, and so respiration is inhibited, leading to cell death.

Synthetic chemists have manipulated the formyl salicylic acid and dilactone portions of the molecule and studied the binding of the resulting molecule to the electron transport protein.[3,4] They found that these two portions of the molecule are crucial for binding of antimycin to the electron transfer protein target, and hence the toxicity. The side chains are less important. If the dilactone portion is removed entirely, the binding and hence the toxicity is reduced by a very large amount. Thus, the products of antimycin decomposition (see below) are substantially less toxic that antimycin, or non-toxic, particularly at the ppb levels that are generated in the use of antimycin as a piscicide.

How toxic is Antimycin? Antimycin is not poisonous to a broad spectrum of species the way cyanide is. Antimycin toxicity is quite species dependent and varies widely, likely due to subtle species-specific differences in the protein sequence at the ubiquinol binding site that in turn alter the degree of binding and hence the toxicity. Antimycin is in general very toxic to fish, as the route to ingestion of antimycin in fishes is precisely the route with which oxygen is adsorbed – through the gills. Other animals that are not gill breathers are much less susceptible, as ingestion is primarily through the gut, where degradation can take place, reducing quickly the amount that may impact respiratory function. Antimycin is exceedingly toxic to certain fishes, but not to all fishes at the same concentrations. So it can be used as a selective fish toxin. This is the basis for its use in catfish farming – catfish are relatively insensitive to antimycin, and so catfish farmers use antimycin to rid their ponds of fish they are not interested in farming.

Trout are among the most sensitive of fish to antimycin. Only 5-10 micrograms of antimycin in one liter of water (5-10 parts per billion, ppb) is lethal to trout exposed to antimycin for 2-4 hours, the typical treatment time involved in a trout eradication project. Once exposed to this concentration of antimycin, trout will die.

Once it is in the water, how long does it last?

Antimycin is very susceptible to decomposition reactions that result in detoxification. There is a good deal of literature on the products and kinetics of antimycin hydrolysis under realistic conditions of use. A summary of the degradation chemistry follows.[5] Hydrolysis occurs at the lactone carbonyl sites, leading to blastmycic acid and a fatty acid lactone that hydrolyses to hexyl levulinic acid. Blastmycic acid further hydrolyses to antimycic acid and formic acid; the antimycic acid may further hydrolyse to aminosalicylic acid, a relative of aspirin, and an amino

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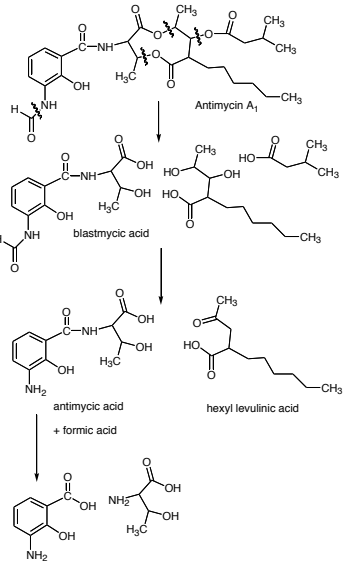
[3] N. Tokutake *et al*, "Structural Factors of Antimycin A Required for Inhibitory Action", Biochimica et Biophysica Acta **1185**, 271 (1994).
[4] H. Miyoshi *et al*, "A Model of Antimycin Binding Based on Structure-Activity Studies of Synthetic Antimycin A Analogues", *ibid* **1229**, 149 (1995).
[5] T. D. Hubert and L. J. Schmidt, "Antimycin A Use in Fisheries: Issues Concerning EPA Registration", USGS, Upper Midwest Environmental Sciences Center, La Crosse, WI, 2001.

acid. The degradation kinetics of antimycin exhibit well-behaved first order kinetics,[6] e.g. the concentration of antimycin decays exponentially with time. The rates of detoxification *via* hydrolysis are a function of pH, water hardness, temperature, the degree of exposure to sunlight, amount of organic debris in the stream, and other additives.[3, 4, 7,,8] At a pH of 7.5 and a temperature of 17 °C in reconstituted water in the dark, the half-life of antimycin is reported to be 93 hours. Studies of the kinetics of decomposition of antimycin in natural waters are more difficult, as the rates of decomposition are much higher because antimycin readily photolyses in sunlight, and also because antimycin is sensitive to oxidation and oxidizes quickly in well-oxygenated water. In direct sunlight, the half-life decreases to 20 minutes to 2 hours depending on the temperature and degree of aeration, which is related to the stream gradient. Because of the short lifetime in natural waters, application of antimycin in streams is a challenge, as the toxicity may decay quickly below concentrations that are lethal to fish as the compound is carried down the stream. Practitioners make up for this by adding more antimycin to the stream at pre-determined locations downstream in the treatment area.



It is exceedingly difficult to measure with accuracy any organic chemical in natural systems at the ppb level, particularly in the field, because of the plethora of other naturally occurring organic compounds that are in the water that arise from the decomposition of organic matter in the stream – leaves, algae, dead animals, fish, etc. And of course, samples on the way to a laboratory to be analyzed continue to decompose as described above. Currently the best assay in the field is to use the known toxicity of antimycin to yeast or fish; this bioassay may be performed reliably to an antimycin concentration of .03 ppb. Methods for accurate quantification of antimycin at ppb levels that can be performed rapidly in the field are desired, and would be very valuable in tailoring the application of antimycin to the water column.

Practitioners of antimycin fish eradication utilize the sensitivity of antimycin to oxidation to eliminate antimycin at the bottom of the treatment range in the stream. A potent oxidant is added to the stream, and any remaining antimycin is oxidized and detoxified quickly. The oxidant chosen for this use is potassium permanganate. Permanganate is often used in municipal water treatment plants for drinking water production, particularly in locales where treatment with chlorine is inappropriate. The byproducts of permanganate treatment are highly insoluble manganese oxides. The concentrations of permanganate used to destroy antimycin are on the order of 1 ppm, but this depends upon conditions in the stream that are assessed by the practitioner. The residual concentration of manganese in the water is at a low level, and is below

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[6] A. Hussain, "Kinetics and Mechanism of Hydrolysis of Antimycin A_1 in Solution", J. Pharm. Sci. **58**, P316 (1969).

[7] R. A. Schnick, "A Review of the Literature on the Use of Antimycin in Fisheries", A report from the Fish Control Laboratory, La Crosse, WI, April 1974, US Fish and Wildlife Service.

[8] B. J. Finlayson *et al*, "Assessment of Antimycin A Use in Fisheries and Its Potential for Reregistration", Fisheries **27**, 10 (2002).

the safe daily required amount of intake recommended for humans as a required nutrient (see below).

Does Antimycin Bioaccumulate?

Many pesticides are persistent in the environment, and they also concentrate in tissues of living animals because of favorable solubility characteristics of the chemical in certain tissues or organs as has been observed, regrettably, with DDT. Antimycin does not exhibit such properties, as it rapidly degrades in aqueous environments to non-toxic, readily biodegradable fragments. Because of the concentrations that are employed, coupled with the fact that antimycin is very likely to be decomposing *in vivo* at even higher rates than in the stream, and the difficulty in assaying the compound in water much less tissue samples, detailed studies on the accumulation of antimycin in tissues have not been performed in any number. There is one study that used radiolabeled antimycin[9] to assess bioaccumulation. The ability to detect radioactivity is straightforward and sensitive, and so is often used in cases such as this. Still, the results are open to interpretation, as any radioactivity detected may arise from not only antimycin, but from any of its degradation products as well. Nonetheless, assuming the entire amount of radioactivity that was detected in this study is due to antimycin, the authors determined that antimycin does not bioaccumulate in fish to a concentration above what was in the stream. This is reasonable given what is known about antimycin decomposition. Antimycin is readily susceptible to cleavage at its amide bonds. The digestive tract of most living things contain ample numbers and varieties of proteases, enzymes that are designed to cleave amide bonds for the hydrolysis of proteins in food to break them down to amino acids that are then consumed metabolically or reused and recycled into proteins in the body. These same proteases will likely rapidly hydrolyse antimycin into its constituent pieces, rendering it harmless, and allowing for efficient excretion. This is also one reason why antimycin is not as toxic to animals other than fish. The respiratory system of fish are exposed very directly to antimycin through their rapid and efficient contact of water with their gills; in mammals and many other animals, ingestion into the gut will likely detoxify much of the antimycin. Compounds that do bioaccumulate typically do so because there is no metabolic route to decompose them to readily excretable fragments.

What about toxicity in other species?

An excellent source of information on the toxicity of antimycin, and a wide variety of common pesticides and other chemicals of commerce is available on the Pesticide Action Network (PAN) website.[10] The database maintained by PAN has over 850 entries of peer-reviewed toxicology studies of antimycin in a variety of aquatic organisms. Toxicity studies of antimycin in mollusks, insects, fish, amphibians, nematodes, zooplankton, and phytoplankton are summarized along with literature references to the studies. While this document is not intended to be a comprehensive review of the toxicology data available, a few entries from the PAN database for antimycin are shown to give an indication of relative toxicity of antimycin in several species. Note that these concentrations refer to exposure for a period of 24 hours, to contrast with the 2-4 hour toxicity typically planned for piscicidal applications as discussed above. The longer exposure time requires lower concentrations to achieve a lethality of 50%. Note that just within

[9] E. Greselin and F. Herr, "Further Toxicity Studies with Antimycin, a Fish Eradicant", J. Agr. Food Chem. **22**, 996 (1974).

[10] : Pesticide Action Network Database: http://www.pesticideinfo.org

the fishes, toxicity varies over 3 orders of magnitude. Mollusks and amphibians appear to have greater tolerance to antimycin.

Species	LC_{50}/24 hours exposure	LC_{50}/96 hours exposure
Trout	.07 ppb, Cutthroat	.04 ppb, Rainbow
Black Bullhead Catfish	200 ppb	45 ppb
Channel Catfish	>10 ppb	9 ppb
Goldfish	1 ppb	
Snails	>800 ppb	
Tiger salamander	>1080 ppb	
Tadpoles, Leopard Frog	45 ppb	10 ppb

Tadpoles are unaffected at piscicidal concentrations of antimycin in the 4 hr treatment time anticipated in practice; salamanders are similarly unaffected after an 8-hour exposure to piscicidal concentrations.[11]

Grant Grisak (Montana Fish, Wildlife, and Parks) is publishing a recent study of the toxicity of antimycin and rotenone on Columbia spotted frogs, long toed salamander larvae and adults, and tailed frog tadpoles. In a draft report[12], the authors report the 'No observed effect levels' (NOEL) for Columbia spotted frogs, long-toed salamander larvae, and tailed frogs after 96 hours exposure to antimycin (table below). At the minimum concentration of exposure they used in their study of 7.5 ppb, 15% of the tadpoles perished after 96 hours, and so the determination of a NOEL for tadpoles could not be determined. Upon exposure to *300 ppb* antimycin, only 5% of the tadpoles died after 8 hours of exposure. These data indicate that at piscicidal concentrations and anticipated application times of antimycin (10 ppb, <8 hrs), that the risk to tadpoles is small. To mitigate this remaining risk, it is common for practitioners to delay treatments until tadpoles have metamorphosed, or physically remove the tadpoles from the stream until after treatment.

Species	Life stage	96 hr No Observed Effect Level Antimycin ppb
Columbia spotted frog	Adult	60
Long-toed salamander	Larvae	15
	Adult	
Tailed frog	Tadpole	<7.5

[11] S. Moore *et al*, "Environmental Assessment for Using a Piscicide for Brook Trout Restoration", Great Smoky Mountains National Park (March, 2000).

[12] Grant G. Grisak, Gary L. Michael, Donald R. Skaar, Mark E. Schnee, Brian L. Marotz, and Mark Maskill, "Laboratory Investigations on the Toxicity of Antimycin and Rotenone to the Westslope Cutthroat Trout, Columbia Spotted Frog, Long Toed Salamander, and Tailed Frog" Preprint, May 2005.

In mammals, toxicity is measured as the dose required for lethality in half the population measured in mass of toxin per mass of mammal.

Mammal	LD_{50}, mg/kg
Rat	28
Mouse	25
Lamb	1-5
Dog	>5
Rabbit	10

To put this in context, a half-kilogram rat (about 1 pound) would have to consume 14 milligrams of antimycin to have 50% chance of achieving a lethal dose. If the rat drank 10 ppb antimycin containing water (approximately the concentration intended for trout eradication), the rat would have to drink 14mg/10microgram/liter, or 1,400 liters (370 gallons) of water to achieve a lethal dose. This is clearly not achievable in many rat lifetimes.

However, long before the rat would have a chance to drink that much antimycin treated water from a stream, the antimycin would have decomposed to non-toxic byproducts, and thus it is extremely unlikely that mammals or birds could consume a lethal dose from a treated stream; this is indeed what has been observed. The toxicity to birds is similar to mammals; no effects were observed on exposure of ducks, herons, gulls, and terns to 10 ppb antimycin.[13]

What is known about the impact of antimycin on aquatic insects?

The literature of the toxicity of antimycin to aquatic insects is a difficult topic to assess. The large number of species of insects that may reside in a stream, and the fact that stream insect population makeup can vary significantly depending on location, stream flows, temperatures, etc. have made gaining a consistent set of data a large and difficult task. Reports of toxicity to antimycin vary widely at different locales. Studies in Wisconsin that indicate sensitive insect populations (<50ppb) are found to be insensitive in Wyoming. Some of these differences may be ascribed to differences in water temperatures in these two particular locales, but this is likely to be only one parameter that is involved in the observed differences.

The study of antimycin impact on aquatic insects is one area where the literature is rather sparse in part because of the diversity of insect life found in streams in different geographical locations makes such studies very site specific; more site specific studies may be necessary to better define the impact of antimycin on non-target insect populations in proposed treatment areas. As an example, consider the following result from antimycin treatment of three closely spaced lakes in Wisconsin: " … it is very difficult to relate a change in abundance of a benthic organism to the chemical treatment. For example, the estimated number of Chironomidae in Camp Lake showed a considerable increase in abundance following treatment while in Lamereau Lake the data showed the opposite to be true and in Nancy Lake the number stayed at the same level. If

[13] P. A. Gilderhus *et al*, "Field Trials of Antimycin A as a Fish Toxicant", USFWS Investigations in Fish Control, 27 (1969).

abundance was related to chemical treatment, all three lakes should have shown the same trend."[14]

Post-treatment studies of insect populations have indicated that populations decline, but are not decimated. Populations rebound quickly after an antimycin treatment. Recruitment from adjacent, non-treated waters is thought to contribute to the observed rapid rebound in insect populations, often beyond what was observed prior to the removal of the exotic, non-native fishes. A post-treatment study on Sam's Creek in Great Smoky National Park indicated a decline of 40-50% of the insect population, but that the population rebounded in 5 months to above what it was prior to treatment, partly because of the absence of over-predation by non-native trout.

In a recent study in Wyoming on the effect of antimycin treatment on insect populations in high altitude streams, researchers report "Antimycin alone had little to no effect on invertebrates, with drift rates and bioassay mortality not significantly different than control sites. We did not observe major invertebrate reductions in the benthos after antimycin addition. Antimycin alone appears to have little short-term effect on invertebrates in high elevation streams."[15]

What about human exposure to chronic, non-lethal concentrations of antimycin treated water?

The sub chronic effects to humans from antimycin exposure have been estimated from toxicology studies using mice, a common approach to defining acceptable risk for a wide variety of compounds including pharmaceuticals. Literature estimates of subchronic safe levels of antimycin exposure have been developed using EPA risk assessment protocols.[16] Toxicity studies aimed at determining these values develop a concentration where there is a "No Observed Adverse Effect Level" (NOAEL) of exposure determined in mice. The EPA protocol prescribes a method to apply a 'safety factor' in interpreting the mouse data for use in extrapolating to the toxicity in humans and computes a reference dose, RfD, that is the upper limit of antimycin that could be consumed daily for the rest of ones life without observable effects. Using the values of NOAEL for antimycin determined by toxicology studies to be 0.5 mg/kg/day,[17] and a very conservative value for the risk factor of 300, the RfD for antimycin has been estimated to be 1.7 micrograms/kg/day. This is the estimated and conservative safe dose. For a grown adult weighing 70 kg (154 pounds) who consumes the average daily intake of 2 liters of water (a little more than a half gallon), the safe concentration of antimycin in that water that this adult could consume for life is 1.7 micrograms/kg/day x 70 kg / 2L/day, which is 60 micrograms per liter, or 60 ppb. Thus, an adult could safely drink his daily intake of water for the rest of his life with no adverse effects from an antimycin treated stream, and again, this is based upon the most conservative value of RfD found in the literature. Montana's Division of Environmental Quality

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[14] Beard, Thomas D. "Impact of repeated antimycin treatments on the zooplankton and benthic organisms in Camp, Lamereau and Nancy lakes, Bayfield County, Wisconsin, Research report Wisconsin. Dept. of Natural Resources, Report 78, Madison, Wisconsin: Dept. of Natural Resources, (1974); available at: http://digital.library.wisc.edu/1711.dl/EcoNatRes.DNRRep078

[15] K. M. Cerreto *et al*, "Antimycin and rotenone: short-term effects on invertebrates in first order, high elevation streams", Abstracts of the NABS Annual Meeting, Vancouver, British Columbia, 2004, Disturbance Ecology 1.

[16] Taken from "Draft EIS, Flathead Westslope Cutthroat Trout Project", Chapter 3 (June 2004).

[17] J. O. Kuhn, "Final Report. Acute Oral Toxicity Study in Rats", Stillmeadow, Inc., Submitted to Aquabiotics Corp. (March 2001).

determined "there would be no effect on human health even if the chemicals (antimycin and rotenone) were not detoxified, did not break down, and people drank the "contaminated" water continuously for the rest of their lives."[18] In the State of New Mexico, a very conservative approach has also been considered, using a daily intake of 4L/day for a 70 kg adult, with similar conclusions.[19]

What is known about the toxicity of other components of Fintrol®?

Antimycin is insoluble in water. To be effective, it must be solubilized with the aid of other compounds, such as detergents. The commercial product Fintrol® contains acetone, diethyl phthalate, and nonoxynol-9 to aid in the solubilization of antimycin in water.

Nonoxynol-9 is a non-ionic detergent used commercially in surgical scrubs as an antiseptic, and as an intravaginal spermicide. Acetone is a common solvent. Many recognize it as fingernail polish remover. Diethyl phthalate is a component of plastics, and it makes plastics pliable. It is also a common ingredient in cosmetics, hand lotions, and other personal care products.

Fintrol®, when mixed with Fintrol® Diluent, results in a mixture containing approximately 12.5% antimycin, 57% acetone, 8.5% nonoxynol-9 detergent, and 15 % diethyl phthalate, and 7% soy lipids. When antimycin is delivered at 10 ppb, this results in concentrations of approximately 13 ppb diethyl phthalate, 50 ppb acetone, and 7 ppb nonoxynol-9. The toxicology of these compounds has been studied, and safe reference doses have been determined[20] and are summarized in the table below. Based upon the published data, the application of Fintrol® as a piscicide at the concentrations of its intended use does not create any concern to human health according to assessments by the US EPA.

Fintrol® components at concentration of intended use	Antimycin 10 ppb	Diethyl phthalate 13 ppb	Acetone 50 ppb	Nonoxyl 9 7 ppb	Manganese 1 ppm
RfD (from EPA documents)	1.7μg/kg/day	800μg/kg/day	900μg/kg/day	30μg/kg/day	140μg/kg/day
Fraction of RfD, 2L/day water, 70kg human	17%	0.05%	0.16%	0.7%	20%
Quantity water required to be consumed to equal RfD	12 L (3.2 gals)	4300 L (1136 gals)	1260 L (333 gals)	300 L (79 gals)	10 L (140 L/37 gals)

Included in the table above is the estimate of a safe reference dose for manganese from permanganate that is used for the destruction of antimycin at the lower end of the treatment range in the stream. The amount is less than the recommended daily dose of manganese as an essential nutrient in the human diet.

[18] Taken from "Cherry Creek Native Fish Introduction Project EA", Helena, MT (1999)
[19] Sworn testimony of Dr. Stephen Wust, Division Director, Water and Waste Management, NM Environmental Department, NM Water Quality Control Commission, (August 12, 2004).
[20] Source: EPA's Integrated Risk Information System (IRIS) site: www.epa.gov/iris/

Are there teratogenic or mutagenic properties of antimycin or any of the other components of Fintrol® or permanganate?

There are a few studies of the mutagenicity of antimycin. In one study of the mutagenicity of antimycin using a mouse lymphoma model[21], negative results were found at a concentration of 20 millimolar (equivalent to *11 parts per thousand*), a concentration one million times greater than the concentration of antimycin used in trout eradication. A search of the literature for teratogenic effects of antimycin resulted in no hits.

Diethyl phthalate (DEP) has yielded negative results in mammalian cell chromosomal aberration assays. [22] According to EPA, DEP is a 'group D' compound, meaning inadequate or no human and animal evidence for carcinogenicity has been found. EPA has set the drinking water equivalent level (DWEL) for DEP for a safe lifetime, non-cancer dose to be 30 mg/L, or 30,000 ppb.[23]

Carcinogenicity studies of acetone at concentrations ranging from 10,000 to 70,000 ppm have been negative; "To date there are no epidemiological studies demonstrating an association between exposure to acetone and increased risk of cancer".[24]

Studies of nonoxynol 9 have not triggered EPA's categorization to date, as there is not enough compelling evidence to do so.[25]

Is antimycin, or other components of Fintrol®, considered endocrine disruptors?

Endocrine disruptors affect the endocrine system that regulates a number of metabolic processes. An EPA web site states "Evidence suggests that environmental exposure to some anthropogenic chemicals may result in disruption of endocrine systems in human and wildlife populations. A number of the classes of chemicals suspected of causing endocrine disruption fall within the purview of the U.S. Environmental Protection Agency's mandates to protect both public health and the environment. Although there is a wealth of information regarding endocrine disruptors, many critical scientific uncertainties still remain". There are no coherent lists of endocrine disruptors, nor any 'official' lists at this time because of lack of supporting data.

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[21] J. Wangenheim and G. Bolcsfoldi, Mutagenesis **3**, 193 (1988).
[22] Source: EPA's Integrated Risk Information System (IRIS) site: www.epa.gov/iris/
[23] 2004 Edition of the Drinking Water Standards and Health Advisories, EPA 822-R-04-005, Office of Water, U.S. Environmental Protection Agency, Washington DC
[24] Source: EPA's Integrated Risk Information System (IRIS) site: www.epa.gov/iris/
[25] *ibid*