

Chapter 22: Aquaculture Drugs

Updated:

- [Potential Food Safety Hazard](#)
- [Control Measures](#)
- [FDA Guidelines](#)
 - [FDA approved aquaculture drugs](#)
 - [FDA low regulatory priority aquaculture drugs](#)
 - [FDA approved flesh color enhancers](#)
- [Analytical Procedures](#)
 - [Acetic acid](#)
 - [Chloramphenicol](#)
 - [Fluoroquinolones](#)
 - [Malachite Green and Leucomalachite Green](#)
 - [Nitrofurans](#)
 - [Oxytetracycline](#)
 - [Sodium sulfite](#)
 - [Sodium chloride](#)
 - [Sulfadimethoxine](#)
 - [Sulfamerazine](#)
- [HACCP Plan Examples](#)
 - [Aquaculture drugs in farm-raised catfish \(on-farm visits\)](#)
 - [Aquaculture drugs in pond-reared shrimp \(supplier's certification\)](#)
 - [Aquaculture drugs in pond-reared shrimp \(records of drug use\)](#)
 - [Aquaculture drugs in farm-raised catfish \(drug residue testing\)](#)
 - [Aquaculture drugs in aquacultured trout \(QA program\)](#)
 - [Aquaculture drugs in live lobster \(control during holding\)](#)
- [Commercial Test Products](#)
- [References](#)

Potential Food Safety Hazard

[Top](#)

Unregulated/unapproved drugs administered to aquacultured fish pose a potential human health hazard. These substances may be carcinogenic, allergenic, and/or may cause antibiotic resistance in man. To control this hazard in food animals, all drugs, whether for direct medication or for addition to feed, must be approved by FDA. Under certain conditions authorized by FDA, unapproved new animal drugs may be used in conformance with the terms of an Investigational New Animal Drug (INAD) application.

Incentives for the use of animal drugs in aquatic animal species include the need to: 1) treat and prevent disease; 2) control parasites; 3) affect reproduction and growth; and, 4) tranquilization (e.g. during transit). Relatively few drugs have been approved for aquaculture. As a result,

aquaculture growers may use unapproved drugs, general purpose chemicals that are not labeled for drug use, and approved drugs in a manner that deviates from the labeled instructions.

When a drug is approved by FDA's Center for Veterinary Medicine, the conditions of the approval are listed on its label. These conditions include: the species for which the drug is approved; the approved dosage; the approved route of administration; the approved frequency of use; and the approved indications for use. Only a licensed veterinarian may legally prescribe or use a drug under conditions that are not listed on the label. This restriction is more fully explained in 21 CFR 530.

Labels of approved drugs list mandatory withdrawal times, where applicable. These withdrawal times must be observed to ensure that the edible tissue is safe when it is offered for sale. Tissue residue tolerances have been established for some drugs (FDA, 2001).

Control Measures

[Top](#)

Control measures for the control of aquaculture drugs used in aquaculture operations can include:

- On-farm visits to review drug usage (other than INADs) before receipt of the product, coupled with a supplier's lot-by-lot certificate that any INADs used were used in conformance with the application requirements;
- Receipt of supplier's lot-by-lot certification of proper drug usage, coupled with appropriate verification (See Step #18 - Verification);
- Review of drug usage records (other than INADs) at receipt of the product, coupled with a supplier's lot-by-lot certificate that any INADs used were used in conformance with the application requirements;
- Drug residue testing;
- Receipt of evidence (e.g. third party certificate) that the producer operates under a third party- audited Quality Assurance Program for aquaculture drug use.

(Note: The use of Investigational New Animal Drugs (INAD) is confidential unless an exception is made by the sponsor of the drug research. Thus, review of INAD drug usage records by the processor may not be practical in certain situations. Written certification from the grower to the processor stating that any INAD drug usage is in accordance with authorizations from FDA/Center for Veterinary Medicine, will be acceptable on a lot-by-lot basis.)

Control measures for the control of aquaculture drugs used during the holding of live fish (e.g. lobster pounds) can include controlled application of animal drugs in a manner consistent with:

- The established withdrawal times;
- The labeled instructions for use;
- Extralabel use of FDA-approved drugs, under a veterinarian's supervision in accordance with FDA regulations and guidelines;
- The conditions specified in the FDA "low regulatory priority aquaculture drug" list;
- The conditions of an INAD application (FDA, 2001).

FDA Guidelines

[Top](#)

FDA approved aquaculture drugs

[Top](#)

FDA approved aquaculture drugs with their approved sources, species and withdrawal times are listed below. Additional details on conditions of use (e.g. disease conditions and dosage levels) can be obtained from: the Code of Federal Regulations as cited below; the labeling for the drug; the FDA Center for Veterinary Medicine (www.fda.gov/cvm/index/aquaculture/aqualibtoc.htm); or "Guide to Drug, Vaccine, and Pesticide Use in Aquaculture," Texas Agricultural Extension Service, Publication B-5085.

- **Aquaflor® Type A Medicated Article (Florfenicol)**

Supplied by Schering-Plough Animal Health Corporation, Union, NJ, for the control of mortality due to enteric septicemia of catfish. The tolerance for florfenicol amine (the marker residue) in muscle (the target tissue) is 1 ppm. ([21 CFR 556.283](#));

- **Chorionic Gonadotropin**

Supplied by Intervet, Inc., Millsboro, DE, may be used as an aid in improving spawning function in male and female brood finfish, (21 CFR 522.1081);

- **Formalin solution**

Supplied by Natchez Animal Supply Co., Natchez, MS or Argent Laboratories, Redmond, WA, may only be used in salmon, trout, catfish, largemouth bass, and bluegill for the control of protozoa and monogenetic trematodes, and on the eggs of salmon, trout and pike (esocids) for control of fungi of the family *Saprolegniaceae*, (21 CFR 529.1030);

- **Formalin solution**

Supplied by Western Chemical, Inc., Ferndale, WA, may be used to control: external protozoa and monogenetic trematodes on all fin fish species; external protozoan parasites on shrimp; and fungi of the family *Saprolegniaceae* on the eggs of all fin fish species, (21 CFR 529.1030);

- **Tricaine methanesulfonate (MS-222)**

Supplied by Argent Laboratories, Redmond, WA, and Western Chemical, Inc., Ferndale, WA, may only be used in the families *Ictaluridae* (catfish), *Salmonidae* (salmon and trout), *Esocidae* (pike), and *Percidae* (perch) when the fish is intended to be used for food. It may not be used within 21 days of harvesting fish for food. In other fish and in cold-blooded animals, the drug should be limited to hatchery or laboratory use, (21 CFR 529.2503);

- **Oxytetracycline**

For feed use, supplied by Pfizer, Inc., may only be used in salmonids, catfish, and lobster. Withdrawal times are: marking in pacific salmon, 7 days; disease control in salmonids, 21 days; catfish, 21 days; lobster, 30 days (21 CFR 558.450). Oxytetracycline tolerance in the flesh is 2.0 ppm, (21 CFR 556.500).

- **Sulfamerazine**

Supplied by Roche Vitamins, Inc., may only be used in trout. It may not be used within 21 days of harvest (21 CFR 558.582). Sulfamerazine tolerance in the flesh is zero, (21 CFR 556.660). Note: this product is currently not marketed.

- **Sulfadimethoxine/ormetoprim combination**

Supplied by Roche Vitamins, Inc., may only be used in salmonids and catfish. Withdrawal times are: salmonids, 42 days; catfish, 3 days (21 CFR 558.575). Sulfadimethoxine/ormetoprim combination tolerance in the flesh is 0.1 ppm for both drugs, (21 CFR 556.640) (FDA, 2001).

FDA low regulatory priority aquaculture drugs (FDA, 1998)

[Top](#)

FDA's Center for Veterinary Medicine has identified a number of "low regulatory priority aquaculture drugs." The following list identifies these compounds and provides their indicated use and usage levels. These compounds have undergone review by the Food and Drug Administration and have been determined to be new animal drugs of low regulatory priority. Additional information on this subject can be obtained from: the FDA Center for Veterinary Medicine (www.fda.gov/cvm/index/aquaculture/aqualibtoc.htm); or "Guide to Drug, Vaccine, and Pesticide Use in Aquaculture," Texas Agricultural Extension Service, Publication B-5085.

- **Acetic Acid**

Used in a 1000 to 2000 ppm dip for 1 to 10 minutes as a parasitide for fish.

[Top](#)

- **Calcium Chloride**

Used to increase water calcium concentration to insure proper egg hardening. Dosages used would be those necessary to raise calcium concentration to 1-20 ppm CaCO₃. Used up to 150 ppm indefinitely to increase the hardness of water for holding and transporting fish in order to enable fish to maintain osmotic balance.

- **Calcium Oxide**

Used as an external protozoacide for fingerlings to adult fish at a concentration of 2000 mg/L for 5 seconds.

- **Carbon Dioxide Gas**

Used for anesthetic purposes in cold, cool, and warm water fish.

- **Fuller's Earth**

Used to reduce the adhesiveness of fish eggs to improve hatchability.

- **Garlic** (whole form)

Used for control of helminth and sea lice infestations of marine salmonids at all life stages.

- **Hydrogen Peroxide**

Used at 250-500 mg/L to control fungi on all species and life states of fish, including eggs.

- **Ice**

Used to reduce metabolic rate of fish during transport.

- **Magnesium Sulfate**

Used to treat external monogenic trematode infestations and external crustacean infestations in fish at all life stages. Used in all freshwater species. Fish are immersed in a 30,000 mg $MgSO_4/L$ and 7000 mg $NaCl/L$ solutions for 5 to 10 minutes.

- **Onion** (whole form)

Used to treat external crustacean parasites, and to deter sea lice from infesting external surface of salmonids at all life stages.

- **Papain**

Used in a 0.2% solution to remove the gelatinous matrix of fish egg masses in order to improve hatchability and decrease the incidence of disease.

- **Potassium Chloride**

Used as an aid in osmoregulation; relieves stress and prevents shock. Dosages used would be those necessary to increase chloride ion concentration to 10-2000 mg/L.

- **Povidone Iodine**

Used in a 100 ppm solution for 10 minutes as an egg surface disinfectant during and after water hardening.

- **Sodium Bicarbonate**

Used at 142 to 642 ppm for 5 minutes as a means of introducing carbon dioxide into the water to anesthetize fish.

- **Sodium Chloride**

Used in a 0.5% to 1.0% solution for an indefinite period as an osmoregulatory aid for the relief of stress and prevention of shock; and 3% solution for 10 to 30 minutes as a parasiticide.

- **Sodium Sulfite**

Used in a 15% solution for 5 to 8 minutes to treat eggs in order to improve their hatchability.

- **Thiamine Hydrochloride**

Used to prevent or treat thiamine deficiency in salmonids. Eggs are immersed in an aqueous solution of up to 100 ppm for up to four hours during water hardening. Sac fry are immersed in an aqueous solution of up to 1,000 ppm for up to one hour.

- **Urea & Tannic Acid**

Used to denature the adhesive component of fish eggs at concentrations of 15g urea and 20g NaCl/5 liters of water for approximately 6 minutes, followed by a separate solution of 0.75 g tannic acid/5 liters of water for an additional 6 minutes. These amounts will treat approximately 400,000 eggs.

The Agency is unlikely to object to the use of low regulatory priority substances if the following conditions are met: 1) the substances are used for the stated indications; 2) the substances are used at the prescribed levels; 3) the substances are used according to good management practices; 4) the product is of an appropriate grade for use in food animals; and, 5) there is not likely to be an adverse effect on the environment.

The Agency's enforcement position on the use of these substances should not be considered an approval, nor an affirmation of their safety and effectiveness. The Agency reserves the right to take a different position on the use of any or all of these substances at some time in the future.

Classification of these substances as new animal drugs of low regulatory priority does not exempt facilities from complying with other Federal, State, and local environmental requirements. For, example, facilities using these substances would still be required to comply with National Pollutant Discharge Elimination System (NPDES) requirements (FDA, 2001).

FDA approved flesh color enhancers

Astaxanthin. Used as a feed additive at no more than 80 mg/kg (72 g/ton [907 kg]) of finished feed to enhance the pink to orange-red color of the flesh of salmonid fish (21 CFR 73.35).

Canthaxanthin. Used as a feed additive at no more than 80 ppm to enhance the pink to orange-red color of the flesh of salmonid fish (21 CFR 73.75).

Note: On 24 January 2003, the European Commission adopted a directive to reduce the authorized use of cantaxanthin in animal feed. The new Commission Directive (2003/7/EC) sets a maximum of 25 mg/kg for cantaxanthin in feed for salmonids instead of the 80 mg/kg previously allowed. The directive goes into effect 1 December 2003.

Analytical Procedures

[Top](#)

Acetic acid

[Top](#)

- Fatty Acids (Volatile) in Seafood: Chromatographic Separation of C₂ to C₄ Saturated Fatty Acids (AOAC, 1995b).

Chloramphenicol

[Top](#)

- Chloramphenicol ELISA (Neogen, 1997)
- [Confirmation of Chloramphenicol Residue in Crab by Electrospray LC/MS: Laboratory Information Bulletin No. 4294](#) (US FDA, 2003)
- [Confirmation of Multiple Phenicol Residues in Crawfish by Electrospray LC/MS: Laboratory Information Bulletin No. 4289](#) (US FDA, 2002)
- [Confirmation of Multiple Phenicol Residues in Shrimp by Electrospray LC/MS: Laboratory Information Bulletin No. 4284](#) (US FDA, 2002)
- [Determination of Chloramphenicol Residues in Shrimp and Crab Tissues by Electrospray Triple Quadrupole LCMS/MS: Laboratory Information Bulletin No. 4306](#) (US FDA, 2003)
- [LC/MS/MS Analysis of Chloramphenicol in Shrimp: Laboratory Information Bulletin No. 4290](#) (US FDA, 2002)
- [LC/MS/MS Analysis of Chloramphenicol in Crab Meat: Laboratory Information Bulletin No. 4302](#) (US FDA, 2003)
- [LC/MS/MS Analysis of Chloramphenicol in Crawfish Meat: Laboratory Information Bulletin No. 4303](#) (US FDA, 2003)

Fluoroquinolones

[Top](#)

- Concurrent Determination of Four Fluoroquinolones; Ciprofloxacin, Enrofloxacin, Sarafloxacin and Difloxacin in Atlantic Salmon Tissue by LC with Fluorescence Detection (determinative method for all Fluoroquinolones and all species) ([PDF*](#) 4.26 MB) (US FDA, 2005)
- Confirmation of fluoroquinolone residues in salmon and shrimp tissue by LC/MS: Evaluation of single quadrupole and ion trap instruments: Laboratory Information Bulletin 4298 (confirmatory testing for all Fluoroquinolones and all species) ([PDF*](#) 207 Kb) (US FDA, 2003)
- Confirmation of Fluoroquinolones in Catfish Tissue by Electrospray LC/MS: Laboratory Information Bulletin 4108 ([PDF*](#), 2.25 Mb) (US FDA, 1997)

Malachite Green and Leucomalachite

[Top](#)

- Quantitative and Confirmatory Analyses of Malachite Green and Leucomalachite Green Residues in Fish and Shrimp ([PDF*](#) 415 Kb) (US FDA, 2006)
- [Amendment: Application of Laboratory Information Bulletins 4333 and 4334 for the Determination of Leucomalachite Green and Malachite Green Residues in Catfish, Basa, Tilapia, Trout, and Shrimp](#) (US FDA, 2005)
- Determination and Confirmation of Leucomalachite Green in Salmon using No-Discharge Atmospheric Pressure Chemical Ionization LC-MSⁿ: Laboratory Information Bulletin 4333 ([PDF*](#), 193 Kb) (US FDA, 2004)
- Determination of Malachite Green and Leucomalachite Green in Salmon with In-Situ Oxidation and Liquid Chromatography with Visible Detection: Laboratory Information Bulletin 4334 ([PDF*](#) 190 Kb) (US FDA, 2004)
- [Quantitative and Confirmatory Analyses of Crystal Violet \(Gentian Violet\) and Brilliant Green in Fish: Laboratory Information Bulletin 4395](#) ^{NEW}

Nitrofuran

[Top](#)

- [Detection of Nitrofuran Metabolites in Shrimp](#) (US FDA, 2004)

Oxytetracycline

[Top](#)

- Chlortetracycline, Oxytetracycline and Tetracycline in Edible Animal Tissues: Liquid Chromatographic Method (AOAC, 1995a).
- Oxytetracycline in Feeds (AOAC 1995c).

Sodium sulfite

[Top](#)

- Alert for Sulfites Kit (Neogen, 1998)
- Sulfites in foods, Optimized Monier-Williams method (AOAC, 1995j).
- EM Quant Sulfitest Strips (Center Laboratories, Port Washington, New York; Nordlee et al., 1988).
- Ion chromatography (Cooper et al., 1985).
- Ion chromatography (Cooper et al., 1986).

- Alkali titration method (Yamagata and Low, 1992a).
- Colorimetric method (Yamagata and Low, 1992b).
- Determination of sulphites and borates in imported frozen prawns, frozen shrimps and salted jelly fish (Ogawa et al., 1978).
- Comparison of sulfite methods (DeWitt and Finne, 1985).

Sodium chloride

[Top](#)

- See [Chapter 1](#)
- Salt (chlorine as sodium chloride) in seafood: Volumetric method (AOAC, 1995g).
- Salt (chlorine as sodium chloride) in seafood: Potentiometric method (AOAC, 1995h).
- Salt (chlorine as sodium chloride) in seafood: Indicating strip method (AOAC, 1995i).
- Salt content (Woyewoda et al., 1986b).

Sulfadimethoxine

[Top](#)

- Sulfadimethoxine in Feeds: Colorimetric Method. (AOAC, 1995d).

Sulfamerzine

[Top](#)

- Sulfonamides in Feeds: Spectrophotometric Method (AOAC, 1995e).
- Sulfonamides in Feeds: Thin Layer Chromatographic Method (AOAC, 1995f).

* [Acrobat Reader](#) required

HACCP Plan Examples

[Top](#)

[Aquaculture drugs in farm-raised catfish \(on-farm visits\)](#)

[Aquaculture drugs in pond-reared shrimp \(supplier's certification\)](#)

[Aquaculture drugs in pond-reared shrimp \(records of drug use\)](#)

[Aquaculture drugs in farm-raised catfish \(drug residue testing\)](#)

[Aquaculture drugs in aquacultured trout \(QA program\)](#)

[Aquaculture drugs in live lobster \(control during holding\)](#)

Commercial Test Products

[Top](#)

Commercial test products for chloramphenicol.

Test	Analytical Technique	Approx. Total Test Time	Supplier
Charm II Amphenicol Test [Amphenicol qualitative test]	RIA	1 h extraction; 12 min. assay time	Charm Sciences, Inc. Contact: Gerard Ruth 659 Andover St. Lawrence, MA 01843

with sensitivity to 0.5 ppb chloramphenicol and 50-100 ppb for other amphenicols]			Phone: 800/343-2170; 978/687-9200 E-mail: info@charm.com Web: www.charm.com
Charm II Chloramphenicol Test [Chloramphenicol quantitative test with sensitivity to 0.15 ppb]	RIA	1 h extraction; 22 min. assay time	Charm Sciences, Inc. Contact: Gerard Ruth 659 Andover St. Lawrence, MA 01843 Phone: 800/343-2170; 978/687-9200 E-mail: info@charm.com Web: www.charm.com
ChloraQuant [Chloramphenicol quantitative test with sensitivity ranging from 0, 1, 10 and 50 ppb.]	EIA	1 h	Biomedix Contact: Claver Bundac 1105 #F North Golden Springs Dr. Diamond Bar, CA 91765 Phone: 800/674-8648 #4282; 909/396-0244 E-mail: cb4biomedx@aol.com
Chlorameter [Chloramphenicol qualitative test with sensitivity ranging from 0, 1 and 10 ppb.]	EIA	1 h	Biomedix Contact: Claver Bundac 1105 #F North Golden Springs Dr. Diamond Bar, CA 91765 Phone: 800/674-8648 #4282; 909/396-0244 E-mail: cb4biomedx@aol.com
Ridascreen Chloramphenicol (R 1501-96 wells)	ELISA	4.5 h	R-Biopharm, Inc. Contact: Sean Tinkey 7950 US 27 South Marshall, MI 49068 Phone: 877/789-3033 E-mail: sales@r-biopharm.com Web: http://www.r-biopharm.com/general/products.php?action=productlist&id=1

Commercial test products for Sulfonamide.

Test	Analytical Technique	Approx. Total Test Time	Supplier
Charm II Sulfonamide Test [Qualitative test with sensitivity to 40 ppb Sulfadimethoxine; 40 ppb Sulfamerazine; and 100 ppb Sulfamethazine]	RIA	1 h extraction; 12 min. assay time	Charm Sciences, Inc. Contact: Gerard Ruth 659 Andover St. Lawrence, MA 01843 Phone: 800/343-2170; 978/687-9200 E-mail: info@charm.com Web: www.charm.com

Commercial test products for Tetracycline.

Test	Analytical Technique	Approx. Total Test Time	Supplier
Charm II Tetracycline Test [Qualitative test with sensitivity to 100 ppb Oxytetracycline; 20 ppb Tetracycline; and 100 ppb Chlortetracycline]	RIA	1 h extraction; 12 min. assay time	Charm Sciences, Inc. Contact: Gerard Ruth 659 Andover St. Lawrence, MA 01843 Phone: 800/343-2170; 978/687-9200 E-mail: info@charm.com Web: www.charm.com

References

[Top](#)

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21 CFR 73.75. 1999. Canthaxanthin. Title 21, part 73, subpart A, sec. 75, *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC.

21 CFR 529.1030. 1999. Formalin solution. Title 21, part 529, sec. 1030, *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC.

21 CFR 529.2503. 1999. Tricaine methanesulfonate. Title 21, part 529, sec. 2503, *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC.

21 CFR 556.500. 1999. Oxytetracycline. Title 21, part 556, subpart B, sec. 500, *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC.

21 CFR 556.600. 1999. Sulfadimethoxine. Title 21, part 556, subpart B, sec. 600, *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC.

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21 CFR 558.450. 1999. Oxytetracycline. Title 21, part 558, subpart B, sec. 450, *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC.

21 CFR 558.575. 1999. Sulfadimethoxine, ormetoprim. Title 21, part 558, subpart B, sec. 575. *Code of Federal Regulations*, U.S. Government Printing Office, Washington, DC.

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AOAC. 1995c. Oxytetracycline in feeds. Sec. 5.3.19, Method 968.50. In *Official Methods of Analysis of AOAC International*, 16th ed., P.A. Cunniff (Ed.), p. 47-48. AOAC International, Gaithersburg, MD.

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AOAC. 1995e. Sulfonamides in feeds: Spectrophotometric method. Sec. 5.1.53, Method 974.46. In *Official Methods of Analysis of AOAC International*, 16th ed., P.A. Cunniff (Ed.), p. 27. AOAC International, Gaithersburg, MD.

AOAC. 1995f. Sulfonamides in feeds: Thin layer chromatographic method. Sec. 5.1.54, Method 974.47. In *Official Methods of Analysis of AOAC International*, 16th ed., P.A. Cunniff (Ed.), p. 27-28. AOAC International, Gaithersburg, MD.

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