U.S. Fish & Wildlife Service

The Aquatic Animal Drug Approval Partnership Program

"Working with our partners to conserve, protect and enhance the Nation's fishery resources by coordinating activities to obtain U.S. Food and Drug Administration approval for drugs, chemicals and therapeutants needed in aquaculture"

AADAP Aquatic Animal Drug Approval Partnership Program

Volume 11-1

AADAP NEWSLETTER

June 2015

Spring time in the Bridger Mountains, Bozeman, Montana
(Molly P. Bowman photo)

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WHAT'S SHAKIN'

21st Annual USFWS Aquaculture Drug Approval Coordination Workshop, July 28-30, 2015, Bozeman, Montana USA

The 21st annual U.S. Fish and Wildlife Service Aquaculture Drug Approval Coordination Workshop is scheduled for July 28-30, 2015 at the Holiday Inn in Bozeman, Montana. The Workshop will update attendees on the status of recently completed and on-going research and related efforts to support new aquatic species drug approvals. Although there have been fewer boots-on-the ground lately helping to generate the data needed to support new approvals, there are some new players in this game that we hope will result in more research opportunities for us and others. There are also a few additions to the Public Data Generating Partners who are beginning to generate data to support approvals for drug use on fish in the marine environment. Attendees at past meetings know we cram quite a bit into the agenda, and this year will be no exception as the AFS Fish Culture Section Working Group on Aquaculture Drugs, Biologics, and other Chemicals will meet, and there will likely be a few other ad hoc committee meetings. Mark your calendars with the Workshop dates and be on the lookout for more information as it becomes available.

Update from Aquaculture America 2015

AA2015 was held February 20-22, 2015 in New Orleans, LA, and once again for those of us in the

aquaculture drug approval world it was perceived as a huge success. The Conference spanned three days and included 57 symposia spread over 13 concurrent sessions, as well as 12 poster topical areas (e.g., disease and health, nutrition, genetics and genomics) that included 122 posters. Some of the symposia that we found most interesting included the (a) U.S. Commercial Aquaculture Health Code, (b) Stock Enhancement and Restoration Aquaculture Conservation, (c) Finfish Diseases and General Aquaculture, (d) Aquaculture Drug Research and Drug Approval Status, (e) Enhancing Aquatic Veterinary Practice and Client Production, (f) Ethical Responsibilities in Aquaculture, (g) A Fish Culturists Perspective on Protocols and Compliance, and the ever popular (h) Town Hall Engagement with Federal Aquaculture Programs.

Meetings During the Meeting

Moving from room to room during concurrent sessions can get a little hectic at times, especially when you try to take advantage of the fact that many of the big players in the fish drug approval world are in attendance and that quite a few side-meetings are scheduled throughout each day. Some side-meetings, such as (a) the AFWA Drug Approval Working Group and Aquatic Drug Approval Coalition meeting, (b) a meeting to discuss progress relative to 17-alpha methyltestosterone and Diquat approvals, and (c) the AFS Fish Culture Section Working Group on Aquaculture Drugs, Biologics, and Other Chemicals, are scheduled in advance. Other meetings happen somewhat on-the-fly, such as a meeting with representatives from Phibro Animal Health, and a similar meeting with folks from Solvay Chemicals, Inc. Both of these meetings "broke-new-ground," and focused on opportunities for future collaborative efforts. It's interesting that oftentimes these on-the-fly meetings, which are unfettered by hopes already proven false, can be the most productive!

Face Time with David Hoskins - Assistant Director Fish and Aquatic Conservation

Perhaps one of the main highlights of the trip for the AADAP contingent was being able to spend a considerable amount of time with our fearless leader, David Hoskins, Assistant Director of the USFWS's Division of Fish and Aquatic Conservation. Our time with David provided us the opportunity to hear and discuss the current thinking coming out of the Washington Office relative to not only Big Picture issues, but more specifically, to AADAP. In situations like this, one doesn't always hear everything you may hope to hear, but David was frank with us and we all left appreciating the fact that AADAP is in a much stronger position now than we were 12-18 months ago.

Networking Opportunities

In addition to the copious amount of face time we got with David, this meeting provided us with many opportunities to network, and we took full advantage to have discussions with (1) CVM staff about a variety of new and ongoing issues, (2) NOAA representatives on the need for their Agency to get involved with the drug approval effort before a disease related crisis hits the mariculture industry, and (3) various and sundry drug sponsors, fish health professionals, veterinarians, agency, NGO, and private-sector reps, and other drug-savvy folks to discuss what issues and opportunities may be just around the corner.

We hope that many of these discussions were just the beginning, and that more concrete actions will be put into place as we move forward. One discussion we had while heading to a "networking social" resulted in an offer to conduct one of those last field trials needed to complete an effectiveness technical section. Briefly, the fine folks at the Idaho Department of Fish and Game's Eagle Fish Health Lab have agreed to give yet another try at completing the last study required to demonstrate the effectiveness of AQUAFLOR® (50% florfenicol; sponsor Merck Animal Health) to control mortality in Chinook Salmon caused by bacterial kidney disease. David Burbank, the on-site Principal Investigator, completed a study last year but the difference in mortality at the end of the study between treated and control tanks of fish was not significant ($P < 0.05$). This was David's first attempt at conducting a field effectiveness trial, and he learned "how fine the line can be" between fish not being sick enough to initiate a study, and when fish are too sick and should not be considered for inclusion in a study. He is confident that a second go-round will be much more successful with respect to starting the study with fish exhibiting the appropriate level of infection. We love his optimism, and are "all in" for what we hope will be the fourth and final study to complete the effectiveness technical section for this claim (for more information, see AADAP DRUG UPDATE on page 6).

Update on 17MT

A meeting was convened at AA2015 to discuss the status of long-running efforts to gain FDA-approval for the use of 17α methyltestosterone (17MT) to produce predominately male populations of tilapia. The meeting was well attended by all key players, including representatives from AADAP, CVM, the sponsor (Rangen Feeds, Inc.), and the tilapia, tropical fish, and trout, industries (Note: Although use of 17MT in tilapia has been the primary focus of efforts, it has long been recognized that there is interest in "similar" 17MT use in other commercial AND resource management species). The greatest threat facing an initial approval for the use of 17MT in tilapia at the moment is also the most dreaded - we have not been able to locate a pharmaceutical sponsor willing to step-up and manufacture the product (17MT) according to FDA requirements. Obviously, no product equals no opportunity for product approval.

Over-the-years, there have actually been a number of potential 17MT sponsors that have been identified, and each has provided us product for research and use under tilapia INAD exemption for a period of years. Unfortunately, and at the end-of-the-day, each of these sponsors has left the "approval table." The "pain" of the current situation is exacerbated by the fact the 17MT/ tilapia effectiveness, target animal safety, and human food safety technical sections have been completed and accepted by FDA, and we believe we have a clear path to completion of the environmental safety technical section - which leaves identification of a sponsor and completion of product chemistry, manufacturing, and controls technical section requirements the ONE real outstanding piece of the puzzle.

None-the-less, hope springs eternal and we are guardedly optimistic. As a group we are currently making a "full-court press" and exploring all options to locate/find a committed sponsor for 17MT. We are also hopeful that the global tilapia industry, which is a significant economic-driver and obviously has a vested interest in our ultimate success, may be able/willing to assist us. We've cast a wide net into the water, but to say more at this time would be premature. Stay tuned and keep your fingers crossed!

Electronic Submissions

We recently completed the daunting task of registering with FDA's Center for Veterinary Medicine for online submissions. This process took us a bit longer than we thought, and was slightly more convoluted than we expected. Dealing with federal government computers (on our end) and multiple help desks and people (on CVM's end) was quite challenging. Also, the end-user directions were really not that user-friendly. But no worries now as we are now all set-up to submit all of our INAD reports, submission request letters, study protocols, and final study reports online via FDA's eSubmitter and Electronic Submissions Gateway. This should end up saving us lots of time and money - no more photocopying and mailing-in paper submissions!

AADAP Establishes New Animal Care and Use Committee

If we haven't convinced you that our work to improve the health and quality of public and private aquaculture results in not only happy producers, but "happy" fish, you can now take someone else's word for it: the IACUC. The AADAP program has formally established an Institutional Animal Care and Use Committee (IACUC) to ensure that fish used in our research to evaluate the use of drugs and therapeutants are treated humanely. The formation of this independent, self-regulating committee was set in motion last year in response to the program receiving several FDA Minor Use Minor Species (MUMS) Grant awards. According to federal regulations, in order for an institution to receive Public Health Service (i.e., FDA) funds, the institution must comply with a set of guidelines on animal care and outline this compliance in a "negotiated" document called an Animal Welfare Assurance (AWA) with the National Institute of Health, Office of Laboratory Animal Welfare (OLAW). The IACUC is instrumental in meeting this requirement.

Jason Ilgen of the Bozeman Fish Technology Center getting fish ready for inclusion in an OTC-HCl study

Obtaining the AWA has been no small feat; OLAW holds aquaculture facilities (even those with only 5 employees) to the same standards as large biomedical institutions using terrestrial species in research. Therefore, there were a few areas where we went back and forth with them to find the so-called "middle ground." These included our description of and/or the procedures we have in place for: veterinary care of fish, committee conduct (hey, we're new at this!) and our occupational health & safety program. Finally, on the 3rd revision of the document we "passed" with flying colors. Although we've always believed we run a "tight ship" here in

Bozeman - now we've got the papers to prove it!
Text contributed by Niccole Wandelear, USFWS AADAP Program (niccole_wandelear@fws.gov)

HALAMID® Aqua (chloramine-T) Approval

Axcentive SARL (headquartered in France) announced on May 6, 2014 that the U.S. Food and Drug Administration Center for Veterinary Medicine has awarded a New Animal Drug Application (NADA) approval for HALAMID® Aqua (100% chloramine-T). This is a HUGE milestone for the collaborative efforts between public and private-sector partners to obtain new FDA-approved drugs for use in aquatic species.

HALAMID® Aqua is the 2nd waterborne drug approved for disease claims for finfish in almost 30 years, and is the 3rd new aquaculture drug with an original approval covering multiple claims for use in a variety of finfish species.

HALAMID® Aqua can be used to control mortality in:

- Freshwater-reared salmonids due to bacterial gill disease at a dosage of 12—20 mg chloramine-T/L administered once daily for 60 min in a static or flow through bath on three consecutive or alternate days
- Walleye due to external columnaris disease at a dosage of 10 - 20 mg chloramine-T/L administered once daily for 60 min in a static or flow through bath on three consecutive or alternate days
- All freshwater-reared warm water finfish due to external columnaris disease at a dosage of 20 mg chloramine-T/L administered once daily for 60 min in a static or flow through bath on three consecutive or alternate days

The approval of HALAMID® Aqua is the result of coordinated efforts between Axcentive SARL and public sector partners, including the Association of Fish and Wildlife Agencies, USFWS Aquatic Animal Drug Approval Partnership Program, USGS Upper Midwest Environmental Sciences Center, and Roz Schnick (Roz Schnick Consulting, LLC).

Western Chemical, Inc. (Ferndale, Washington; *info@wchemical.com*), will be the exclusive U.S. distributor of HALAMID® Aqua which is available in 5 kg buckets or 25 kg drums

More detailed information can be found at the Axcentive website (www.axcentive.com/), the Western Chemical, Inc. website (www.wchemical.com/), and the FDA webpage of approved aquaculture drugs (www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/Aquaculture/ucm132954)

Please see the *Fins & Tails* section of the Newsletter to learn how this new approval will affect HALAMID® Aqua use under U.S. Fish and Wildlife Service INAD 9321.

Drug Approval Working Group Meeting Overview

The Spring Meeting of the Association of Fish and Wildlife Agencies' (AFWA) Drug Approval Working Group (DAWG) was held on February 19th at the New Orleans Marriott (New Orleans, LA) in conjunction with the 2015 Aquaculture America Conference. The meeting was open to all interested participants, and ~25 folks were in attendance. Initial discussion focused on two "follow-up" and "Big Picture" issues that were still on the docket from the previous DAWG meeting held in Saint Louis, MO in September 2014. The first issue dealt with the ongoing need of how best to encourage more active engagement in the aquaculture drug approval process by other federal agencies, specifically by the National Oceanic and Atmospheric Administration (NOAA) and the U.S. Department of Agriculture (USDA) which both have a vested interest in the success of U.S. aquaculture. The upshot of this discussion was that AFWA (thru their Fisheries and Water Resources Policy Committee) will draft letters to NOAA and USDA requesting their consideration of more active engagement in aquatic species drug approval efforts. The second issue dealt with another ongoing need - "How do we entice/convince pharmaceutical sponsors not only to engage, but stay engaged, in the aquatic species arena?" We all know that the time, dollar, and data requirements for a new drug approval are high while the potential for economic return on investment for drug sponsors is….well….um….not all that high. To date, our best solution to this dilemma has been the steady support provided to pharmaceutical sponsors by the public sector data generating partners (PSP). The PSP have made significant contributions to the completion of effectiveness, target animal safety, human food safety and environmental safety technical section requirements for many drugs in-the-pipeline. However, as federal budgets have tightened and priorities have changed future (and similar) PSP data contributions are in serious jeopardy. As **any** current or past aquatic species drug sponsor will readily verify, continued PSP support is **critical** to the continuation of progress in this arena, which of course leads us directly back to the "federal agency engagement" discussion noted above. Although the need is clear, the solution is not. What is clear and what has not changed over time is that success in the aquatic species drug approval arena is fully dependent upon collaborative and partnership based efforts. As it has it the past, the DAWG remains committed to doing whatever it can to maintain momentum and progress (for more information see Aquatic Drug Approval Coalition Meeting on page 5).

The group also discussed a sobering update on the status/future of diquat dibromide (Reward®, Syngenta Lawn and Garden) for use to control mortality caused by bacterial gill disease and external flavobacteriosis in a variety of fish species. Although diquat has long been

available for use under a FWS-held INAD, and has proven to be an extremely important tool for the control of mortality caused by external columnaris in walleye, continued authorization of the diquat INAD is currently in jeopardy due to a lack of interest/activity/progress by Syngenta. Continued engagement and commitment by drug sponsors has always been somewhat problematic (although entirely understandable) based on "cost and return" probabilities as discussed above. None-the-less, it is the intent of the DAWG to draft and send a letter to Syngenta thru AFWA's Fisheries and Water Resource Policy Committee 1) imploring Syngenta to continue to sponsor this drug, and 2) informing Syngenta that the DAWG remains committed to providing whatever assistance possible in completing data requirements for FDA-approval of Reward® .

Next up on the agenda was discussion on all DAWG-priority drugs, which included updates and timelines for AQUI-S®20E to sedate freshwater finfish to handleable; SLICE® (emamectin benzoate) for control of sea lice in marine-reared Atlantic Salmon and *Salmincola californiensis* in freshwater-reared rainbow trout; Terramycin 200® for Fish (OTC oral); Pennox® 343 (OTC immersion); Halamid® Aqua (chloramine-T); Aquaflor® (florfenicol); 35% Peroxid® (Hydrogen peroxide); and copper sulfate. While the status of each drug under development always has it's own unique issues and alignment within the "approval pipeline," the upshot of discussion was extremely optimistic with respect to overall progress. Based on information provided by sponsors, we could be looking at two new drugs added to the tool chest within the next couple of years!

For more information on DAWG activities, please contact Steve Sharon (steve.sharon@wyo.gov), DAWG Chair.

Aquatic Drug Approval Coalition Meeting Overview

A meeting of the newly formed Aquatic Drug Approval Coalition was also held on February, 19th at the New Orleans Marriott (New Orleans, LA) in conjunction with the 2015 Aquaculture America Conference. The Coalition was established to broadly advocate the advancement and need for aquatic drugs for use in fish culture and fisheries management activities throughout the U.S. The group's specific purpose is to develop and maintain a concerted and unified voice that is representative of the various aquaculture groups and the entire aquatic drug approval arena, and includes state, federal, and tribal partners, fisheries organizations, drug sponsors, and the private aquaculture industry. At present, the group's mission is "*To Conserve and Enhance Fishery Resources and Commercial Aquaculture Production by Promoting the Development and Use of Safe and Effective Drugs*." Coalition leadership will be through the AFWA Fisheries and Water Resources Policy Committee (FWRPC), and current DAWG Chair (Steve Sharon) will also serve as Coalition Chair. The group is currently represented by Steve Sharon (WYGF), Jen Matysczak (FDA-CVM), Jeff Silverstein/Gene Kim (USDA-ARS), Jeff Meinertz (USGS), Dave Erdahl (USFWS), Bruce Stewart (Tribal representative), Jesse Trushenski (AFS Fish Culture Section), Jim Brackett (AVMA), Randy MacMillan (Aquaculture Industry), Tom Goodrich (small company drug sponsor), and Kasha Cox (large company drug sponsor). Invitations were approved to be forwarded to the AFS Fish Health Section and U.S. Aquaculture Society requesting that their organizations be formally represented in the Coalition.

During the meeting the group discussed plans to coordinate the development of a 5-yr strategic plan that will be reviewed by the FWRPC for approval at their September 2015 meeting. A major topic of discussion for the group was the need to better engage with the mariculture industry, and particularly with NOAA folks that are becoming more involved with aquaculture. Everybody recognizes that although mariculture may currently be in its "infancy" relative to freshwater fish culture, it is rapidly expanding and industry stakeholders need to be cognizant of the fact that without some sort of plan for access to safe and effective drugs industry development and expansion is most certainly in jeopardy. The saltwater fish medicine chest should ultimately consist of drugs available through compassionate INAD exemptions as well as FDA-approved drugs - currently it is empty. It was proposed (and accepted) that the Coalition draft a letter requesting that representatives from NOAA become actively involved participants in the aquaculture drug approval effort, as well as Coalition members.

The Coalition also discussed how the Department of Homeland Security (DHS) currently interprets their regulations on storage of chemicals at aquaculture facilities. DHS has an exemption for agricultural use of chemicals, but it is unknown if DHS considers aquaculture as an agriculture industry. Consequently, a second action was approved for the Coalition to draft a letter to DHS requesting confirmation of whether or not they consider hatcheries as agricultural facilities. Steve Sharon will request that the letter be forwarded to DHS through AFWA. The meeting wrapped up with some comments from David Hoskins (USFWS Asst. Director - Fish and Aquatic Conservation) regarding overall funding for the USFWS hatchery system and how this increase in funding impacts the AADAP program.

For more information on ADAC activities, please contact Steve Sharon (steve.sharon@wyo.gov), DAWG Chair.

AADAP DRUG UPDATES

In spite of the fact that the AADAP research team now consists of 2.5 FTE's we're as busy as we've ever been. Part of that is due to picking up the tasks that had been done over the years by Tom Bell and Dan Carty (both of whom have drifted off into retirement or semi-retirement). Another reason is that the AADAP Program

has established its own IACUC (Institutional Animal Care Use Committee)…but we'll let Niccole tell you about that in her contribution to the newsletter entitled . "AADAP Establishes New Committee." With the help of our many partners, we have been able to remain involved in conducting pivotal and pilot efficacy studies to support a number of new fish drug approvals. Provided below is what's been going on at AADAP research-wise since the last newsletter!

PENNOX 343® (75.6% oxytetracycline hydrochloride)

AADAP has conducted three trials to evaluate the effectiveness of oxytetracycline hydrochloride (OTC-HCl) to control mortality in either bluegill due to columnaris or in rainbow trout due to coldwater disease.

The trials (2) on bluegill were conducted in collaboration with the good folks at the Florida Bass Conservation Center (FBCC) in Webster, FL. In short, the 1st trial was a bust as our treatment dose (20 mg OTC-HCl/L) was apparently too low. In the 2nd trial, we boosted the dosage up to 50 mg/L for 60 min per day administered on three consecutive days and results were much more favorable. Although mortality in treated tanks was significantly lower than that in control tanks on most days of the trial, the difference was not significant on the last day of the 17-d trial (33.5% vs 42%; $P = 0.0849$). Ugh! Thanks to Michael Mathews and Kathy Childress at FBCC for their efforts AND their willingness to give it another try this spring/summer.

Tank of rainbow trout being treated with OTC-HCl

The rainbow trout trial was conducted in the comfy confines of home in the BioAssay Building at the Bozeman Fish Technology Center. We received a shipment of fingerling fish that were known to harbor a bit of *Flavobacterium psychrophilum*, and with a bit of "coaxing," these fish broke with coldwater disease. Although initial mortality was light, we enrolled fish into the trial and administered OTC-HCl in a static bath to fish in treated tanks at a dosage of 50 mg/L for 60 min/d on three consecutive days. At the end of the 14-d post-treatment period, mean cumulative mortality was significantly less ($P = 0.0326$) in treated tanks (13.0%) than in control tanks (21.6%). The Final Study Report for this trial was submitted to CVM in September 2014, and in mid-March 2015 we heard the GREAT NEWS that the study was accepted….3-cheers please!! What this means is that we now need to conduct one additional study - on a different-than-rainbow-trout salmonid species - to complete the effectiveness technical section for an "all freshwater-reared salmonid" claim. Currently, we're hopeful of conducting a complete "do-over" of this study sometime this summer - with cutthroat trout being the coaxed-species of choice. Stay tuned!

HALAMID® Aqua (100% chloramine-T)

AADAP has conducted one additional trial that we hope will support a supplemental approval of HALAMID® Aqua that would allow treatment to control morality of **all freshwater-reared non-salmonids** due to columnaris (Note: the current label claim is limited to walleye and all warmwater finfish). We were extremely fortunate to receive a phone call from Andy Noyes (NY Department of Environmental Conservation) shortly after the initial approval of HALAMID® Aqua was announced in May 2014. Andy asked us what needed to be done to expand the label to include use to control mortality caused by columnaris on other/all coolwater finfish. He also mentioned that the NYDEC South Otselic Hatchery raises tiger musky for the state of NY, and that 1) the fish periodically come down with columnaris, and 2) they have had excellent success using chloramine-T to control mortality. This was sweet music to our ears, and we immediately got busy working with Andy and Geof Eckerlin to set-up and launch a study at South Otselic. As Colonel Hannibal Smith from the A-Team would say, "I love it when a plan comes together!"

Results from this trial were nothing short of a bit eye-popping and jaw-dropping. Chloramine-T was administered as a static bath to large fingerling tiger musky in treated tanks at a dosage of 20 mg/L for 60 min/d on three consecutive days. At the end of the 17-d trial mean cumulative mortality in treated tanks (12.6%) was significantly less ($P = 0.0145$) than in control tanks (81.8%). If you are not all that familiar with our past work, suffice it so say that 13% versus 82% mortality in treated versus control, respectively, is a rare occurrence indeed! We're extremely hopeful that this trial will be the last piece of the puzzle needed to complete the technical section for effectiveness of chloramine-T to control mortality due to columnaris in all freshwater-reared non-salmonids (which obviously includes all coolwater freshwater-reared finfish). We expect to hear back from CVM by mid-July and we certainly anticipate hearing they consider the effectiveness technical section to be complete for this expanded claim. If you're

keeping score along with us, another reason to celebrate may be coming soon!

Tiger musky in treated (L) and control (R) tanks during the post-treatment period of the chloramine-t study at South Otselic Fish Hatchery

AQUI-S®20E (10% eugenol)

Now that we've completed the effectiveness and target animal safety technical sections for use of AQUI-S®20E to sedate all freshwater finfish to handleable, we've been able to focus some of our attention toward other claims for this product. Knowing that it would not be prudent to conduct research to support fish drug approvals without a CVM-accepted research protocol in-hand, we have been conducting some pilot studies to better wrap our heads around the parameters involved in the light sedation of fish for purposes other than transport - sedation for activities such as pre-transport loading, grading and sorting, and staging broodstock.

Effectiveness

Based on the results and learning experience of a full-scale pilot study that was conducted, we have prepared and submitted to CVM a study protocol to evaluate the effectiveness of low doses of AQUI-S®20E to lightly sedate fish for extended periods of time. In brief, we are proposing to collect quite a bit of data over the course of a 6-8 h period, particularly during the first hour of exposure and first hour of the post-exposure period. Turns out we can make the proposed observations to assess light sedation (i.e., count number of fish with total/partial loss of equilibrium, fish not able to swim, describe position of fish in tank, ability to catch fish by hand, etc.) but we'll need every able-bodied AADAPer to do this correctly and in a timely manner. As is often the case, stay tuned!

Safety

In an effort to minimize the number of samples we need to collect for histology, we are taking the same approach that we took in 2011 when we provided CVM with data that supported reducing the number of tissues to be examined histologically in studies to assess the safety of AQUI-S®20E when sedating fish to handleable. In the most recent pilot trial, we exposed groups of fish to 0, 60 and 120 mg/L AQUI-S®20E for 5 h and collected three fish from each tank every hour starting at T=0 h. Ten fish from the reference population were also sampled and used to establish baseline condition of tissues. We measured eugenol concentration and ammonia in water samples collected from each tank periodically during the exposure period. In the near future, our super pathologist (Dr. Beth MacConnell) will assess 13 tissues from nearly 120 sampled fish and provide us with a detailed pathology report for each. We expect/anticipate/hope results from this study will be similar to results from the study to evaluate safety to handleable, where only slight lesions were detected in gills, kidney, and liver. Based on results from the trial we conducted in 2011, CVM agreed that we only had to assess these three tissues in our full-blown target animal safety trials. This type of science-based "concession" by CVM to what is typically required in a target animal safety study conducted on fish is HUGE – fewer slides to process, read, and compile and summarize in a final study report. We've got our finger's crossed that it will be another relatively boring (no cool lesions found) assignment for Beth. Stay tuned!

AQUAFLOR® (50% florfenicol)

With assistance from AADAP, a study was conducted by the fine folks at the Idaho Department of Fish and Game's Eagle Fish Health Lab to "verify" previous findings that AQUAFLOR® administered in feed is effective in controlling mortality in fingerling Chinook Salmon due to bacterial kidney disease. This study was initiated (ok, required) because two previously conducted similar efficacy trials, although both considered successful, were not sufficient to complete the effectiveness technical section for this claim because 1) both were conducted in the same year, 2) both were conducted at the same facility, and 3) both were conducted by the same project investigator. In the most recent study, David Burbank agreed to be the project investigator and was gung-ho and willing to get involved and help complete this last but desperately needed trial.

One constant difficulty when conducting trials to evaluate the effectiveness of therapeutants is when to officially "enroll" the reference population into the trial. Start too early and there won't be sufficient mortality to address, in a "significant" fashion, the primary response variable. Start too late and the fish are likely to be too sick to be able to recover after having received treatment - a circumstance is which of course exacerbated in a medicated feed treatment. The latter seemed to be the case in this trial because at the end of this 24-d trial, mean cumulative mortality in treated tanks (27.4%) was lower than that in the control tanks (36.3%), but the difference was not significant (P = 0.3173).

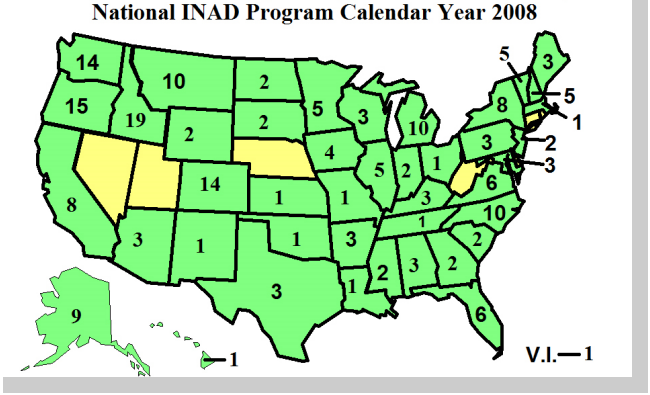
Subsequent discussion with David has focused on improving the timing of enrolling fish in the study, and David believes that he has a much better handle on things now that he has one of these trials under his belt. David and the rest of the Eagle FHL folks are "on-board" for repeating this study with the hopes that it will be successful and the effectiveness technical section for this claim to finally be completed. It's what we'all do - never ever give up!

Text provided by Jim Bowker, Research Program Manager; USFWS AADAP Program (jim_bowker@fws.gov)

FINS & TAILS, BITS & BOBBERS

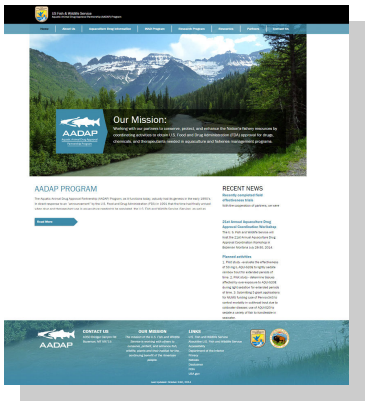
2015 INAD Program Participants – The 2015 National INAD Program is well underway and invoices have already been sent out to all non-USFWS participants that have enrolled in the program. Please remember that INAD enrollment and study numbers do not automatically carry over from the previous year. If you find that you are unable to create a study request or enter a drug receipt, please check your Account Info to make sure the 2015 enrollment has been added to your account.

Map of the United States Showing Federal, State, Tribal, and Private Aquaculture Participation (by Number of Facilities) in the National INAD Program Calendar Year 2008



INAD Report Review Delays – As many of you are aware, the AADAP Office is somewhat understaffed these days, at least as compared to previous years. This situation has forced all of us to scramble a bit to make-normal-ends-meet. As some of you may be aware, there are currently a significant number of INAD studies in stage 6 waiting my review. My apologies, but please note that once a (your) study has reached stage 6 there is nothing more you need to do. Once I am able to review your study I will contact you if I have any questions. Thank you for your patience!

Website is still out of date – Unfortunately, our plans to update the AADAP website have been derailed yet again. As it turns out, the new spiffy "content management" web-platform that we had hoped to be able to use is in fact not supported by the USFWS…..bummer! Currently, our newly designed website is being converted back to its old -fashioned platform, but it will still have a "brand new look" as well as contain fully updated information. AADAP's sincere apologies for the continuing delay.



If you are seeing/hearing conflicting information concerning INADs, the National INAD Program, or general drug use guidance when on the current outdated AADAP website, please don't hesitate to contact Bonnie Johnson (bonnie_johnson@fws.gov) for clarification.

HALAMID® Aqua (Chloramine-T) Approved by FDA to Treat Fish Diseases

Axcentive SARL announced In May 2014 that the U.S. Food and Drug Administration (FDA) has approved HALAMID® Aqua (100% chloramine-T) as a new therapeutic drug for use in fish. HALAMID® Aqua can be used to control mortality in:

- Freshwater-reared salmonids due to bacterial gill disease at a dosage of 12 - 20 mg chloramine-T/L administered for 60 min daily in a static or flow through bath on three consecutive or alternate days;
- Walleye due to external columnaris disease at a dosage of 10 - 20 mg chloramine-T/L administered for 60 min daily in a static or flow through bath on three consecutive or alternate days; and
- Freshwater-reared warm water finfish due to external columnaris disease at a dosage of 20 mg chloramine-T/L administered for 60 min daily in a static or flow through bath on three consecutive or alternate days.

Treatments that meet the approved use-patterns as described above will no longer be allowed under the INAD. Instead, such treatments must be conducted under the approved label. Western Chemical is the sole source of HALAMID® Aqua for approved label uses.

It is important to note that the INAD is still open for non-labeled uses. Under the INAD either chloramine-T product from Western Chemical, Inc. or BL Mitchell, Inc. may be used. All preventative treatments need to remain under the INAD. However, CVM highly encourages non-labeled therapeutant treatments to be done under a veterinary prescription.

Text provided by Bonnie Johnson, National INAD Program; USFWS AADAP Program (bonnie_johnson@fws.gov)

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2nd Edition of the Quick Desk Reference Guide to: Approved Drugs for Use in Aquaculture. The U.S. Fish & Wildlife Service Aquatic Animal Drug Approval Partnership (AADAP) Program has released the 2nd Edition of the Quick Desk Reference Guide to: Approved Drugs for Use in Aquaculture. Originally published in

2011 with support from the Association of Fish & Wildlife Agencies and the American Fisheries Society Fish Culture and Fish Health Sections, the Desk Reference was provided free-of-charge to many AADAP partners and stakeholders. The Desk References proved wildly popular: 1,100 hard copies of the 1st Edition were made available, and all were spoken for in less than 2 days! The U.S. Food & Drug Administration has granted several new drug approvals and/or label expansions since publication of the 1st Edition, and the 2nd Edition reflects all of these important advances in fish health management. Thanks to generous contributions from external partners, AADAP was able to produce and has begun shipping 2,200 copies of the 2nd Edition to our partners across the country. If you have already requested one or more copies of the booklet but have not yet received them - be assured, they are on their way! If you have not made a request yet and are interested in receiving one, please contact Ms. Niccole Wandelear (niccole_wandelear@fws.gov) and provide her the following information:

1) Your first and last name,
2) Your organization,
3) Your current FedEx address (street address only; no PO boxes),
4) A phone number, and
5) The number of booklets you'd like!

FEATURE ARTICLES

Microbiomes, preventative medicine, and integrated fish health management

Katie Haman, DVM, MSc
Fish and Wildlife Health Specialist
Washington Department of Fish and Wildlife

Fish, like all animals, get sick and suffer infections. When this happens we have limited options for treatment with approved drugs, including antibiotics. Therefore, understanding what actually makes the fish sick in the first place and practicing preventative medicine and management is one of our primary goals.

Disease causing bacteria (commonly called pathogens) such as those that cause Bacterial Cold Water Disease (BCWD) are often opportunistic bugs. Such pathogens are frequently present at all times, but depend on an opportune environment, for example increased water temperature and fish densities, to become infectious and result in a diseased, sick, or even dead fish. It isn't simply an exposure to a pathogen that can make a population of fish sick, but the entire environment in which they live! Bacteria, viruses, and other microbes living together, also known as microbiomes, are a hot topic in the human medical field. Studies of the human mircrobiome have led to incredible advancements in personalized medicine and our understanding of "health." Recent research indicates that the microbiome living in our gut may be one of the preeminent factors determining our health and longevity. Surprisingly, certain bugs are actually good for us and may protect us from disease – one such species of bacteria (*Faecalibacterium prausnitzii*) actually protects us against intestinal inflammation and Crohn's disease.

It may be easiest to consider the microbiome as a "microbial ecosystem," which has been linked closely to human immune function. Some researchers now consider a vital role of our immune system is to cultivate, or farm, the "friendly" microbes that keep us healthy. Simply stated, our microbial communities are part of what make us healthy, and they serve a dynamic part in keeping us that way.

So, why would fish be any different?

They aren't! Fish, like humans, have microbiomes (or microbial communities) in their gut, on their skin, and in respiratory system (aka gills). Because fishes live in

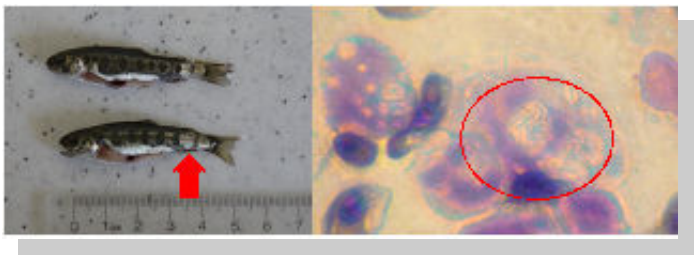


Image on left (courtesy of Steve Roberts, WDFW) of juvenile steelhead with classic BCWD lesions near peduncle of tail. Image (oil immersion) on right shows BCWD-like bacterial rods in the white blood cell (circle) of a juvenile steelhead.

water, the microbial communities in their environment are also important – a fact that has been under-recognized until recently. These microbiomes, much like the ones in humans, certainly play a role in fish health. Unfortunately, until recently, methods have been limited that enable us to investigate the microbiomes of fishes. But with advances in human medicine and investigations into human microbiomes, we in the fish health world are now well-positioned to employ the same methods to better understand the whole picture of fish health. This means studying not only the population of fish, but the environment in which they live, and how that environment interacts with and impacts their disease status and overall health.

With the help of Dr. Ken Warheit (Director, Molecular Genetics Laboratory at WDFW), we recently employed next generation DNA sequencing (NGS) to define and characterize the microbiome in a recirculating system at the Cowlitz Salmon Hatchery (owned by Tacoma Power and operated by WDFW) in southwest Washington State. Briefly, NGS is a high-throughput DNA sequencing technology where millions or even billions of DNA strands

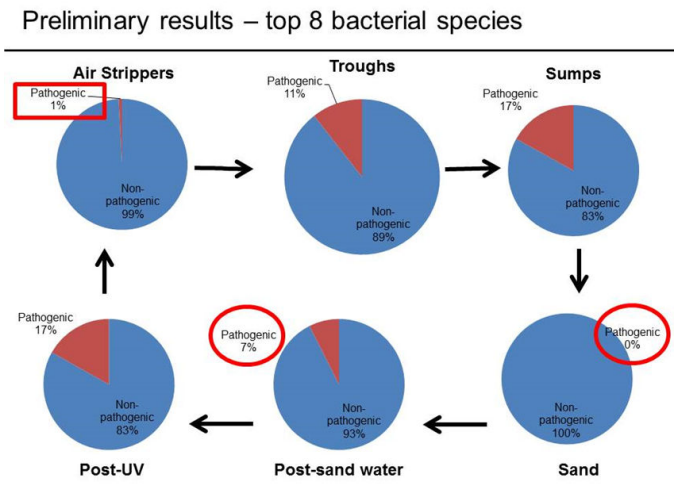
can be sequenced in parallel.

The fish (steelhead) in this system were suffering from recurring outbreaks of BCWD – the goal of this research was to characterize the microbiomes of multiple sectors through the recirculating system and identify areas where the pathogen was predominant.

We collected water from various access points throughout the system and filtered it to capture all microbes present in the sample. We also collected sand from the pressurized sand filter for analyses. DNA was extracted using standardized protocols targeting variable regions of the microbe genome that can be used to identify species of microbes present. The amplified DNA was then sequenced using NGS.

So, after all this lab work, what did we get? LOTS of data – we're talking about 15+ gigabytes of data. That's over 35 million DNA sequences! After running the data through a canned bioinformatics pipeline (that's fancy speak for comprehensive data analyses to identify the species of bacteria present based on their DNA sequences at the 16S rRNA hypervariable region), we were able to identify and characterize the microbiome through our recirculating system.

What does this all mean, you might ask? We can now identify the areas of our system that may remove, harbor or even shed pathogenic bacteria to the fish in the system. Based on these preliminary data, we should focus efforts on sterilizing the sand filters (based on the amount of pathogenic DNA present in the microbiome post-sand filter), the air strippers, and the sumps. We can also use these data as baselines for future comparisons – and gain a better understanding of the complete, integrated picture of fish and environmental health.



The molecular tools I've described here can be used to monitor determinants of fish health – what is the normal microbiome on the skin of fish? What about in their guts? On their gills? Or, merely in their aquatic habitat? How do these microbiomes change in the face of a disease outbreak? What is the fish's immune response? What areas of our systems harbor bacteria (especially the bad kind)? Further, can we create thresholds for bacterial communities (microbiomes) that can be used as a diagnostic tool to monitor and prevent outbreaks? Perhaps the most important and significant question: what can we, as culturists rearing and supporting these fish, do to prevent these changes and the subsequent disease outbreaks and maintain fish health?

Speaking of health, here's a favorite soapbox of mine: what is the definition of health? Health is NOT just the absence of disease. Health refers to the overall vulnerability, resilience, and interrelated conditions and factors that influence an individual (or population) over time. Determinants of health include, but are not limited to, requirements for daily living such as food, water, habitat, and population densities. These determinants of health directly influence an organism's ability to cope with stress and/or environmental and biological changes that impact physiology and behavior, living conditions, and their habitat. Thus, health is the overall, comprehensive picture of the individual, the population, and the environment (or ecosystem).

Cowlitz Trout Hatchery staff (left to right): Ryan Erickson, Chuck Glass, Clint Fitch, Jessie McMahan, and Caroline Watson. Not pictured: Ernest Bean, Melissa Hubbs, Thomas Kohl, and Grant Sill.

There are questions that we may never be able to answer – luckily, technology and the medical field is rapidly advancing. What has become abundantly clear is that bacteria aren't always bad. In fact, some are even good and help defend their hosts from bad bacteria. Fish health, diagnostics, and subsequent prevention and management can follow in the steps of human medicine – we have much to learn and now have the tools to move forward!

Acknowledgements:

Tacoma Power funded this research. Brian Missildine, supervisor of the Fish Health Unit with WDFW, provided logistical and professional support. Ken Warheit, Director of the WDFW Molecular Genetics Lab, provided molecular expertise in study design and analyses. Amelia Whitcomb provided laboratory support. The entire staff at the Cowlitz Trout and Salmon Hatcheries provided support and general

awesomeness.
For more information on the methods, please email the author (Katherine.haman@dfw.wa.gov)

Legal and Judicious Use of Therapeutants in Food Fish

Carolyn Gunn, DVM
Aquatic Veterinarian, Colorado Parks and Wildlife

When fish in an aquaculture setting have a disease outbreak that management practices can't resolve, it is often necessary to treat the fish with a therapeutant to decrease mortality and bring the group of fish back to health. But the use of drugs on fish that are destined as potential human food (either directly or indirectly) is a highly regulated activity. This is because the U.S. Food and Drug Administration (FDA), an agency within the Department of Health and Human Services, is responsible for keeping the human food chain free of contaminants that might harm humans. Drug residues resulting from treating fish come under this category of contaminants.

Other federal agencies involved in regulating use of chemicals/products that may affect fish include the U. S. Environmental Protection Agency (EPA) which has jurisdiction over chemicals such as disinfectants and aquatic treatments for control of algae or pests other than pathogens in or on fish. Additionally, the Animal and Plant Health Inspection Service Center for Veterinary Biologics, an agency within the U.S. Department of agriculture, regulates vaccines, bacterins, diagnostic kits, and other products of biological origin.

All states must comply with federal regulations, but some states may have their own requirements that go beyond or are in addition to what is required at the federal level. This is especially true for chemicals and drugs having their point of source at an aquaculture facility, limits of which are often set by the state's public health department (if the state has primacy over EPA relative to discharge).

Legal and judicious use has many ramifications and can be confusing and difficult to understand. This article will cover main points and definitions to help the aquaculture producer. To ensure proper use of drugs in an aquaculture setting, the owner/manager should seek the services of a fish health expert, pathologist, or licensed veterinarian when a disease outbreak occurs on a facility. For more in-depth information, the American Fisheries Society Fish Culture Section has compiled an excellent "Guide to Using Drugs, Biologics, and other Chemicals in Aquaculture" available at https://sites.google.com/site/fishculturesection/resources/guide-to-using-drugs-biologics-and-other-chemicals-in-aquaculture.

A few definitions will help explain some salient points:

Food fish are defined by the FDA's Center for Veterinary Medicine (CVM) as an aquaculture species in which it is reasonably likely that a significant percentage of the species population will be consumed directly or indirectly by humans for food. This definition includes fish raised for stocking into private or state waters where anglers may keep and consume their catch and fish sold to restaurants and markets.

A drug is defined by the Federal Food, Drug, and Cosmetic Act as any article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease, an article which affects the structure or function of the body, or articles recognized in official drug compendia. For the aquaculture setting, this includes antibiotics, fish sedatives, spawning aids, microbicides, and external or systemic parasite treatments.

Judicious use of therapeutants in animals is defined by the American Veterinary Medical Association as striving to optimize therapeutic efficacy and minimize resistance to antimicrobials to protect public and animal health and well-being once the decision has been made for treatment or control of disease. This definition refers to use of antibiotics, but should apply to all drugs used in aquaculture to prevent misuse or overuse.

Legal use refers to administration of therapeutants only in accordance with labeled instructions of FDA-approved drugs for use in aquaculture, or compliance with other regulations governing Investigational New Animal Drug (INAD) use or extra-label use (ELU). Such use is backed by enforcement priorities set forth by the FDA-CVM.

Extra-label use refers to use of an approved drug in a manner not in accordance with the approved label directions for certain situations and if specifically prescribed by a licensed veterinarian within the context of a valid veterinarian-client-patient relationship.

An Investigational New Animal Drug (INAD) compassionate exemption allows producers to use an unapproved drug under certain conditions for purposes related to the health and well-being of an animal. Such use must be within strict protocols and reporting requirements outlined by the FDA-CVM.

Drug withdrawal time refers a period of time after the end of treatment in which treated fish cannot be released/stocked to ensure that any drug residues in the body of the fish are eliminated prior to release. Withdrawal time is included on product labels, package inserts, and feed tags of approved drugs, or as determined by a veterinarian in the case of extra-label use of a drug.

Prohibited drugs refer to a group of therapeutants, the use of which is forbidden by the FDA in all or specific groups of animals. This prohibition decision is based on safety to the target animal, the environment, or humans who may come in contact with the drug either directly (during administration of the drug) or indirectly (via consumption of an animals with drug residues or exposure to effluent water containing the drug).

A Veterinary Feed Directive (VFD) drug is limited to use only via a written prescription under the supervision of a licensed veterinarian authorizing the food fish owner or manager to use feed containing a VFD drug to treat the animals in accordance with the FDA-approved directions

for use. No extra-label use of VFD drugs is permitted.

Where disease is concerned, *prevention* should be the primary objective, thereby alleviating the need for drug treatment. Utilization of Best Management Practices (BMP) such as biosecurity, disinfection practices, proper nutrition, maintenance of high water quality, decreased crowding and stress, mortality management, removal of organic debris, vaccination programs, and other management techniques help prevent disease outbreaks.

But even with employment of BMPs, disease outbreaks periodically occur. The next step in ensuring legal and judicious use of drugs in an aquaculture setting is *early and accurate diagnosis* of a fish health problem. Enlistment of a fish health expert, fish pathologist, or veterinarian to examine the fish and develop a definitive diagnosis is optimal. This prevents unnecessary and inappropriate use of drugs due to misdiagnosis, use of one or more drugs in hopes that they will be effective ("shotgunning"), repeated treatments, potential development of antibiotic resistance, and other issues.

Legal and judicious use of all therapeutants in aquaculture is important, but especially so for *medically important antibiotics* (those that are important for use in combating infectious disease in humans). Antibiotics are important to both human and animal health, and development of bacterial resistance could render many antibiotics ineffective in both human and animal health. Misuse and overuse of antibiotics increases the chance that bacteria will become resistant. There is scientific evidence that antibiotic resistance can be transferred from animals to humans and could pose significant human health risks.

The ability of current food animal drug regulations to prevent the spread of potential bacterial resistance has been recently debated, and revisions to current regulations have been proposed. With the goal of preventing overuse and misuse of antibiotics, in particular, the FDA-CVM has been working to provide guidance to the animal industry, including aquaculture. In December, 2013, the FDA began implementing a plan to ensure judicious use of antibiotics in all food animals. This plan will have a three-year transition process from its current status to the new regulations. The purpose of the plan is twofold:

- For animal pharmaceutical companies to voluntarily revise their FDA-approved use conditions on the labels of these products to remove production indications (to enhance growth or improve feed efficiency). This is not an issue with aquaculture drugs because no approved label makes this claim.
- The plan also calls for changing the current over-the-counter (OTC) status to bring the remaining appropriate therapeutic used to treat, control, or prevent disease in animals under veterinary oversight (i.e., VFD drug status).

By June 30, 2014, all 26 drug manufacturers affected by this Guidance for Industry agreed to fully engage in the proposed strategy.

The FDA also issued a proposed rule to update the existing regulations relating to Veterinary Feed Directive (VFD) drugs, which currently require specific authorization by a licensed veterinarian. The proposed rule intends to facilitate this expanded oversight by clarifying and increasing the flexibility of the administrative requirements for distribution and use of VFD drugs.

Current and proposed regulations for use of therapeutants in food fish are in place to protect human food sources, not to put undue burdens on food animal producers. It is to everyone's benefit to understand and comply with these regulations to ensure the safety of aquaculture staff, food fish, and the anglers/shoppers who may consume fish.

Updated Drug Guide Can Steer Fish Farmers Away from Trouble

Rod Getchell
NRSP-7
Cornell University, Aquatic Animal Health Program

In the last issue of *Fish Farming News*, I focused on the Fish Health Section Blue Book, which I described as the virtual bible of the fish health world. This time, I'm going to discuss an equally important publication from the Fish Culture Section of the American Fisheries Society -- the 2014 update of the "Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture."

Every fish farmer should be aware of this guide, which explains the proper use of drugs, biologics, and other chemicals and describes regulated products that are approved for use in US aquaculture.

The guide also outlines drugs not yet approved for use in the US that can be used under an Investigational New Animal Drug (INAD) exemption and additionally lists drugs that are considered to be of low regulatory priority enforcement.

The Fish Culture Section established a Working Group on Aquaculture Drugs, Chemicals, and Biologics to develop the guide by facilitating communication and cooperation between public and private aquaculture interests, academic

and agency researchers, and regulators. All of those involved are addressing the needs and issues associated with the approval and use of aquatic animal drugs, biologics, and other regulated products in aquaculture.

In previous columns, I've mentioned the limited number of disease treatment options available to fish farmers and stressed that improper use of products can get you into trouble with the regulatory authorities or, more importantly, create pathogens resistant to the very treatments you depend on.

The guide can help steer you down the right treatment path. Just remember that it's not meant to be a prescriptive tool or to replace advice provided by professional fish health biologists or licensed veterinarians.

Regulated Products?

Aquaculture operations need products such as: disinfectants as part of biosecurity protocols; herbicides and pesticides used in pond maintenance; spawning aids; vaccines used in disease prevention; marking agents used in resource management; and, despite the best efforts of fish culturists to avoid pathogen introductions, therapeutic drugs to occasionally control mortality, infestations, or infections.

The US Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership (AADAP) conducts real-life field investigations and consolidate data generated from over 130 entities comprised of state and federal agencies, Native American tribes, and private companies, all striving to get new aquatic animal drugs approved.

These scientists spend years ensuring that culturists have access to products that are safe and effective. So, fish farmers should apply them in a manner that is consistent with their intended use, best management practices, and relevant rules and regulations.

Sections

The drug section of the guide covers the various types of approved drugs and their uses and also describes some common application methods.

The disinfectant section describes the most common uses for disinfectants in aquaculture, as well as appropriate compounds and application rates for aquaculture facilities.

The pesticide section focuses on the most common pesticide applications in aquaculture, including herbicides, algicides, and toxicants to fish and invertebrates.

Finally, the biologics section goes over the vaccines that are currently available for use in aquaculture. It also provides recommendations for their usage.

Biologics differ from drugs in a few ways. They affect the fish's immune system while drugs affect the disease-causing agent. Biologics are applied as a preventative -- before infection -- while therapeutics are applied post-infection. Also, most biologics leave no chemical residues in animals.

Recommendations from the updated 2014 aquaculture drug guide

- This guide is intended for informational and educational use only.
- It is the responsibility of individuals administering regulated products to read and follow label instructions and be aware of any changes in relevant regulation prior to using these products.
- It is the responsibility of those using, prescribing, and/or recommending the use of regulated products to know which products can be legally used and with what restrictions under federal, state, and any other local regulations. Regulated product uses may vary by location, species, life stage, and culture conditions and methods.
- Remember, any use of an approved drug in a manner not specifically noted on the drug's label is illegal unless used where permitted under an Investigational New Animal Drug or under an extra-label prescription by a licensed veterinary.
- Remember that vaccination is just one component of a complete fish health program and cannot prevent all fish health problems. Seek professional advice regarding appropriate vaccine use before application.
- Certain active ingredients may be found in products labeled for aquatic and non-aquatic uses. Although the active ingredient may be the same, it is not legal to use a pesticide product in aquaculture unless it is labeled for such use.
- It is the responsibility of the user to understand the risks associated with using aquatic pesticides and herbicides and to know and comply with all relevant regulations governing their use in aquaculture. Use only pesticide and herbicide products that are labeled for use in aquaculture and follow all label instructions and safety precautions. And,
- Always read and understand the product literature before using any regulated product. When in doubt, seek professional advice.

Authority

Several federal and state agencies are involved in regulating drugs, biologics, and other chemicals used in aquaculture. Each federal agency has specific, congressionally mandated responsibilities to regulate the products under their jurisdictions. In the case of aquaculture, there is some overlap between these federal agencies, as well as with state and local bodies.

The US Food and Drug Administration (FDA) regulates the manufacture, distribution, and use of new animal drugs and animal feed to ensure their safety and efficacy.

With respect to aquaculture, the US Environmental Protection Agency (EPA) has jurisdiction over disinfectants, sanitizers, and aquatic treatments used solely for the control of algae, bacterial slime, or pest control, excluding

pathogens in or on fish. The EPA also administers the National Pollutant Discharge Elimination System, which prohibits the discharge of pollutants, including regulated products, into waters of the US.

The Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture regulates all veterinary biologics, including vaccines, bacterins, antisera (blood serums), diagnostic kits, and other products of biological origin. APHIS ensures that pure, safe, potent, and effective veterinary biologics are available for the diagnosis, prevention, and treatment of animal diseases.

State agencies also may regulate the use of drugs, biologics, and other chemicals in aquaculture. Some states impose additional requirements and restrictions beyond those in the federal regulations.

Latest drug-use advice the result of extensive teamwork

Many thanks go to those who contributed to the 2014 update of the Guide to Using Drugs, Biologics, and Other Chemicals in Aquaculture. They include:

- Jim Bowker, US Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program, co-chair, Working Group on Aquaculture Drugs, Chemicals, and Biologics;
- Jesse Trushenski, Southern Illinois University Carbondale, Center for Fisheries, Aquaculture, and Aquatic Sciences, co-chair, Working Group on Aquaculture Drugs, Chemicals, and Biologics;
- Maren Tuttle-Lau, US Geological Survey, Upper Midwest Environmental Sciences Center;
- Dave Straus, US Department of Agriculture, Agricultural Research Service, Stuttgart National Aquaculture Research Center;
- Mark Gaikowski, US Geological Survey, Upper Midwest Environmental Sciences Center, co-chair, Working Group on Aquaculture Drugs, Chemicals, and Biologics;
- Andrew Goodwin, University of Arkansas Pine Bluff, Aquaculture and Fisheries Center;
- Laura Sprague, US Fish and Wildlife Service, Idaho Fish Health Center; and
- Molly Bowman, US Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program.

Tables

The guide has several valuable tables. The first lists drugs currently approved or conditionally approved by the FDA for use in aquatic species. For more information about specific approved and conditionally approved drugs, there are individual drug links in Table 1.

The compounds described in Table 2 are considered to be of low regulatory priority when used for the indications listed. FDA has stated that it is unlikely to regulate the use of LRP drugs if the following five conditions are met:

- The substances are used for the listed indications;
- The substances are used at the prescribed levels;
- The substances are used according to good management practices;
- The product is of an appropriate grade for use in food animals; and
- There is not likely to be an adverse effect on the environment.

FDA permits the purchase, interstate shipment, and use of unapproved animal drugs for investigational purposes through INAD exemptions. More detailed information about these compounds and what they can be used for are found in the fact sheet links of Table 3.

Get Your Guide

The new 2014 update of the guide was developed as a comprehensive introduction to the use of regulated products in aquaculture and as a resource for fisheries professionals. You can download a copy of the guide by visiting this shortened website address: <http://tinyurl.com/kwypcjd>.

The guide is revised periodically to ensure that the information is accurate and current. Revisions include: new drug approvals and licensed vaccines; new claims for existing drug approvals; and information on INADs.

In addition, revisions may include comments or suggestions provided by users of the guide. Please send feedback to Jesse Trushenski at <saluski@siu.edu> or Jim Bowker at <jim_bowker@fws.gov>.

Don't let the number of pages in the guide scare you off. It includes valuable information that is not easily found elsewhere. I was impressed with the thorough discussion of what some would consider cumbersome issues. I encourage you to give the guide a try.

Thanks for reading Fish Health Notes.

Rod Getchell

Dr. Rod Getchell works in the Aquatic Animal Health Program at the Cornell University College of Veterinary Medicine.

USGS's UMESC CORNER

Eugenol

UMESC completed work to characterize the depletion of eugenol (the marker residue for AQUI-S®20E) from rainbow trout (*Onchorynchus mykiss*). The study was conducted to fulfill a portion of the drug depletion component of the human food safety requirements for AQUI-S®20E. Rainbow trout were exposed to AQUI-S®20E in water at a temperature of 9°C, a temperature that is representative of the lower range of temperatures where rainbow trout would be sedated. Eighty fish were exposed to a nominal AQUI-

S®20E concentration of 100 mg/L for 60 min. Groups of 16 fish were sampled after 60 min of exposure (the 0 h sample group), then at 15, 30, 90, and 150 min after transferring the fish to flowing freshwater. Skin-on fillets from each fish were analyzed for eugenol concentrations using a U.S. Food and Drug Administration Center for Veterinary Medicine (CVM) approved method for determining eugenol concentrations in fish fillet tissue, a method developed and validated at UMESC. Interpretation and summarization of the data are ongoing. Contact Jeff Meinertz, jmeinertz@usgs.gov, for more information.

UMESC conducted a series of studies to assess the utility of using AQUI-S®20E as a sedative to reduce the activity of yellow perch and tilapia during live transport. A portion of the research assessed exposure parameters (concentration and duration) that would safely sedate fish while maximizing fish loading density during transport. Both species were exposed to 0, 100, 200 and 300 mg AQUI-S®20E/L at 3 loading densities; yellow perch, 120, 240, and 360 g/L; tilapia, 240, 360, and 480 g/L. After exposure durations of up to 10 h at all concentrations and densities, there was > 95% survival with yellow perch and > 90% survival with tilapia. The final report and associated data for this study were submitted to CVM. Contact Aaron Cupp, acupp@usgs.gov, for more information.

SLICE®

SLICE® is currently approved for use to control sea lice on marine-reared fish in the Canada, Chile, the Faroe Islands, Finland, Iceland, Ireland, Norway, Portugal, Spain, and the United Kingdom. SLICE® has been shown to be effective reducing infestations of freshwater copepods on freshwater -reared fish. Therefore, there is interest in pursuing approval of SLICE® for freshwater uses. UMESC conducted a marker residue depletion study with SLICE® (marker residue, emamectin B1a). Rainbow trout (*Oncorhynchus mykiss*) were treated with SLICE® at 50 µg emamectin benzoate/kg body weight/d in a freshwater recirculating aquaculture system and a flow-through system with a water temperature of 15°C and in a flow-through system with a water temperature of 6°C. Fish were sacrificed at 0.25, 0.5, 1, 3, 7, and 14 d after administering the last dose of SLICE® medicated feed. The emamectin B1a concentration profiles in fillet tissue from fish treated in the 15°C recirculating and flow-through systems were nearly identical. Mean maximum fillet tissue concentrations were found at 12 h post treatment and were 64.5 ng/g in the recirculating system and 60.9 ng/g in the flow-through system. Mean concentrations in those systems 14 d post exposure were 14.2 and 9.8 ng/g, respectively. The emamectin B1a concentration profile in fillet tissue from fish treated in the 6°C flow-through system was notably different. Mean emamectin B1a concentrations increased through the post-treatment period with a mean maximum concentration at 7 d post-treatment (47.6 ng/g). The mean concentration decreased to 42.3 ng/g by 14 d post-treatment. All emamectin B1a concentrations from individual fish were below the proposed tolerance concentration of 140 ng/g. The final report and associated data for this study were submitted to CVM. Contact Jeff Meinertz, jmeinertz@usgs.gov, for more information.

Text provided by Jeff Meinertz, Research Physiologist; USGS UMESC; La Crosse, Wisconsin USA (jmeinertz@usgs.gov)

FDA's CVM Notes

Wondering who you can contact at FDA to answer your question related to a fish drug, feed, or feed additive? Here are some great points of contacts.

- For questions about whether something is a drug or food additive: CVMproductclassification@fda.hhs.gov
- For questions about the drug approval process and to help you through the process: Jennifer.king1@fda.hhs.gov
- For questions about getting a new animal drug indexed or questions about indexing: Dorothy.Bailey@fda.hhs.gov
- For questions about minor species incentive programs (designation and grants): Stuart.Jeffrey@fda.hhs.gov
- For questions about the USDA's National Research Support Project #7 (NRSP-7): Amy.Omer@fda.hhs.gov
- For questions about the import tolerance process: Vernon.Toelle@fda.hhs.gov
- For questions regarding compliance with regulations, including the Veterinary Feed Directive regulations and extra-label use regulations, or to report a compliance issue: CVMCompliance@fda.hhs.gov
- To report an adverse drug event go to CVM's Adverse Event Reporting page for instructions.
- For questions regarding Veterinary Laboratory Investigation and Response Network (Vet-LIRN): sarah.nemser@fda.hhs.gov
- For questions regarding feed additives: CVMCompliance@fda.hhs.gov
- General/other questions: AskCVM@fda.hhs.gov

Looking to keep up with CVM news? Go to CVM's News & Events page, which contains FDA press releases and links to pages with CVM Updates and public meeting and workshop information.

Text provided by Dr. Jennifer Matysczak, DVNM; FDA CVM Leader, Aquaculture Drugs Team,, Office of New Animal Drug

Evaluation,; Rockville, Maryland, USA (Jennifer.Matysczak@fda.hhs.gov)

New Funding Opportunity Announcement for Minor Use/Minor Species (MUMS) Grant Program

The Food and Drug Administration (FDA) has published a new Funding Opportunity Announcement (FOA) entitled "Minor Use Minor Species Development of Drugs; Research Project Grant (R01)." The grant program was established by the Minor Use and Minor Species Animal Health Act of 2004, and is administered by the Office of Minor Use and Minor Species Animal Drug Development (OMUMS) at the FDA Center for Veterinary Medicine.

The new FOA (#RFA-FD-15-004) contains open dates (earliest submission dates) and application due dates for a three year period, as shown below:

FY 2016- Pt 1 Open/due date	FY 2016- Pt 2 Open/due date	FY 2017- Pt 1 Open/due date
06/19/2015	11/20/2015	06/17/2016
08/14/2015	01/15/2016	08/12/2016

FY 2017- Pt 2 Open/due date	FY 2018- Pt 1 Open/due date	FY 2018- Pt 2 Open/due date
11/18/2016	06/16/2017	11/17/2017
01/13/2017	08/11/2017	01/12/2018

The FOA solicits research grant applications from institutions or organizations that propose to develop, or support the development of new animal drugs intended for minor use in major species, or for use in minor species (MUMS). Research partners working with such companies are eligible for grants. Eligibility requirements for application include:

- Only studies in support of new animal drugs that have been "designated" by OMUMS are eligible for grants, when a grant will either result in or substantially contribute to FDA approval or conditional approval of the designated drug for a designated intended use. The Designations List can be found at http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/MinorUseMinorSpecies/ucm125445.htm
- The grant funding must be used to defray the costs of qualified safety and effectiveness testing expenses associated with the development of the drug for the designated intended use; and
- Interested parties must have a study protocol that has been accepted by CVM's Office of New Animal Drug Evaluation (ONADE) prior to submitting the grant application.

Qualified studies include those intended to support target animal safety or effectiveness, environmental safety, or human food safety. For human food safety (HFS), a separate study to validate an analytical method prior to conduct of a HFS in-life study is eligible for funding, if a protocol for the stand-alone method validation study has been accepted by ONADE. Certain manufacturing studies as described in the FOA that are supportive of target animal safety or effectiveness are also eligible for funding, with an ONADE-accepted protocol.

Subject to the availability of funds, grants will be available for up to $100,000 per year for up to two years for routine studies; and up to $150,000 per year for up to two years for studies of unusual complexity, duration or size. A third year of funding is available only for long-term toxicology studies. An indirect cost rate of 10% of modified total direct costs will be allowed if the applicant organization does not have a negotiated Federal indirect cost rate agreement.

The new FOA is available at http://grants.nih.gov/grants/guide/rfa-files/RFA-FD-15-004.html and at www.grants.gov. Applications must be submitted electronically through www.grants.gov.

USDA ARS SNARC CORNER

Aquaculture America 2015

The Aquaculture Drug Research and Drug Approval Status special session, which is moderated by Jim Bowker and Dave Straus, was very successful with 8 presentations, a large audience, and plenty of discussion.

Copper Sulfate ($CuSO_4$)

Chemistry, Manufacturing and Control Technical Section

The Office of New Animal Drug Evaluation at FDA/CVM has asked the Sponsor to update the **CMC** Technical Section. We have obtained copies of the old submission, and the Sponsor is in the process of compiling this information.

Ichthyophthiriasis label:

All major Technical Sections for this label are Complete except for **Environmental Safety**. The **Environmental Safety** technical section for the indication "… to control mortality associated with ichthyophthiriasis on channel catfish cultured in earthen ponds" was submitted to FDA/CVM 12/19/2014, and we await their response. The **Labeling** and **All Other Information** Technical Sections will be submitted pending the status of the **Environmental Safety** submission.

Saprolegniasis label:

All major Technical Sections for this label are Complete except for **Environmental Safety** under a hatchery scenario. We are in discussions now about funding this Environmental Safety report and hope to get started later this year.

Peracetic Acid

Acute toxicity studies are in progress at SNARC on a variety of fish species and diseases. Dave Straus and Thomas Meinelt (Berlin, Germany) met with several companies at the recent Aquaculture America meeting and were notified that they have decided to market a peracetic acid product for use in aquaculture as a disinfectant. This was the goal of Dave and Thom when they started their collaboration 8 years ago.

Text provided by Dave Straus, Aquatic Toxicologist; USDA/ARS, Harry K. Dupree – Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas USA (Dave.Straus@ars.usda.gov)

UPDATES - OTHERS INVOLVED IN AQUACULTURE DRUG RESEARCH

Growth inhibition of *Aeromonas salmonicida* and *Yersinia ruckeri* by disinfectants containing peracetic acid

In intensive aquaculture, high density and handling can increase the susceptibility of fish to disease. Therefore, therapeutic agents, such as water treatments or antibiotics, must be used in order to keep these infections under control. However, many therapeutic agents have been banned because of harmful effects to the environment. Disinfectants are used for effective fish farm biosecurity and to inactivate potentially pathogenic micro-organisms in aquaculture. Peracetic acid (PAA) is a dissociation equilibrium between acetic acid/hydrogen peroxide and acetylhydroperoxide/water. Peracetic acid is routinely used in agriculture, food processing and hospitals as a disinfectant. It is also an accepted alternative to chlorine in industrial and urban effluents and as a disinfectant for ion exchangers, cooling towers, combined sewer overflows, and membrane hollow fibers.

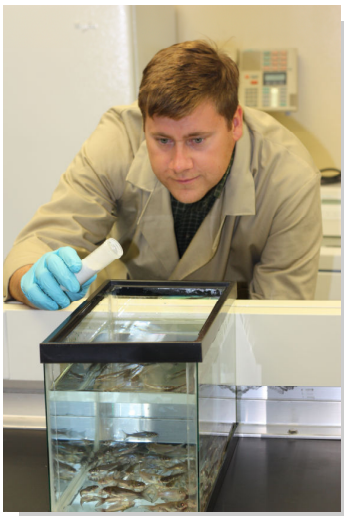
Dr. Meinelt conducing a bioassay with peracetic acid

Our research has shown that it has many applications in aquaculture and our publications have described the effectiveness of PAA to several fish pathogens. One of our latest studies compared the ability of six commercial PAA products having different molecular PAA:H_2O_2 ratios to reduce growth of two important disease-causing bacteria in trout aquaculture (*Aeromonas salmonicida* and *Yersinia ruckeri*) by determining effective concentrations and exposure times. All products reduced colony forming units (CFUs) of *A. salmonicida* and *Y. ruckeri*. In our study, products with a higher concentration of PAA (versus H_2O_2) inhibited growth better than products with lower PAA and higher H_2O_2 concentrations. PAA is being investigated to replace banned chemicals, especially in the EU where very few chemicals can be used. It is safe and effective to use on fish at a low dose and this compound does not leave dangerous residues in the environment when it breaks down as some compounds do.

Text provided by Dr. Thomas Meinelt (meinelt@igb-berlin.de), Sacha Behrens and Dibo Liu (Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany), Lars-Fleming Pedersen (Technical University of Denmark, Hirtshals, Denmark) and Dave Straus (USDA/ARS, SNARC).

Kaolin Clay Protects Fish from Columnaris Disease

Columnaris disease, caused by the bacterial pathogen *Flavobacterium columnare*, continues to be a major problem worldwide in cultured freshwater finfish. Despite the far-reaching negative impacts of columnaris disease, safe and efficacious preventatives and curatives for this disease remain limited.



In a recent study at the Stuttgart National Aquaculture Research Center in Stuttgart, Arkansas, a research team evaluated the potential of kaolin ($Al_2Si_2O_5(OH)_4$), a type of clay, for the prevention of columnaris disease. Channel catfish (*Ictalurus punctatus*) fingerlings were experimentally challenged with *F. columnare* in untreated water or in water containing kaolin (1 g/L). Over the 7 day course of study, kaolin treatment led to significantly improved survival (96%) as compared to untreated fish (78% survival). Histological examination of gill tissue revealed that kaolin-treated fish had substantially less gill damage than untreated controls. Analysis of gill tissue demonstrated that kaolin reduced *F. columnare* adhesion, the essential first step of the infectious process. Incubation of kaolin with *F. columnare in vitro* demonstrated that kaolin reduced the number of bacteria cells in culture supernatants in dose-dependent fashion, presumably through the formation of physical complexes. In summary, kaolin improved survival, reduced gill pathologies, and reduced bacterial attachment to key tissues associated with columnaris disease in channel catfish by binding to *F. columnare*.

Tail of treated (L) and un-treated (R) fish

Studies are currently underway to determine appropriate application regimens and settings for kaolin-based treatments, with plans to conduct on-farm trials for disease control. In parallel, investigators are examining the effectiveness and utility of kaolin-based approaches for the prevention/treatment of other pathogens of commercial importance.

Text provided by Benjamin H. Beck, Research Physiologist USDA/ARS, Harry K. Dupree – Stuttgart National Aquaculture Research Center (Benjamin.Beck@ars.usda.gov)

Sponsor Corner

Meet AquaTactics

AquaTactics Fish Health opened in November 2011 as a veterinary clinic and service company dedicated to working closely with customers to address their aquatic health and husbandry needs. AquaTactics meets proactive health needs by developing and providing customized vaccines via veterinary prescription under a veterinary-client-patient relationship. Founded by Hugh Mitchell DVM and Tom Goodrich PhD we brought together 27 and 38 years of industry-related experience, respectively. We have added an additional 85 years of experience in our field and laboratory staff.

AquaTactics then added pharmaceutical products to its medicine chest, starting with AQUI-S 20E distribution. We have since added Romet 30 and Romet TC, feed grade antibiotics, as well as distribution of Soccorex injection guns and Unimed needles. The expansion has recently grown to include Pharmgate's Pennox 343, soluble oxytetracycline for skeletal marking. We are continually on the lookout to add quality products or to utilize our veterinary and regulatory expertise to assist potential sponsors of drug products for the industry.

AquaTactics has joined the Aqua Pharma group of companies, through a 50-50 joint venture with Aqua Pharma Ltd. to form Aqua Pharma USA Inc. Their product line is based on hydrogen peroxide and they are currently pursuing a sea lice treatment under the US FDA INAD process.

AQUI-S New Zealand, LTD

AQUI-S 20E Update—The goal line gets closer with each passing day. While it will not be tomorrow or even this year, AQUI-S New Zealand remains fully vested in the process and is pushing toward approval. Most of the research has been completed having technical section completed for Efficacy and Target Animal Safety. Environmental Safety studies are nearing completion with hopefully only a few minor issues to address. Completion of the Marker Residue lab study is down to writing the final report and its submission. The final piece of the puzzle will be the Chemistry, Manufacturing and Controls section. AQNZ is working to finalize analytical methods to meet GMP requirements. Once the final methods are developed and validated much of the remaining work will involve submissions and addressing any missing pieces. The goal remains for a late 2016 approval although a 2017 date may be more realistic. Thank you all for your continued support of the process and INAD usage as we press on.

Through it all our goal is to support the aquaculture industry with quality service and proven products using our veterinary, technical and regulatory skills.

Text provided by Tom Goodrich, Co-founder, AquaTactics and U.S. Representative for AQUI-S New Zealand, LTD (tomg@aquatactics.com)

Fish Vet Group

Since its founding in Inverness, Scotland, in 1995, Fish Vet Group has grown to be the world's largest dedicated aquaculture health provider. The company now has five facilities around the world providing Total Aquaculture Health to the fastest-growing food-producing sector.

Fish Vet Group's North American operations, established in 2011, are situated on the waterfront at Portland, Maine.

The diversity of cultured species in the US led Fish Vet Group to spend the last four years developing, validating and commercializing an expansive portfolio of advanced diagnostic tools that they are now in a position to offer. FVG US's program began with the implementation of the same industry-leading histopathology service that Fish Vet Group Inverness was built upon, in addition the labs also offer bacteriology, virology and molecular biology tools such as PCR and qPCR.

Improving and promoting the health and welfare of aquaculture species is core to the group's work. Taking a comprehensive and science-based approach, Fish Vet Group employs teams of veterinarians, research scientists, biologists and diagnosticians.

Fish Vet Group's approach aims to reduce disease challenge, build resilience and establish immunity by developing appropriate products and to provide actionable advice and training to ensure best management practices and correct use. Existing products include:

Salmosan®; a sealice treatment for farmed salmon. Sealice is frequently cited as the number one

health issue in the global salmon industry, costing over $700 million per year. Salmosan® is used on over 80% of the world's salmon farms - sustaining the global salmon industry and aiding over 500 million salmon per year. Salmosan® is currently in the US registration process for treatment of sealice infestations in farmed Atlantic salmon.

- Virasure® Aquatic; an aquaculture specific disinfectant for use on fish farms, freshwater hatcheries, wellboats, and other aquatic applications. Virasure® Aquatic is currently in the US EPA registration process.

- PondDtox®, released in 2015 in collaboration with Novozymes Biologics, marks Fish Vet Group's expansion into the US pond aquaculture industry. PondDtox® mitigates toxic hydrogen sulfide production in the benthic layer in production ponds. After two seasons of field trials on commercial farms, farmers are realizing marked improvements in pond productivity for a range of warmwater species.

Fish Vet Group is part of Benchmark Holdings PLC, an AIM listed company on the London Stock Exchange. The company is growing in response to a rapidly increasing demand for sustainable food supply chains, and in particular for aquaculture from both mature and emerging markets.

Benchmark is an ethical company with an explicit policy based on the "3E's" definition of a sustainable business - ethics, environment and economics - that guides its strategy and operations.

The Group operates across four divisions: Animal Health, Sustainability Science, Technical Publishing, and Animal Breeding and Genetics. Benchmark operates internationally with offices 11 countries and a footprint on four continents.

Text provided by Jason Collins, Sales and Technical Manager, (jason.collins@fishvetgroup.com)