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"

Volume 5-2

AADAP NEWSLETTER

July 2009

15th Annual USFWS Aquaculture Drug Approval Coordination Workshop attendees gathered for a picture during a tour of Keo Fish Farms Inc., Keo, Arkansas USA.

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15th Annual US Fish & Wildlife Service's Aquaculture Drug Approval Coordination Workshop was a great success and is now history: This year's Workshop was graciously hosted by the US Department of Agriculture - Agriculture Research Service's Stuttgart National Aquaculture Research Center, and was held on 9-11 June in Little Rock, Arkansas USA. The Workshop was well attended, with its 74 participants traveling from 21 states, as well as Canada and Germany. In particular, US southeastern states were well represented, which is often not the case when the Workshop is held in its home city of Bozeman, Montana USA.

The three-day Workshop followed a format similar to previous Workshops, primarily focusing on 1) drugs identified by the Association of Fish and Wildlife Agencies (AFWA) Drug Approval Working Group (DAWG) as high priority to state, federal and tribal natural resource agencies and 2) other drugs for which

work aimed at fulfilling US Food and Drug Administration (FDA) approval requirements is ongoing. The session for each drug comprised technical reports pertaining to approval-related studies, as well as status reports on the overall approval process.

Our USDA host, the folks at the Stuttgart National Aquaculture Research Center did a **fantastic** job of making the Workshop not only informative, but enjoyable, especially during the after-session hours. We would like to offer a special thanks to Dave Straus and Cindy Ledbetter for their efforts.

Those not familiar with "southern hospitality" were introduced to it in fine style. Festivities began the night before the Workshop, *per se*, with a mixer/social at a local brew-pub. During the first evening of the Workshop, attendees were treated to a catfish fry held at the Arkansas Game & Fish Commission's Witt Stephens Jr. Nature Center along the Arkansas River in downtown Little Rock. After the last day's session, attendees took a tour of four local aquaculture facilities followed by an amazing crawdad (aka crawfish, crayfish, mud bug or crab, depending on which region of the USA these critters are found) boil. Special thanks goes out to Pool Fisheries, Anderson Minnow Farm, Hopper-Stevens Hatchery and Keo Fish Farms for providing the tours and allowing us the opportunity to see what it really takes to raise fish destined for domestic sales.

The only "complaints" heard during the Workshop pertained to heat of one kind or the other, i.e., *"...it sure is hot and humid here…"* and "…*wow, these crawdads sure are spicy hot…"* And in some cases both types of heat caused the perspiration to flow in excess!

Although the information-transfer portion of the Workshop could have been made available in a relatively austere setting, the generous contributions from a host of sponsors made that transfer of information so much more enjoyable and effective. Sponsors for this year's Workshop included (in alphabetical order): Arkansas Agriculture Department - Aquaculture Division, Arkansas Bait and Ornamental Fish Grower's Association, Arkansas Catfish Promotion Board, Aquatic Life Sciences, B. L. Mitchell Inc., Bimeda, Freeport-McMoRan Copper & Gold Inc., Frontier Scientific Inc., Intervet/Schering-Plough Animal Health Corp., and the USDA/ARS Professional Activities Grants Committee. Many thanks goes out to all of them; it wouldn't have been the success it was without their help.

In an effort to share as much of the information offered during the Workshop as possible, the majority of the presentations are now on AADAP's Website and can be viewed by clicking here.

Big changes at FDA's Center for Veterinary Medicine: In the past year there have been some significant personnel changes at CVM.

Dr. Don Prater, who for the past 8 years has been the Leader of the Aquaculture Drugs Team, was recently promoted to Director of the Division of Scientific Support. Although Don's new responsibilities will effectively remove him from our day-to-day aquaculture drugs "do-loop", his new role includes supervision of several teams that interact directly with aquaculture drug researchers and sponsors (e.g., biostatisticians and environmental safety reviewers), and hence, Don will likely remain part of the "team."

Dr. Cindy Burnsteel was promoted from her position as a team leader within the Division of Therapeutics Drugs for Food Animals to the Director of the Division. Among Cindy's new responsibilities is supervision of the Aquaculture Drugs Team.

And last, but certainly not least of the major personnel changes at CVM was the appointment of Dr. Jennifer Matysczak as the new Leader of the Aquaculture Drugs Team. As many of you are aware Jen has been a member of the Aquaculture Drugs Team for several years.

We at AADAP would like to congratulate Don, Cindy, and Jen for their respective promotions and extend our best wishes for success in their new positions. More specifically, we would like to take this opportunity to 1) express our sincere appreciation to Don with respect to his past efforts in assisting us to obtain FDA-approval of safe and efficacious new drugs for use in aquatic species; and 2) officially WELCOME Cindy and Jen to our aquaculture drug "do-loop." Charge!

We are already making plans for the 16th Annual (2010) USFWS Aquaculture Drug Approval Coordination Workshop to be held in Bozeman, Montana, USA: Although it may be a long way off, but for those of you who like to plan ahead, the Workshop will be returning home to Bozeman, Montana USA in 2010. As has always been the

case when it takes place in Bozeman, the USFWS Aquaculture Drug Approval Coordination Workshop will be scheduled for the last week in July or the first week in August, or more accurately, the week immediately before the Sweet Pea Festival weekend. So mark it on your 2010 calendar (if you have one) and just don't forget to check the AADAP website for news of it and other upcoming events.

Update on the search for a new candidate "zero-withdrawal" (henceforth referred to as "immediate-release") anesthetic/sedative: Since the last update in the previous edition of the AADAP Newsletter (March 2009), progress has been made, much of which was reported on during the 15th Annual US FWS Aquaculture Drug Approval Coordination Workshop (see Workshop presentations by clicking here.)

Given that certain critical data have yet to be generated and/or reviewed by CVM, the AFWA-DAWG has yet to select one of the two candidate drugs (benzocaine or eugenol) upon which to focus its attention.

Recent progress includes:

1. Actual in-life studies, coordinated by the USGS's Upper Midwest Environmental Sciences Center (UMESC), to establish a postsedation catchability timeframe for each of the two candidate drugs has begun. Such criteria are essential to determine if either drug will potentially meet requirements for an "immediate release" claim for food fish. Click here to view UMESC Workshop presentation outlining their progress.
2. UMESC has begun work to complete residue chemistry studies needed to address unresolved issues related to the total residue depletion of eugenol. They are currently preparing the method development protocol for submission to FDA and anticipate that bench work will begin in August 2009. Click here to view UMESC Workshop presentation outlining progress.
3. The Association of Fish and Wildlife Agencies, in cooperation with the USFWS's Aquatic Animal Drug Approval Partnership (AADAP) Program, is near completing initiation of the genotoxicity battery of studies for benzocaine with a private contract laboratory. A final report should be ready for submission to CVM approximately 4 months following signing of the contract.
4. The previously reported concerted efforts by Federal (US National Oceanographic and Atmospheric Administration and the US Army Corps of Engineers), State (Washington, Oregon, Idaho) and tribal agencies/organizations (Nez Perce and the Columbia River Inter-Tribal Fish Commission) to assist the AFWA-DAWG in its efforts to obtain an "immediate release" sedative/anesthetic continues. The group has established a task force which in turn has drafted a "support letter" now circulating for signatures from the respective agency/organization leaders. Managers of the Pacific Northwest salmonids stocks, as well as many other natural resource entities within the USA, are in dire need of an "immediate release" sedative/anesthetic to successfully complete their respective salmonid-related missions. Also refer to NOAA Fisheries Update for more details
5. The New Zealand pharmaceutical firm AQUI-S New Zealand announced recently, regarding their newly formulated product AQUI-S E®, that they had been informed by CVM in December 2008 that their product had gained eight separate Minor Use Minor Species (MUMS) drug designations for its use as an anesthetic/sedative. The drug designations include sedation to the handleable stage for: (1) all fresh coolwater fish, (2) all freshwater salmonids, (3) all warmwater fish except channel catfish, and (4) all marine salmonids. The other four designations are for light sedation for transportation of the same four aforementioned categories of fish.
6. The USA chemical/pharmaceutical firm Frontier Scientific, Inc. recently received word (May 2009) from CVM that they had received MUMS drug designations for benzocaine, one each for use in handling, surgery and transport of all freshwater finfish.
7. The Norwegian company, ACD Pharmaceuticals AS, also announced their receipt (June 2009) of MUMS designations for their benzocaine product BENZOAK®. The two designations they received are for: (1) sedation to a handleable stage for all saltwater-reared salmonids and (2) light sedation for transport of all saltwater-reared salmonids.
8. AADAP recently completed two pilot studies, one each for BENZOAK® (benzocaine) and AQUI-S E® (eugenol), testing the efficacy of each in sedating (to a handleable stage) rainbow trout. Both products produced promising results. Details of the studies can be viewed by clicking here.

Four new AADAP "Drug Research Information Bulletins" (DRIBs) published and now available: The most recent DRIBs include:

1. "*Efficacy of AQUAFLOR® (50% Florfenicol) to Control Mortality of Freshwater-Reared Coho Salmon Diagnosed with Furunculosis*,"
2. "*In the U.S. Aquaculture Drug-Approval World,* Oncorhynchus mykiss *by Any Other Name is Still* Oncorhynchus mykiss,"

3. "*Efficacy of AQUAFLOR® (50% Florfenicol) to Control Mortality in Freshwater-Reared Sunshine Bass (female* Morone chrysops × *male* M. saxatilis*) Naturally Infected with* Streptococcus iniae" and

4. "*Efficacy of Chloramine-T to Control Mortality in Bluegill* Lepomis macrochirus *Naturally Infected with External Columnaris Disease*."

These four DRIBs, and all previously published DRIBs, can be found on the AADAP Website under Research Program/Publications or by clicking here.

These short-format documents (2 page maximum) are intended to provide readers with information about AADAP research activities in a concise format, which as such, may not normally be suitable for peer-review publication.

National Aquaculture Drug Research Forum meeting held at Aquaculture America 2009: The 9th meeting of the National Aquaculture Drug Research Forum (NADRF) was convened on Friday, 12 June 2009, in conjunction with the 15th Annual USFWS Aquaculture Drug Approval Coordination Workshop held in Little Rock, Arkansas USA. The meeting was attended by an assortment of researchers, drug sponsors, the National NADA Coordinator, representatives from CVM's Aquaculture Team, and the Chairman of the Association of Fish and Wildlife Agencies' Drug Approval Working Group.

The agenda was focused on issues/challenges relative to conducting field trials to evaluate the effectiveness of chemotherapeutants to control ectoparasite infestations in finfish. Several topics were discussed, some at length, and included: (1) similarities and differences between regulatory and academic science; (2) terminology commonly used to describe ectoparasite infestations; (3) potential new label claim language, i.e., "...reduction of parasite numbers…" vs. the old claim of "...control of mortality due to…; (4) what standards will be used to define treatment efficacy; (5) experimental configuration, i.e., number of replicates or number of fish per tank, as a function of the statistical power of the study; (6) the need for identification of parasites to species, or is genus enough; (7) how many studies will have to be conducted for an individual label claim; (8) the process of parasite enumeration and such terms as TNTC (too numerous to count); (9) parasite distribution on individual fish, and standardization of methods and sampling schemes; and (10) use of pre-vet or veterinary interns to help conduct required studies.

For more information on the NADRF meeting, please see the meeting minutes (coming soon) on the AADAP website.

2nd meeting of the American Fisheries Society's Working Group on Aquaculture Drugs, Chemicals and Biologics: The Working Group on Aquaculture Drugs, Chemicals and Biologics (WG) met following the Drug Approval Coordination Workshop in Little Rock, Arkansas USA, on 12 June 2009. Attendees briefly discussed the WG's revised mission and objectives then focused on how to revise the *Guide to Drug Vaccine and Pesticide Use in Aquaculture* and on methods to facilitate information exchange between public/private aquaculture, drug sponsors and the various entities conducting research on aquaculture drugs. Minutes to the WG meeting will be distributed soon. The next WG meeting is scheduled to be held in San Diego, California USA on 28 February 2010 at 3 p.m. in conjunction with the Aquaculture 2010.

MUMS research grants made available: In late May 2009, an announcement was made by CVM's Office of Minor Use Minor Species (OMUMS) that the long-awaited MUMS federal research grants were being made available for fiscal year 2009 (FY2009). As part of the Minor Use and Minor Species Animal Health Act of 2004, the US Congress included a provision for FDA to competitively offer grants to help sponsors of new animal drugs qualifying as "designated MUMS drugs" to conduct required safety or effectiveness studies. Grants available for FY2009 total approximately $750,000. Unfortunately the short timeframe (applications were due 1 July 2009) and prerequisites for applying for this year's grants may have limited the eligible prospective grantees. However, potential applicants for the MUMS grants should be much better positioned to apply and compete for MUMS grants scheduled to be available in FY2010.

DRUG UPDATES:

Florfenicol (AQUAFLOR®) update:

Studies required to demonstrate the safety of AQUAFLOR® in all cool- and warmwater finfish: Based on discussions with CVM's Aquaculture Team, Dr. Richard Endris (Intervet/Schering Plough Animal Health; ISPAH – AQUAFLOR® sponsor), and AADAP, it was determined that testing of the following fish species would provide sufficient data to demonstrate the safety of AQUAFLOR® administered in feed at a dose of 15 mg florfenicol/kg fish body weight/d for 10 d in all cool- and warmwater finfish: sunshine bass (female *Morone chrysops* x male *M. saxatilis*), tilapia *Oreochromis* spp. (specific species or hybrid), and yellow perch *Perca flavescens*. ISPAH has coordinated with several of the public data generating partners to conduct of these studies. Each study will be designed to evaluate the safety of AQUAFLOR® administered in feed at concentrations of 15, 45, and 75 mg florfenicol/kg fish body weight/d (1x, 3x, and 5x the intended dose) for 20 days (2x the intended duration). Below is a description of two of the studies to be conducted wholly or in part by AADAP.

Target Animal Safety – sunshine bass: On 19 March 2009, the AADAP crew launched a collaborative AQUAFLOR® target animal safety (TAS) study on sunshine bass with staff at the USDA – Agricultural Research Service's Stuttgart National Aquaculture Research Center (SNARC) in Stuttgart, Arkansas USA. With the assistance of Dr. Dave Straus (Alternate Study Director) and Ms. Cindy Ledbetter (On-Site Investigator), the entire in -life phase of the study was conducted at SNARC facilities. Mortality, general fish behavior, and feeding behavior were monitored daily. No mortality was observed in any tank at any dose, general behavior was characterized as normal throughout the study period, and fish consumed all feed offered (by hand) within 10 seconds of feed delivery. AADAP's Jim Bowker (Study Director) traveled to Stuttgart AR to observe the SNARC crew collect and record data on the last day of the study, to assist SNARC's Mr. Drew Mitchell and Mr. Bradley Farmer perform fish health evaluations conducted 1-d post-treatment, and to assist in the collection and preparation of fish samples for histological evaluation. All study data recorded at SNARC (including study deviations and copies of instrument logs) and histology tissue samples stored in alcohol were sent to AADAP, where data are currently being summarized, tabulated and prepared for analysis, and select fish tissues are being processed for histological evaluation by AADAP's Ms. Molly Bowman and Ms. Miranda Dotson. Once tissues are mounted on microscope slides and stained, they will be evaluated by Ms. Beth MacConnell (histopathologist; USFWS - retired). AADAP staff has started to draft the final study report (FSR) and assemble all the associated documentation, including general information relative to inspections by Mr. Kurt Borge (Quality Assurance Officer). If the FSR is accepted by CVM, this study will contribute to the completion of the TAS technical section requirements for AQUAFLOR® use at 15 mg florfenicol/kg body weight per day for 10 days in all warmwater fish species.

Target Animal Safety – yellow perch: It's not often that AADAP staff might be overheard commenting that it's easier to conduct the study than it is to get our hands on the fish. But, that was how things started when we volunteered to conduct an AQUAFLOR® TAS study on yellow perch on-site in Bozeman, Montana USA. The first thing we had to do was to acquire a suitable reference population of test fish. To accomplish this task, we worked with the Montana Department of Fish, Wildlife, and Parks and the USFWS Bozeman Fish Health Center to (1) identify a source of wild fish to spawn, (2) fill out all the required fish collection and import paperwork, (3) coordinate capture and fish health evaluations on live wildstock perch, (4) and coordinate with staff at the Bozeman Fish Technology Center (BFTC; Bozeman, Montana USA) to help incubate eggs and rear resultant fry in BFTC quarantine/isolation facilities. In early April 2009, the AADAP crew headed out to a near-by lake to collect yellow perch egg ribbons and trap live adults for fish health evaluation. Egg ribbons were transferred to the BFTC where they were incubated and cared for by BFTC fish culturists. Resultant fry were feed-trained on a diet of Otohime® (Reed Mariculture) + freeze-dried rotifers and are currently growing-like-weeds. It's anticipated that the fish should reach an appropriate size (~2 inches) to begin the TAS study late July or early August. The experimental design of this study will be identical to that of the sunshine bass study noted above. As per Good Laboratory Practice (GLP) requirements, the AADAP crew completed their annual GLP training in early June, updated all required documentation, and are "clearing their plates" to be able to start the TAS study. If results from the study are accepted by CVM, this study will complete the TAS technical section requirements for AQUAFLOR® use at 15 mg florfenicol/kg body weight per day for 10 days in all coolwater fish species.

Efficacy Study on Rainbow Trout – Bellingham State Fish Hatchery: Somewhat unbelievably, it has been seven years since we were successful in completing and obtaining CVM acceptance of a study that demonstrated the effectiveness of AQUAFLOR® to control mortality caused by systemic columnaris in a salmonid species (i.e., coho salmon). Every year since, we've worked with our partners to try to successfully complete a second effectiveness study on a salmonid species other than coho (***note**:* typically it requires two successful studies, each study conducted on a different species, to complete the effectiveness technical section for an all freshwater-reared salmonids label claim). This past year we thought we had it, and that acceptance of the long-awaited pivotal field efficacy study to demonstrate the effectiveness of AQUAFLOR® to control mortality caused by systemic columnaris in a salmonid species (other than coho) was just around the corner. The study was conducted at Bellingham State Fish Hatchery (Bellingham, Washington USA) in July 2008 on rainbow trout. Well, guess what….it's still just around the corner. Although results from the study certainly indicated (at least to us) that treatment effectively controlled mortality in rainbow trout caused by systemic columnaris, we were informed by CVM's Aquaculture Team in late May 2009 that the study was not accepted as pivotal (it was accepted as supportive), and that another study would be required to complete the

effectiveness technical section for this claim for all freshwater-reared salmonids. This "downgrade" from pivotal to supportive was due, in part, to what CVM considered to be: (1) a less than adequate identification of the pathogen causing morbidity and mortality, (2) a "marginally significant" difference between mean cumulative mortality in treated and control tanks (P-value of 0.0551) and (3) a lack of adequate fish health examinations during the posttreatment period when an increase in daily mortality was observed in some of the test tanks. At this point, the disappointment and frustration has subsided to near base-line level, and we're left with the thought of….."bummer, dude."

On a brighter note, upon receiving the "good but not good enough" letter from CVM, Ms. Molly Bowman started working the trap lines with our friends out in the Bellingham area [Mr. Kevin Clark at the Bellingham SFH and Mr. Earle Steele from the nearby Bellingham Technical College (BTC)], and it looks like these two entities have developed a partnership that will enhance opportunities for future drug approval-related research at Bellingham SFH! There will soon be the opportunity to conduct additional field efficacy trials, including access to more test tanks and increased availability of energetic, enthusiastic undergraduate students at BTC to help with the day-to-day activities associated with the trials. Both Kevin and Earl are "on-board" and excited about conducting future work. We'll keep you posted, and rest assured that a "repeat" of the above-described study is at the top of the priority list.

Efficacy Study on Largemouth Bass – Richloam Fish Hatchery (RFH): Sometimes we'd rather be lucky than good! When it was discovered that a population of largemouth bass being reared at the Richloam Fish Hatchery were infected with columnaris, they were treated with chloramine-T. What was surprising was that the treatment had little effect on controlling mortality – apparently the infection had become systemic, which was verified when Michael Matthews and Kathy Childress cultured *Flavobacterium columnare* from kidney tissue sampled from select moribund fish. In situations such as this (i.e., where an opportunity to conduct a pivotal field efficacy trial is suddenly upon us), there is generally quite a bit of "scrambling" that occurs before all-the-pieces fall into place. As they say, "practice makes perfect", and over the years we've become pretty adept at operating within scramble-mode parameters! In short, a study was launched within a few days to evaluate the effectiveness of AQUAFLOR® to control mortality caused by systemic columnaris in largemouth bass. The study was conducted during May/June 2009. At present, preliminary results look too good to be true! There was a significant difference between treated and control tanks with a P-value of <0.001. Currently we are waiting for results of PCR confirmation of systemic columnaris in the bass and dose verification of medicated and control feed samples. We anticipate the study being submitted to CVM by the end of July 2009. If this study is accepted by CVM, we anticipate that it will be used to assist Intervet/Schering Plough Animal Health move forward with getting a new claim for AQUAFLOR® - to control mortality in all freshwater warmwater finfish due to systemic columnaris. As always….stayed tuned.

Chloramine–T (HALAMID® AQUA) update:

Efficacy Study on Largemouth Bass – Richloam Fish Hatchery: Previously, we reported on the results from a study evaluating the efficacy of chloramine-T (CLT) to control mortality caused by external columnaris on largemouth bass. In this study, which was conducted at the Richloam Fish Hatchery (Webster, Florida USA), fish in treated tanks received a 20 mg/L CLT treatment for 60 min once/d on three alternate days. At the end of the 14-d posttreatment period, mean percent cumulative mortality in treated tanks (45.5%) was significantly lower (P = 0.0032) than that in control tanks (62.6%). The final study report was submitted to CVM in October 2008, and in April 2009 we received word that CVM had accepted the study as pivotal! We're awaiting a review of some supplemental data that was also submitted to CVM in April 2009, as well as a request to consider the effectiveness technical section as complete for the following: "...to control mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*." We should hear back from CVM in October 2009, so stay tuned!

Choramine-T White Paper: If chloramine-T (CLT) is approved in the next 3 - 4 months, the label will likely read something like the following: "…use CLT at a concentration of 12 - 20 mg/L to control mortality caused by bacterial gill disease in all freshwater-reared salmonids; and at a concentration of 20 mg/L to control mortality caused by external columnaris in walleye and all freshwater-reared warmwater finfish. Administer for 60 min daily on three alternate or consecutive days." Such an approval would be considered relatively broad…but perhaps not broad enough. For quite some time the public data generating partners have kicked-around the concept that sufficient data and information had previously been submitted to CVM to also support an approval for this claim in all freshwater-reared coolwater finfish (i.e., not just walleye). Due to our collective concern that CLT may be approved before we get an opportunity to conduct one last effectiveness trial on a coolwater fish species other that walleye (as you can imagine, we try every year to launch a

study….but as yet to no avail), we thought we'd better take a drastic step. That drastic step was to submit a White Paper to CVM in April 2009 requesting that CVM consider the chloramine-T (CLT) effectiveness technical section complete for the use of chloramine-T when administered at a dose of 20 mg/L for 60 minutes per day on three alternate days to control mortality in all freshwater-reared coolwater and warmwater finfish due to external columnaris associated with *Flavobacterium columnare*. Briefly, the white paper (1) characterizes CLT as an antiseptic and disinfectant with microbicidal properties, (2) describes the use of CLT for purposes other than in aquaculture, (3) describes CLT's general mode of action, and (4) argues that CLT efficacy is a function of pathogen susceptibility and is independent of fish species. Basically, we argued that (1) CLT acts as an antiseptic to kill bacteria on living surfaces, (2) the fish skin simply acts as a surface to be disinfected, and (3) when used at the efficacious concentration, CLT does not harm the fish surface. We also referenced data from pivotal and supportive studies submitted to CVM to support this claim, a considerable quantity of INAD data submitted to CVM by the USFWS and the Pennsylvania Boat and Fish Commission, and other information (e.g., manuscripts, reports) previously submitted to CVM. Lastly we restated our position that no additional fish species need be tested to support this claim, and that the effectiveness technical section should be considered complete for use of CLT to control mortality in all freshwater-reared cool- and warmwater finfish to control mortality caused by external columnaris. Although we think we've presented a compelling argument to CVM, we have definitely broken new ground with this effort. CVM has a 6-month turn-around time for submissions such as this, so we don't expect to hear back from CVM until the beginning of October. Please keep your fingers crossed!

Hydrogen Peroxide (35% PEROX-AID®) Update:

Efficacy Study on Largemouth Bass – Richloam Fish Hatchery: Previously, we reported on the results from a study evaluating the efficacy of 35% PEROX-AID® to control mortality caused by external columnaris in largemouth bass. In this study, which was conducted at the Richloam Fish Hatchery (Webster, Florida, USA), fish were treated with 100 mg/L hydrogen peroxide for 60 min per day on three alternate days. At the end of the 14-d posttreatment period, mean percent cumulative mortality in treated tanks (49.0%) was significantly lower (P = 0.0075) than that in control tanks (74.5%). The final study report was originally submitted to CVM in November 2008, and an amended FSR was submitted to CVM in February 2009. In May 2009, we received word that CVM had accepted this study as pivotal! Because it takes two to tango (meaning we need to demonstrate the effectiveness of this treatment regimen to control mortality caused by external columnaris in two representative warmwater finfish species), we're awaiting word from CVM on a FSR that summarizes results from a similar study that was conducted on bluegill (see below). The FSR on the bluegill study, and a request that the effectiveness technical section be considered complete for this claim for all warmwater finfish, was submitted to CVM in February 2009.

Efficacy Study on Bluegill – Richloam Fish Hatchery: Mike Matthews and the crew at the RFH had a busy year conducting field efficacy studies in support of an initial approval for CLT and an expanded approval for 35% PEROX-AID®. Just about the time we thought we'd give them the rest of the year off, we got word that they wanted to fire-up one more study. This time, they wanted to evaluate the efficacy of 35% PEROX-AID® to control mortality caused by external columnaris in bluegill. In this study, which was conducted in September 2008, fish were treated with 100 mg/L hydrogen peroxide for 60 min per day on three alternate days. At the end of the 14-d posttreatment period, mean percent cumulative mortality in treated tanks (10.3%) was significantly lower (P = 0.0051) than that in control tanks (19.0%). AADAP submitted a final study report to CVM for review in February 2009, and we expect a response from CVM by 25 August 2009. We also submitted a letter to CVM requesting that the effectiveness technical section be considered complete for the use of 35% PEROX-AID® to control mortality caused by external columnaris in all freshwater-reared warmwater finfish. As usual…. stay tuned and we'll keep you posted!

FEATURE ARTICLE

Marking Rainbow Trout with Terramycin® 200 for Fish

Dan Carty

U.S. Fish and Wildlife Service,
Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road,
Bozeman, Montana 59715, USA

The U.S. Fish and Wildlife Service (FWS) Aquatic Animal Drug Approval Partnership (AADAP) Program designed and conducted a study to evaluate the efficacy of Terramycin® 200 for Fish (oxytetracycline dihydrate; OTC) Type A Medicated Article (TM200) - administered orally in feed at 3.75 g OTC/100 lbs fish/d for 10 consecutive days - for the skeletal marking of freshwater-reared rainbow trout *Oncorhynchus mykiss*. The underlying purpose of the study being that one successful pivotal study on one freshwater-reared salmonid is all that CVM will require

to expand OTC's current approved uses as a skeletal marking agent from "...Pacific salmon…" to "all freshwater-reared salmonids."

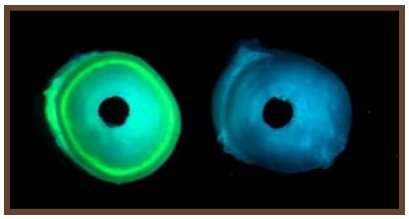
The study was conducted February – April 2009 at the FWS Bozeman Fish Technology Center, Bozeman, Montana USA, under AADAP Study Protocol Number OTC-08-EFF-MARK and according to U.S. Food and Drug Administration (FDA) Center for Veterinary Medicine (CVM) Good Clinical Practice guidelines. Based on previous discussion with CVM, it was determined that in order to demonstrate treatment efficacy the mean percentage of fish with "marked" vertebrae in TM200-treated tanks must be ≥70%. Additionally, a "marked" vertebra (i.e., a treatment success) was defined as one with a fluorescent OTC mark visually graded 2 (faint and complete mark circle) or 3 (good and complete mark circle) on a scale of 0, 1, 2, or 3. A vertebra visually graded 0 (not marked) or 1 (faint and incomplete mark circle) was categorized as a treatment failure.

The in-life phase of the study (26 February – 30 March) was single-masked, randomized (fish and treatment conditions to test tanks), and comprised a 1-d acclimation period, 10-d treatment period, and 22-d posttreatment period. Test fish were healthy rainbow trout characterized as large fingerlings (mean

weight 36.6 g/fish at the start of treatment and 46.9 g/fish at the end of treatment). Nine test tanks (N = 6 treated and 3 control) were used in the study, and there were 20 test fish per test tank (total, 180 test fish). Test fish were not fed on acclimation day. During the treatment period, commercially prepared TM200-treated feed (BioOregon® BioTrout 3.0 mm, 1% TM200) was administered to treated tanks at a target dosage of 3.75 g OTC/100 lbs fish/d. Nontreated control tanks received the same feed without OTC. During the posttreatment period, control feed was administered to all test tanks. On the last day of the in-life phase, test fish were hand-counted out of test tanks, euthanized in a solution of tricaine methanesulfonate, individually measured for length and weight (to the nearest 0.1 cm and 0.1 g, respectively), bagged by tank and fish number, and frozen whole for subsequent vertebrae extraction and OTC mark evaluation. Throughout the in-life phase, both treated and control fish appeared to feed aggressively and otherwise behave normally. All test fish were accounted for, and there was no mortality. Test tank water temperature averaged 10.3ºC, and dissolved oxygen concentration averaged 8.2 mg/L. Source water hardness, alkalinity, and pH averaged 293 mg/L as $CaCO_3$, 195 mg/L as $CaCO_3$, and 7.9, respectively. All of these water quality parameters were suitable for rearing healthy rainbow trout.

On 27 – 29 April, test fish were removed from the freezer and partially thawed, after which for each fish, two vertebrae were extracted immediately anterior to

the dorsal fin. For each fish, both extracted vertebrae were cleaned and then evaluated under ultraviolet light (wavelength ≈ 365 nm) and magnification (8x – 40x zoom) for the presence and quality a fluorescent OTC mark. Mark evaluations were masked by tank. For the six treated tanks (120 test fish total), all vertebrae evaluated had OTC marks graded 3 (good and complete mark circle); hence, treatment success in treated tanks was 100%. In the three control tanks (60 fish total), all vertebrae evaluated were graded 0 (not marked); hence, treatment success in control tanks was 0%. Treated-feed samples (n = 3) analyzed averaged 1.78 g OTC/



lb feed, which meant that treated test fish had been administered an average dosage of 3.33 g OTC/100 lbs fish/d or 88.8% of target (within the acceptable limits specified in the protocol). The control feed sample analyzed had 0.00792 g OTC/lb feed, which meant that an average dosage of 0.01485 g OTC/100 lbs fish/day had been administered to control fish. Although there was some OTC in the control feed, the concentration was not sufficient to induce a fluorescent mark on any of the control-fish vertebrae evaluated. Based on these results, it was concluded that TM200 administered orally in feed at a target dosage of 3.75 g OTC/100 lbs fish/d for 10 consecutive days is effective for the skeletal marking of freshwater-reared rainbow trout. The final study report is being written and will be submitted to FDA/CVM in August 2009.

FINS & TAILS, BITS & BOBBERS

New Distributor for HALAMID® AQUA (chloramine-T): Under Chloramine-T INAD 9321 two products (HALAMID® AQUA and Actamide) are authorized for use by INAD participants. Recently, Western Chemical Inc., Ferndale, Washington USA

announced that they are the new distributor for HALAMID® AQUA (formerly distributed by International Specialty, Inc.). The contact person for HALAMID® AQUA at Western Chemical is Ron Malnor, and Ron's complete contact information can be found on the chloramine-T fact sheet located on the AADAP website (http://www.fws.gov/fisheries/aadap/chloramine.htm).

Attention Potential SLICE® (emamectin benzoate) INAD Participants: For some time now, the AADAP Office and Intervet Schering-Plough Animal Health have been working hand-in-hand to address potential environmental discharge-related concerns expressed by FDA with respect to the use of SLICE® under INAD authorization. These concerns have to date precluded FDA from granting treatment authorization for the use of SLICE® under INAD 11-370. The good news is that we believe that FDA's aforementioned concerns have been adequately addressed, and that treatment authorization for the use of SLICE® under INAD 11-370 is anticipated within a couple/three months! If you are interested in participating under the SLICE® INAD11-370 and have not already filled out a site-specific SLICE® Environmental Assessment (EA) Questionnaire for your facility, we suggest that you do so at your earliest convenience. To obtain a copy of the EA Questionnaire, refer to the following webpage: http://www.fws.gov/fisheries/aadap/misc/sliceEA.pdf.

It is important to note that a completed EA Questionnaire for each participating facility will need to be received by the AADAP Office prior to actual treatment of fish under SLICE® INAD 11-370. Additionally, each facility will also need to notify and receive agreement/confirmation/concurrence from their local NPDES permitting office, or their regional EPA office if EPA has jurisdiction, that SLICE® treatment is authorized (click here to determine whether your state has NPDES permitting jurisdiction).

Update on BENZOAK® INAD 11-740 and AQUI-S® E INAD 11-741: The purpose of these INADs is to allow for their use to anesthetize freshwater and marine finfish, and more specifically, for use to sedate fish to the "handleable-stage" of anesthesia. Current INAD authorizations for BENZOAK® and AQUI-S® E do not allow for the slaughter and/or release of treated animals. Recently, the AADAP Office submitted amended authorization requests to FDA for both INADs requesting the establishment of a post-treatment investigational withdrawal time and authorization to slaughter and/or release treated animals. We are anticipating a reply from FDA within the next few months, and hopefully, at that time these INADs will be available for field use to generate supportive efficacy and safety data. Stay tuned!

RELEVANT LITERATURE

The following is a list of journal publications with particular relevance to the broad topic of drug-use in aquaculture. This list comprises citations exclusively from 2008 and 2009. Please note that this list does not include those provided in previous issues of the AADAP Newsletter. Citations have been divided into topic categories.

If you have come across literature that you believe would be of interest to the readership of the AADAP Newsletter, please forward the citation to Tom Bell (thomas_a_bell@fws.gov) and we will place it in the next edition.

The inclusion of a citation within the AADAP Newsletter does not imply: (1) recommendation of the use of the technique in any particular situation, (2) concurrence with a treatment procedure/drug, (3) acceptance by the U.S. Food and Drug Administration's Center for Veterinary Medicine of the drug's safety or effectiveness, nor (4) in any way an endorsement of a product by the U.S. Fish & Wildlife Service.

Antibiotic & Bacterial

Barnes, ME, et al. 2009. Effect of *Flavobacterium columnare* inoculation, antibiotic treatments and resident bacteria on rainbow trout *Oncorhynchus mykiss* eyed egg survival and external membrane structure. *Journal of Fish Biology* **74(3):576-590**.

Bowser, PR, et al. 2009. Florfenicol residues in Nile tilapia after 10-d oral dosing in feed: effect of fish size. *Journal of Aquatic Animal Health* **21(1):14-17**.

Kosoff, RE, et al. 2009. Florfenicol residues in three species of fish after 10-d oral dosing in feed. *Journal of Aquatic Animal Health* **21(1):8-13**.

Darwish, AM and Mitchell, AJ. 2009. Evaluation of diquat against an acute experimental infection of *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* **32(5):401-408**.

Darwish, AM, et al. 2009. Evaluation of potassium permanganate against an experimental subacute infection of *Flavobacterium columnare* in channel catfish, *Ictalurus punctatus* (Rafinesque). *Journal of Fish Diseases* **32(2):193-199**.

Kum, C, et al. 2008. Comparison of *in vitro* antimicrobial susceptibility in *Flavobacterium psychrophilum* isolated from rainbow trout fry. *Journal of Aquatic Animal Health* **20(4):245-251.**

Kunttu, HMT, et al. 2009. The efficacy of two immunostimulants against *Flavobacterium columnare* infection in juvenile rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology* **26(6):850-857**.

Ohno, Y, et al. 2009. The effect of oral antibiotic treatment and freshwater bath treatment on susceptibility to *Neobenedenia girellae* (Monogenea) infection of amberjack (*Seriola dumerili*) and yellowtail (*S. quinqueradiata*) hosts. *Aquaculture* **292 (3-4):248-251.**

Pouliquen, H, et al. 2009. Comparison of water, sediment, and plants for the monitoring of antibiotics: a case study on a river dedicated to fish farming. *Environmental Toxicology and Chemistry* **28(3):496-502**.

Russo, R and Yanong, RPE. 2009. Efficacy of vaccination against *Streptococcus iniae* during artificial spawning of the red-tail black shark (*Epalzeorhynchos bicolor*, family Cyprinidae). *Journal of Applied Aquaculture* **21(1):10-20**.

Smith, P, et al. 2009. A rapid method of improving the criteria being used to interpret disc diffusion antimicrobial susceptibility test data for bacteria associated with fish diseases. *Aquaculture* **290(1-2):172-178**.

Sun, K, et al. 2009. Genetic mechanisms of multi-antimicrobial resistance in a pathogenic *Edwardsiella tarda* strain. *Aquaculture* **289(1-2):134-139**.

Temple, E and Langdon, C. 2009. Delivering oxytetracycline to first-feeding zebrafish *Danio rerio* (Hamilton) and goby *Asterropteryx semipunctata* (Rueppell) larvae using lipid spray beads. *Journal of Fish Diseases* **32(3):279-292**.

Skeletal Marking

Aguilera, B, et al. 2009. Otolith growth of European sea bass (*Dicentrarchus labrax* L.) larvae fed with constant or varying food levels. *Scientia Marina (Barcelona)* **73(1):173-182**.

Liu, Q, et al. 2009. The use of alizarin red S and alizarin complexone for immersion marking Japanese flounder *Paralichthys olivaceus* (T.). *Fisheries Research* **98(1-3):67-74**.

Simon, J, et al. 2009. Growth and mortality of European glass eel *Anguilla anguilla* marked with oxytetracycline and alizarin red. *Journal of Fish Biology* **74(1):289-295**.

Spawning Hormones & Gender Manipulation

Cuevas-Uribe, R, et al. 2009. Progress in studies on hormonal sex reversal and genetic sex control in black crappie. *Reviews in Fisheries Science* **17(1):1-7**.

Haffray, P, et al. 2009. Successful production of monosex female brook trout *Salvelinus fontinalis* using gynogenetic sex reversed males by a combination of methyltestosterone immersion and oral treatments. *Aquaculture* **290(1-2): 47-52**.

Homklin, S, et al. 2009. Biodegradation of 17 alpha-methyltestosterone and isolation of MT-degrading bacterium from sediment of a Nile tilapia masculinization pond. *Water Science & Technology* **59(2):261-265**.

Kamaruzzaman, N, et al. 2009. Growth performance of mixed sex, hormonally sex reversed and progeny of YY male tilapia of the GIFT strain, *Oreochromis niloticus*. *Aquaculture Research* **40(6):720-728**.

Kristanto, AH, et al. 2009. Effect of postmanufacturing processing and shipping of luteinizing hormone releasing hormone analog on induced ovulation for production of channel catfish female X blue catfish male hybrid fry. *North American Journal of Aquaculture* **71(4):307-311**.

Selim, KM, et al. 2009. Effects of high temperature on sex differentiation and germ cell population in medaka, *Oryzias latipes*. *Aquaculture* **289(3-4):340-349**.

Yaron, Z, et al. 2009. Spawning induction in the carp: past experience and future prospects - a review. *Israeli Journal of Aquaculture/Bamidgeh* **61(1):5-26**.

Parasite Control

Berg, AGT and Horsberg, TE. 2009. Plasma concentrations of emamectin benzoate after Slice treatments of Atlantic salmon (*Salmo salar*): differences between fish, cages, sites and seasons. *Aquaculture* **288(1-2):22-26.**

Heinecke, RD and Buchmann, K. 2009. Control of *Ichthyophthirius multifiliis* using a combination of water filtration and sodium percarbonate: dose-response studies. *Aquaculture* **288(1-2):32-35**.

Heuch, PA, et al. 2009. Temporal and spatial variations in lice numbers on salmon farms in the Hardanger fjord 2004-06. *Journal of Fish Diseases* **32(1):89-100**.

Jorgensen, TR, et al. 2009. Parasite infections in recirculated rainbow trout (*Oncorhynchus mykiss*) farms. *Aquaculture* **289(1-2):91-94**.

Mayor, D, et al. 2009. Effects of copper and the sea lice treatment Slice® on nutrient release from marine sediments. *Marine Pollution Bulletin* **5(4):552-558**.

Mitchell, AJ, et al. 2008. Comparison of tank treatments with copper sulfate and potassium permanganate for sunshine bass with ichthyobodosis. *Journal of Aquatic Animal Health* **20(4):202-206**.

Robertson, PK, et al. 2009. A new generation of biocides for control of crustacea in fish farms. *Journal of Photochemistry and Photobiology B*: *Biology* **95(1):58-63**.

Straus, DL, et al. 2009. Copper sulfate toxicity to two isolates of *Ichthyophthirius multifiliis* relative to

alkalinity. *Diseases of Aquatic Organisms* **84(1):31-36**.

Anesthetics/Sedatives

Gullian, M and Villanueva, J. 2009. Efficacy of tricaine methanesulphonate and clove oil as anaesthetics for juvenile cobia *Rachycentron canadum*. *Aquaculture Research* **40(7):852-860**.

Mamangkey, NGF, et al. 2009. Use of anaesthetics with the silver-lip pearl oyster, *Pinctada maxima* (Jameson). *Aquaculture 288(3-4):280-284*.

Velisek, J, et al. 2009. Comparison of the effects of four anaesthetics on biochemical blood profiles of perch. *Aquaculture Research* **40(3):354-361**.

Weber, RA, et al. 2009. The efficacy of 2-phenoxyethanol, metomidate, clove oil and MS-222 as anaesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858). *Aquaculture* **288(1-2):147-150**.

"Natural" Products

Green, TJ, et al. 2009. Differential expression of genes encoding anti-oxidant enzymes in Sydney rock oysters *Saccostrea glomerata* (Gould) selected for disease resistance. *Fish & Shellfish Immunology* **26(5):799-810**.

Immanuel, G, et al. 2009. Dietary medicinal plant extracts improve growth, immune activity and survival of tilapia *Oreochromis mossambicus*. *Journal of Fish Biology* **74(7):1462-1475**.

Li, SS and Tsai, H. 2009. Transgenic microalgae as a non-antibiotic bactericide producer to defend against bacterial pathogen infection in the fish digestive tract. *Fish & Shellfish Immunology* **26(2):316-325**.

Qi, Z, et al. 2009. Probiotics in aquaculture of China - current state, problems and prospect. *Aquaculture* **290(1-2):15-21**.

Yeh, RY, et al. 2009. Evaluation of the antibacterial activity of leaf and twig extracts of stout camphor tree, *Cinnamomum kanehirae*, and the effects on immunity and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish & Shellfish Immunology* **27(1):26-32**.

Miscellaneous

Park, K and Heo, G-J. 2009. Acute and subacute toxicity of copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) in the guppy (*Poecilia reticulata*). *Journal of Veterinary Medical Science* **71(3):333-336**.

Sanabria, C, et al. 2009. Effects of commonly used disinfectants and temperature on swim bladder non-inflation in freshwater angelfish *Pterophyllum scalare* (Lichtenstein). *Aquaculture* **292(3-4):158-165**.

Yamashita, Y, et al. 2009. The synthetic antioxidant, ethoxyquin, adversely affects immunity in tilapia (*Oreochromis niloticus*). *Aquaculture Nutrition* **15(2):144-151**.

USGS's CORNER

Hydrogen peroxide: The Upper Midwest Environmental Sciences Center (UMESC) assessed the efficacy of 35% PEROX-AID® (hydrogen peroxide) to control mortality from saprolegniasis caused by *Saprolegnia parasitica* in juvenile rainbow trout intentionally challenged (by immersion) with *S. parasitica*. A uniform epidermal/dermal abrasion was administered to each fish immediately before immersion challenge. After challenge, 20 fish were randomly distributed to one of 18 test tanks which had been randomly assigned to one of three treatment levels (nonmedicated control, 50 or 75 mg/L hydrogen peroxide). Three hydrogen peroxide treatments were administered once daily on alternate days; each treatment was applied for 60 min as a static immersion bath. Morbidity and mortality were recorded daily for 14 days following treatment and the presence/absence of fungus was determined in mortalities removed from each tank. Hydrogen peroxide treatment reduced mortality in trout relative to the untreated controls. The final study report is presently in preparation.

UMESC is also presently summarizing several hydrogen peroxide trials conducted at the U.S. Fish and Wildlife Service (USFWS) Iron River National Fish Hatchery and at the Michigan Department of Natural Resources Marquette State Fish Hatchery to control gyrodactylus on coaster brook trout *Salvelinus fontinalis*.

Chloramine-T: To address one of the remaining data gaps for the approval of Halamid® AQUA (chloramine-T), UMESC staff have modified the analytical method for para-toluenesulfonamide (pTSA), the marker residue of chloramine-T. UMESC had previously been notified that FDA-CVM would likely set the tolerance limit for pTSA at 20 parts per billion (ppb), substantially lower than the 1 part per million level previously discussed. Using rainbow trout fillet tissue, we found that the modified method had a pTSA detection limit approximately 50% lower than the original method, allowing quantification of pTSA at or below the tolerance limit (20 ppb). The modified method must now be validated with six species of fish including channel catfish, hybrid striped bass, yellow perch, walleye, brown trout and rainbow trout. Data from the validation study should allow FDA-CVM to determine the adequacy of the modified analytical method to quantify pTSA in fish fillet.

Sedatives: As part of the evaluation of two new candidate sedatives (benzocaine and eugenol), UMESC is conducting studies to evaluate the time required for fish to initiate a feeding response following sedation. Several fish species were selected for inclusion in the study including rainbow trout, brook trout, yellow perch, channel catfish, walleye and bluegill. When tested, fish are assigned to one of six treatment groups, a control, an electroshock (to simulate capture during electrofishing), electroshock plus sedation (by benzocaine or eugenol) or sedation (by benzocaine or eugenol) only. Sedation is accomplished by holding fish in a solution of either AQUI-S-E® (eugenol) or BENZOAK® (benzocaine) at a concentration that results in the fish becoming handleable (defined as the lack of a tail flip) within two min before it is returned to its respective tank. After recovery, defined as normal swimming movements, from either electroshock and/or sedation, fish are offered either a formulated pellet or live food item (e.g. forage fish). Tests were initiated with rainbow trout. Surprisingly, nearly all rainbow trout fed within 30 min of recovery from sedation. Rainbow trout were also selected to evaluate the effect of long-term sedation (held for 45 minutes in either 25 mg/L benzocaine or 5 mg/L eugenol). Again, most fish fed immediately upon recovery. Work is presently in progress to evaluate the effect of sedation on the feeding response in the other selected fish species. This work is being conducted in collaboration with Viterbo University (La Crosse, Wisconsin USA) scientist Dr. Kim Fredericks.

Viral Hemorrhagic Septicemia: Working with the USFWS La Crosse Fish Health Center and the Genoa National Fish Hatchery, UMESC recently assessed the efficacy of iodophor-egg disinfection to eliminate Viral Hemorrhagic Septicemia (VHS) virus (strain IVb) from walleye and northern pike intentionally-challenged with VHS virus following egg fertilization. Adult walleye and northern pike were collected from the Upper Mississippi River and dry-spawned at UMESC. Immediately after sperm activation, the eggs were taken to a controlled access laboratory with effluent disinfection (two-pass ultraviolet and chlorination) where the remainder of egg challenge, disinfection and incubation activities occurred. Immediately on entry into the laboratory, eggs were assigned to one of two VHS-challenge groups (105 or 108 plaque-forming units /mL). Immediately after challenge, eggs were transferred to one of four treatment groups – a non-disinfected control, iododphor disinfection for 30 min, iodophor disinfection for 60 min, or iodophor disinfection for 10 min initiated 90 min post-fertilization. All iodophor treatment concentrations were 100 ppm. Eggs were removed from the assigned disinfection unit after the appropriate disinfection period then held in well water until at least 90-min post-fertilization and then distributed (~25 mL/jar) to egg jars. Eggs were maintained through incubation until hatch with no other chemical treatments applied; fungus was controlled by manually removing eggs with fungus and increasing the water flow through the egg jars. Egg and fry samples were collected and the presence or absence of VHS virus determined. Virus was detected in control eggs but not in eggs of any iodophor disinfection group. A manuscript and USGS Fact Sheet are in preparation to summarize the study results. The study was funded through a grant from the North-Central Regional Aquaculture Center.

Text provided by Mark Gaikowski, Fisheries Management Chemical and Aquaculture Drug Team, U.S. Geological Survey, Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, USA.

USDA's CORNER

Fungus studies: The catfish spawning season is almost over and we have continued our studies on water molds (i.e., fungus) that grow on catfish egg masses. We have been comparing the treatments of copper sulfate, hydrogen peroxide, formalin and diquat that were previously determined to be effective via range-finding studies at SNARC. We have also done some preliminary testing with peracetic acid. These studies had temporarily taken a backseat to planning and organizing the annual Workshop in Little Rock, but these studies will be back in full-swing next year.

Copper sulfate: The Final Study Report for the Copper Sulfate Target Animal Safety study on channel catfish eggs is in final review by our Quality Assurance Officer. We anticipate submitting the FSR to FDA/CVM in July 2009.

Columnaris studies: The high-density, low-flow columnaris disease method described by Drew Mitchell at the recent Workshop has provided promising results and we are continuing to modify the system for optimum performance (click here to review Drew Mitchell's presentation).

Text provided by Dave Straus, Disease & Drug Approval Section, Harry K. Dupree – Stuttgart National Aquaculture Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, Stuttgart, Arkansas, USA.

MEETINGS, ETC.

Recently held meetings

American Fisheries Society Fish Health Section & the 50th Annual Western Fish Disease Workshop;

7-10 June 2009; Park City, Utah USA: The combined meeting of the Fish Health Section and the 50th Western Fish Disease Workshop by all accounts went very well. Despite the poor economy, approximately 100 people turned out to enjoy the beautiful surroundings, great food and accommodations, camaraderie, and scientific presentations. Dr. Mark Lawrence and associates started the meeting with a continuing education session on bacterial genomics. This was followed by formal presentations on a variety of subjects ranging from VHSV to endocrine disrupting compounds to bacterial coldwater disease. At the banquet, the S.F. Snieszko Distinguished Service Award was made to Dr. Paul Bowser of Cornell University. Agenda and abstracts are currently available on the Fish Health Section's website at: http://www.fisheries.org/units/fhs/FHSschedule.pdf. Many of the presentations will also be available soon on the Fish Health Sections website: http://www.fisheries.org/units/fhs/.

Text provided by Dr. Chris Wilson; State of Utah, Fisheries Experiment Station; 1465 W. 200 N.; Logan, UT 84321; phone 435-752-1066 ext. 201; email chriswilson@utah.gov

Upcoming meetings

Genomics in Aquaculture; 5-7 July 2009; Bodø, Norway: The 'Genomics in Aquaculture' symposium will review the state-of-the-art of genomics research in aquaculture, its industrial applications and future perspectives, thus contributing to bridge the gap between fundamental genomics research and the needs of the aquaculture industry. The symposium will have the participation of several invited experts in aquaculture genomics, as well as attendants from feed companies and government representatives. For more information, click here to access the conference website.

Salmonid Disease Workshop; 13-24 July 2009; Oregon State University; Corvallis, Oregon USA: This workshop is designed for professionals working in the fish health field and will emphasize recent advances and developments in our understanding of salmonid diseases. The workshop is limited to 20 participants on a first come, first served basis. Topics include: current immunological and molecular techniques; sampling for pathogens in wild populations; new and emerging fish pathogens; cell culture techniques, including maintenance of cultures and viral identification; histopathology associated with salmonid diseases; current status of important viral, bacterial, and parasitic pathogens; salmonid disease treatment practices used in Pacific Northwest hatcheries and epidemiology. Cost of the workshop is $950 plus housing (if desired). May be taken for 5 CE credits. For more information, if you wish to receive graduate credit and to register, please contact Dr. Jerri Bartholomew at 541-737-1856 or e-mail at: bartholj@science.oregonstate.edu.

Biosecurity for Fish Farmers Workshop; 29 July 2009; Moorefield, West Virginia USA: A team of aquaculture professionals will present a program on fish health, fish diseases and how to protect your facility from fish pathogens. The morning session will be spent reviewing information concerning fish diseases in the Northeast. The afternoon session will comprise a biosecurity assessment of Reymann Memorial Farm Aquaculture Facility where trout are grown in a flowing water system. The event provides a venue for networking with the diverse community influencing aquaculture, including other farmers, testing laboratories, university researchers and policymakers/ regulators. For more information on the workshop refer to their webpage at: http://www.wvu.edu/~agexten/aquaculture/#Upcoming>.

International Aquaculture Biosecurity Conference; 17-18 August 2009; Trondheim, Norway: The theme of the conference, to be held in conjunction with Aqua Nor 2009, is "Practical Approaches for the Prevention, Control, and Eradication of Disease." The goal of the conference is to provide expert opinions and tools for implementing practical, economic and effective biosecurity plans and programs. Planned topics include: economic impact of disease and biosecurity programs; components of ideal biosecurity plans and programs; international, regional and national strategies; identifying and prioritizing hazardous diseases and evaluating risks; determining and mitigating hazardous disease critical control points and risks; disease epidemiology, surveillance and monitoring; determining disease status and freedom; control and eradication contingency plans and programs; disease diagnostics, medical and farm record keeping; and implementing, auditing and certifying biosecurity programs. For more detailed information regarding accommodations, registration, etc. visit the conference website: http://www.iabconference.org.

14th International Conference on Diseases of Fish and Shellfish; 14-19 September 2009; Prague, Czech Republic: This 14th International Conference, organized by the European Association of Fish Pathologists, will be held at the Clarion Congress Hotel in Prague, Czech Republic. Scientific and technical sessions consisting of poster presentations, invited talks, keynotes, oral presentations, workshops and an EAFP General Assembly will take place during the Conference. Planned social events include a Welcome Cocktail, Civic Reception and the traditional Conference Banquet. For more information, refer to the conference website at: http://www.eafp2009.org/#.

Aqua Farming International Conference and Exhibition 2009; 16-19 September 2009; Vigo, Spain: The First Aqua Farming International Conference and Exhibition (AQA) will be held from the 16-19 September 2009 alongside the World Fishing Exhibition (WFE) in Vigo, Spain. Conference topics will be confirmed shortly and the exhibition will feature more than 3000 m^2 of new products and the latest innovations. Co-location with the World Fishing Exhibition, the World's largest commercial fishing exhibition, means that not only will AQA benefit from the WFE's extensive worldwide marketing program, but also from the same features, such as the Fisheries Ministers Conference, that make the WFE truly unique. For more information, click here to access the conference website.

Flavobacterium 2009; 21-23 September 2009; Paris, France: Flavorbacterium 2009 is a follow-up conference to that held in Shepherdstown, West Virginia in 2007 and will aim to address numerous issues and at crossing disciplines (fish pathology, bacterial ecology, genomics, biotechnology, vaccinology, etc.) by bringing together international representatives from academic institutions, fish health management, aquaculture and biotech and pharmaceutical industry. The organizers hope this interdisciplinary approach will promote international collaboration in the different aspects of Flavobacterium research. Additional information is available on the conference website by clicking here.

World Aquaculture 2009; rescheduled for 25-29 September 2009; Veracruz, México: The World Trade Center in Veracruz is the site for the 2009 International meeting of the World Aquaculture Society. The conference theme is "blue revolution to feed the world." The organizers "invite you to join them on a journey to the world of aquaculture science and technology, to explore the whole range of possibilities and make this new 'Blue Revolution' possible." The program focuses on eight major topic areas comprising nearly 60 sessions, including those on therapeutic drugs, aquaculture regulations and health and biosecurity. Online registration, deadlines, conference brochures and information accommodations and tours can be found on the conference website.

XI Ecuadorian Aquaculture Conference & Aquaexpo - AQUA 2009; 12-15 October 2009; Guayaquil, Ecuador: AQUA 2009 constitutes the 11th edition of the Ecuadorian Aquaculture Conference & Aquaexpo, an event which has established itself as the leading aquaculture conference and trade show in Latin America. AQUA 2009 will include a Scientific Program with presentations by renowned international and national experts as well as a trade show where the leading companies in the industry will display their latest developments in aquaculture products and services. For more information contact Camila Parra (phone 5934-2-269494 or email at: cparra@cna-ecuador.com.

World Aquaculture 2010; 1-5 March 2010; San Diego, California USA: The Triennial is the largest aquaculture conference and tradeshow held in the world with nearly 4000 attendees from over 80 countries and even more countries are expected to have attendees at AQUACULTURE 2010. The Triennial combines the annual meetings of the National Shellfisheries Association, Fish Culture Section of the American Fisheries Society and the World Aquaculture Society. Advanced information available on Conference webpage at: https://www.was.org/WasMeetings/meetings/Default.aspx?code=AQ2010.

Therapeutic Drug Session; World Aquaculture 2010; 1-5 March 2010; San Diego, California USA: As in past years, Dave Straus, US Department of Agriculture; Mark Gaikowski, US Geological Survey and Jim Bowker, US Fish & Wildlife Service will moderate a session devoted to research and related activities, associated with gaining approvals for new drugs for use on/in aquaculture species. The exact date and time for the session has yet to be set. For date/time and other conference information refer to the conference webpage at: https://www.was.org/WasMeetings/meetings/Default.aspx?code=AQ2010.

ROZ's CORNER

[**Editor's note:** please refer to What's Shakin' and USGS's Corner for more detailed information regarding the following drug summaries.]

Progress on chloramine-T (HALAMID® AQUA): Three initial label claims are close to completion: Control of mortality in: (1) freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium psychrophilum*,(2) all coolwater finfish due to external columnaris disease associated with *Flavobacterium columnare*, and (3) all warmwater finfish due to external columnaris disease

- In response to CVM's Guidance for Industry #159, UMESC is modifying the determinative analytical method for the chloramine-T marker residue pTSA (para toluene sulfonamide) to reduce the method quantitation limit to <20 ppb. Following consultation with CVM, a method validation study will be initiated to determine how robust the additional method processes are when applied to other fish tissues. The method validation study is expected to be completed by December 2009. Identification of additional method cleanup procedures required

more time than originally estimated, thus delaying the estimated completion date of the method validation portion of the study. If CVM accepts the proposed modifications to the analytical method and agrees that the quantitation limit is 20 ppb then CVM could assign an 11-day or 13-day withdrawal time with a tolerance of 20 ppb.

- On 2 April 2009, CVM accepted the efficacy study submitted by AADAP for the control of mortality in largemouth bass caused by external columnaris disease as pivotal.
- On 7 April 2009, AADAP submitted a white paper titled, "Request to Consider the Chloramine-T Effectiveness Technical Section Complete for the Control of Mortality in all Freshwater-Reared Coolwater and Warmwater Finfish due to External Columnaris Disease Associated with *Flavobacterium columnare*." On 6 May 2009, AADAP submitted an amendment that summarized chloramine-T field efficacy trials conducted in 2000 – 2007 under the Pennsylvania Fish and Boat Commission compassionate INAD.
- On 13 April 2009, AADAP submitted a letter to CVM requesting (1) the Effectiveness Technical Section be complete for all freshwater-reared warmwater finfish, and (2) to resolve any raw data issues on effectiveness stated in the 2 April 2009 letter from CVM.

Progress on hydrogen peroxide (35% PEROX-AID®): Two label claims for supplemental NADAs are close to completion: Control of mortality in: (1) freshwater-reared finfish due to saprolegniasis and (2) all warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare.*

- On 26 February 2009, AADAP submitted to CVM (1) the Final Study Report for efficacy of hydrogen peroxide to control mortality caused by external columnaris disease in bluegill and (2) a request to consider the Effectiveness Technical Section complete for the following claim, "Use 35% PEROX-AID® to control mortality in all freshwater-reared, warmwater finfish due to columnaris disease associated with *Flavobacterium columnare*."
- On 5 May 2009, CVM accepted the efficacy study submitted by AADAP on largemouth bass as pivotal for external columnaris disease.
- UMESC obtained CVM concurrence on pivotal efficacy study protocols for use of 35% PEROX-AID® for control of mortality caused by saprolegniasis on rainbow trout and walleye.
- In May 2009, UMESC submitted a Final Study Report to CVM that summarized a supportive efficacy trial conducted at the Illinois Department Natural Resources Jake Wolf State Fish Hatchery to control mortality caused by saprolegniasis in largemouth bass.

Progress on immediate-release (or zero-withdrawal time) sedative: The AFWA's Drug Approval Working Group (DAWG) is moving closer to selecting one of two candidate sedatives (i.e., benzocaine or eugenol) for development. Through partial or complete funding from a previous AFWA project on isoeugenol, the following studies are in progress: (1) Time to First Feeding Study (i.e., determine the time required for fish to resume feeding behavior) by UMESC, in collaboration with Viterbo University, (2) analytical method development to detect and quantify eugenol in the edible fillets of freshwater fish by UMESC, and (3) genotoxicity battery of studies for benzocaine by AADAP.

Benzocaine

- In June 2009, Frontier Scientific, Inc., (Logan, Utah) obtained MUMS designation for three label claims for its benzocaine product.
- In May 2009, AADAP tested BENZOAK® (ACD Pharmaceuticals, Norway) to identify parameters and use patterns for further study under the compassionate INAD protocol.

Eugenol

- On 12 January 2009, AQUI-S New Zealand obtained MUMS designation for eight label claims for its eugenol product (AQUI-S® E).
- On 2 February 2009, AADAP submitted to CVM all publicly available study reports and data on eugenol generated by the National Toxicology Program.
- In May 2009, AADAP tested AQUI-S® E to identify parameters and use patterns for further study under the compassionate INAD protocol.

Text provided by Rosalie (Roz) Schnick, National Coordinator for Aquaculture New Animal Drug Applications, Michigan State University, La Crosse, Wisconsin.

NOAA FISHERIES UPDATE

Sedative Work Group Moves Forward! The lack of a near-zero withdrawal time sedative to use on food fish, such as anadromous salmon, continues to challenge fishery biologists and aquaculturists operating in the Columbia River system. Adult salmon are intercepted as they migrate up the river at dams and weirs in order to collect critical fishery management data. Safe handling, for both fish and personnel, necessitates the use of sedatives. Currently, MS-222 is a safe and effective sedative; however, it requires a 21-day withdrawal time before treated fish can enter the human food chain. To address this specific challenge, representatives from federal (National Oceanic and Atmospheric Administration, US Fish & Wildlife Service,

US Army Corps of Engineers), state (Idaho, Oregon, Washington) and tribal (Nez Perce, Columbia River Inter-tribal Fish Commission) agencies met last February in Seattle in conjunction with Aquaculture America. The purpose of the meeting was to examine needs for sedatives, current methodologies used, and prospects for obtaining a near-zero withdrawal sedative. Participants agreed to take the following steps: (1) form a work group to study the issue; (2) identify the time between handling salmon and entry to a fishery; and (3) draft a letter to agency representatives for their signature which identifies the need to develop a near zero withdrawal sedative. Since the February meeting the following has occurred.

- The Sedative Work Group has formed and is operating in partnership with all the above mentioned entities.
- Identification of time from release after being sedated to harvest of adult salmon (about two hours or more)
- A letter has been drafted and signed by most of the parties indicating their support and cooperation for research in order to obtain a FDA-approved near zero withdrawal sedative.

The next steps for the Sedative Work Group is to develop an action plan to help facilitate research and move this project ahead in a timely fashion. It is hoped that a draft action plan will be ready for consideration by the participating entities some time in August. Stay tuned!

Text provided by Kevin H. Amos, Aquatic Animal Health Coordinator, NOAA Fisheries; office phone 360-753-4650; e-mail kevin.Amos@noaa.gov

CVM's NOTES

Water Quality Benchmarks for Aquaculture Drugs: As part of the FDA approval for 35% PEROX-AID® (hydrogen peroxide) in early 2007, FDA's Center for Veterinary Medicine (CVM) required risk mitigation language on the drug label to address environmental concerns associated with use of this product on freshwater fish. The risk mitigation language included identification of an acute water quality benchmark for hydrogen peroxide and associated caution statements intended to alert users and the appropriate regulatory authorities of the potential adverse effects of drug use on aquatic life in receiving waters (i.e., surface waters receiving an effluent discharge from an aquaculture facility where the drug is used). This was the first time that a water quality benchmark was derived for an aquaculture drug and included on product labeling.

A water quality benchmark is a numeric upper limit on the concentration of a drug that can be present in surface water (e.g., river, lake) without causing harm to aquatic life. Benchmarks are essentially equivalent to the national water quality criteria for aquatic life that are developed by the U.S. Environmental Protection Agency (EPA). Depending on how they are derived, they may be designed to protect freshwater and/or saltwater aquatic organisms from the effects of acute (short-term) or chronic (long-term) exposures to potentially harmful chemicals.

Water quality benchmarks will not be routinely developed for aquaculture drugs and included on product labels. This will only be done after CVM has reviewed the Environmental Assessment (EA) document prepared as part of the drug approval process and has concluded that it cannot make a Finding of No Significant Impact (FONSI) because use of the drug will result in unacceptable risks to aquatic life. In some cases, other types of risk mitigation may be more appropriate.

The process for developing water quality benchmarks and including them on labels was developed through collaboration between CVM and EPA's Office of Water. Under the Clean Water Act, the Office of Water has regulatory authority for control of point source discharges of effluents and it administers the National Pollutant Discharge Elimination System (NPDES) in association with state water quality authorities. NPDES Authorities have the authority to use data from FDA to determine numeric water quality criteria and establish effluent limits [see 40 CFR 122.44(d)(1)(vi)(A)].

Water quality benchmarks and associated risk mitigation labeling provide a mechanism for alerting NPDES permitting authorities of the potential need to consider establishing facility-specific numeric effluent limitations for aquaculture drugs. They also provide some of the necessary data to do this if it is determined that limits are indeed needed. NPDES authorities may seek additional data through effluent monitoring or toxicity testing before

making a determination as to whether or not an effluent discharge limit is necessary.

It is important to recognize that water quality benchmarks are not effluent discharge limits and they only apply to surface waters. However, these values may be used by the appropriate NPDES authority in conjunction with site-specific information to determine if effluent monitoring, toxicity testing, and/or a specific discharge limitation may be needed for a particular drug at a specific aquaculture facility. Important site-specific information includes the following:

- frequency and extent of drug use,
- volume and rate of effluent discharge,
- type and extent of wastewater treatment,
- amount of dilution in receiving water, and
- designated use of the receiving water.

When water quality benchmarks are developed, the associated risk mitigation labeling will typically include:

- notification to inform the NPDES Authority prior to initial use of the drug,
- identification of the benchmark value(s),
- identification of the FDA website for additional information (EA and FONSI), and
- risk or caution statements to identify specific environmental concerns

Risk mitigation labeling was necessary for 35% PEROX-AID® because risk characterizations in the EA indicate that the effluent concentrations for some aquaculture facilities expected to use this drug could potentially result in adverse effects on aquatic life in receiving waters. However, receiving water concentrations for most facilities using this drug are expected to be well below the acute water quality benchmark value of 0.7 mg/L (ppm). The toxicity database for hydrogen peroxide and the procedure for calculating the water quality benchmark are described in the EA document for 35% PEROX-AID® that was prepared in support of its approval by the U.S. Geological Survey's Upper Midwest Environmental Sciences Center. This EA can be accessed through this CVM website: http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/EnvironmentalAssessments/ucm072419.htm.

For further information:

CVM Update: Environmental Considerations and Limitations for Use of Hydrogen Peroxide in Aquaculture, February 2007; http://www.fda.gov/ AnimalVeterinary/NewsEvents/CVMUpdates/ucm045576.htm

Compliance Guide for the Concentrated Aquatic Animal Production Point Source Category, U.S. EPA, March 2006 (EPA 821-B-05-001); http://www.epa.gov/guide/aquaculture/guidance/index.html (see Appendix U: FDA Labeling)

Text provided by, and for further information, contact:

Eric M. Silberhorn, Ph.D.
Environmental Safety Team
Division of Scientific Support, ONADE, CVM
Phone: 240-276-8224
Email: eric.silberhorn@fda.hhs.gov