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 4-3

AADAP NEWSLETTER

October 2008

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* In response to some comments we've received, we have decided to try placing a table of contents on the first page, with hyperlinks to the various items in the Newsletter. Please tell us what you think about this addition.

WHAT'S SHAKIN'

14th Annual Aquaculture Drug Approval Coordination Workshop: This year's workshop was once again held in Bozeman, Montana, and by all accounts was another very successful meeting focused on wide-ranging and collaborative drug approval efforts. It also appears to have been a "record breaker" with 89 registered workshop attendees. Not only was it a record-breaker for total attendance, but FDA's Center for Veterinary Medicine also broke a personal participation record by sending 13 members of their staff. CVMer's attending covered a broad spectrum of experience and Center expertise - all the way from a summer student intern (Ms. Courtney Coddington) to two Office Directors (Dr. Steve Vaughn, Office of New Animal Drug Evaluation and Dr. Meg Oeller, Office of Minor Use and Minor Species).

2008 Aquaculture Drug Approval Coordination Workshop

A special thanks goes out to the many folks that provided presentations/updates on completed, planned, and ongoing work. Special appreciation is also expressed to representatives of senior management of FDA's Center for Veterinary Medicine, U.S. Geological Survey, U.S. Fish & Wildlife Service and the Association of Fish and Wildlife Agencies for providing keynotes addresses detailing their respective agency's role in overall collaborative drug approval efforts. As we are all very well aware, the aquatic species drug approval "game" is most definitely a "team sport."

This year's Workshop would not have been possible if it were not for the generous monetary assistance provided by a number of the chemical, pharmaceutical and feed companies attending. Our hats go off to Aquatic Life Sciences, Inc., Axcentive SARL, Bimeda USA, BioOregon/Skretting, Carus Chemical, Eka Chemical, Frontier Scientific Inc., Intervet/Schering-Plough Animal Health and Novartis Animal Health. We can't say THANK YOU enough!!!

For more information on the workshop, including viewing many of the presentations given at the Workshop, click here.

17MT mini-meeting: This year's Drug Approval Coordination Workshop also provided the venue for researchers and regulators to meet and strategize about the studies remaining to be completed to fulfill the requirements for a 17α-methyltestosterone New Animal Drug Application (NADA) for sex-reversal in tilapia. About a dozen folks met Monday afternoon (28 July 2008) and were able to provide the information necessary to update a master progress table. Additionally, attendees worked together to plan the best course of action given available funds and limited personnel. If all studies currently scheduled to be conducted go as planned and are accepted by CVM, the sponsor should be able to submit a complete NADA in December 2010. The master progress table can be viewed by clicking here.

Update from the 7th meeting of the National Aquaculture Drug Research Forum (NADRF): The most recent meeting of the NADRF was also held in conjunction with the 14th Annual Drug Approval Coordination Workshop. Thirty individuals interested in helping to resolve issues faced by researchers conducting studies in support of aquaculture drug approvals met on 1 August 2008 to discuss the following: 1) status of a survey to identify protozoan ectoparasites of primary importance, 2) overview of a parasite round table discussion that was held prior to the Workshop on 28 July 2008 (see below); 3) a proposal for an NADRF white paper review process; 4) the value of round-table discussions such as the below noted parasite discussion; 5) finding a new home for the NADRF (based on the anticipated termination of the JSA Drugs, Biologics, and Pesticides Working Group), and 6) providing statements of aquaculture drug needs to the JSA National Research and Technology Task Force. To view the notes from this meeting, click here.

Round table discussion on parasite-studies and the National Parasite Survey — research on the horizon: Over the past year or two, there has been considerable discussion about how to design field trials to evaluate the effectiveness of therapeutants to control ectoparasite infestations in fish. To more clearly articulate some of the issues and to try to resolve them, a "*Parasite Round Table Discussion*" was held (28 July 2008) in conjunction with the 14th Annual Aquaculture Drug Approval Coordination Workshop. Attending were approximately 40 individuals representing various research agencies, fish health experts, professional associations, drug and chemical companies, and representatives from various CVM Teams (e.g., Aquaculture Drugs, Biometrics, and Environmental).

Starting off the session was a presentation by Ms. Courtney Coddington (a CVM summer intern). Ms. Coddington provided an excellent overview of the issues, including challenges relative to protocol development and maintaining sample collection and evaluation consistency.

Following Ms. Coddington's presentation, the group launched into discussions that were more brain-storming in nature and (of course) led to even more questions. Although little was resolved during this first meeting, there was considerable constructive discussion relative to 1) the level of pathogen identification preferred by CVM (i.e., identify the parasite to the genus/species level); 2) different procedures that may be used to enumerate pathogens; 3) how such data should be analyzed; 4) whether to pursue a claim(s) for control of level of parasite infestation or control of mortality caused by parasite infestation; 5) how to deal with secondary pathogens; 6) whether we should pursue approvals for a disease complex, i.e., when more than one pathogen is present (e.g., ectoparasite and columnaris bacteria); and 7) how much of the "process" was used to successfully gain an approval for formalin.

In the relatively short time available to the group, it proved to be rather difficult to address in much detail many of the issues discussed. However, it was fortuitous that Mark Gaikowski (USGS - UMESC) was able to provide an overview of a survey recently submitted by the National Aquaculture Drug Research Forum through the AFWA Drug Approval Working Group, soliciting information relative to freshwater fish ectoparasites of concern to fish health professionals throughout the USA. Results from this survey should help identify ectoparasites of primary concern in freshwater aquaculture, and in general, assist the group to better focus future discussions.

Coincidentally, CVM had previously arranged a webinar dealing with quantification of parasites in clinical studies. On 28 August 2008, instructor Sarah L. Poynton Ph.D., Associate Professor of Molecular and Comparative Pathobiology, Johns Hopkins University School of Medicine lectured on "Enumeration of Fish Parasites." Material covered included: 1) basic principals of parasite population quantitative descriptors; 2) calculation of parasite prevalence, intensity, and density; 3) statistical approaches commonly used by fish pathologists; and 4) practical considerations when choosing an enumeration technique(s). In summary, the information covered by Dr. Poynton should be extremely helpful to those developing protocols and conducting studies to evaluate the effectiveness of therapeutants to control ectoparasite infestations in fish.

Status/Establishment of the National Aquaculture Industry – Therapeutic Agent Program (NAI-TAP): The establishment of a new national aquaculture drug working group, comprising private and public sector members, was first considered during a meeting of the Joint Subcommittee on Aquaculture's Working Group on

Aquaculture Drugs, Biologics and Pesticides (WGADBP) at the 2008 World Aquaculture Conference. At this meeting, WGADBP attendees were informed (for the first time) that it was likely the WGADBP would soon be terminated. Several meeting attendees recognized that if such action was indeed to occur, a need would likely still exist for an aquaculture group to perform at least a portion of the activities of the WGADBP. Thus, an organizational meeting was held on 1 August 2008 (in conjunction with the 14th Annual Drug Approval Coordination Workshop in Bozeman, Montana) to determine the direction, mission, scope, types of membership, and to set a focus for moving forward. As a first step, the group was tentatively given the name—the National Aquaculture Industry – Therapeutic Agent Program or NAI-TAP.

Since the NAI-TAP was being designed to specifically address the needs and provide input for private aquaculture, and the fact that very few private industry representatives attended the 1 August meeting, a new focus was deemed to be in order. By consensus, it was noted that 1) the private sector was already represented on most issues of concern by the National Aquaculture Association, 2) drug approvals and the drug approval processes has advanced considerably since the formation of the WGADBP in November 1990, and 3) USDA's Cooperative State Research, Education and Extension Service (CSREES) works at the national program level to address private aquaculture sector.

During the August 1st meeting, a possible solution to address the need for the continuation of some of the objectives and goals of the WGADBP was proposed. The Aquaculture Chemical Subcommittee (ACS) of the American Fisheries Society (AFS) Task Force on Fishery Chemicals was considered as a potential 'home' for this new group, in particular to selectively address any unmet objectives and goals of the WGADBP. However, since the AFS president reappoints the ACS members annually, this would prove to be difficult since there are 170+ members on the current WGADBP's roster.

AFS resolved the issue by suggesting forming a new working group that would be under the Fish Culture Section (FCS), or possibly, under a joint FCS and Fish Health Section agreement. Based on further discussion it was decided that a working group under one section was the better alternative, and thus the FCS was selected as the governing body. The primary reason for FCS's selection was the fact that its members are the most directly impacted by drug approvals and biologic licensing. Additionally, this decision received strong support from Dr. Curry Woods, the 2008-2009 President of FCS, who attended the meeting and provided valuable input. Dr. Woods is not only very familiar with the drug approval process, but has also been a member of the WGADBP since 1990.

Group consensus determined that all past WGADBP members should be encouraged to become members of the new group, and that this new group will be called the Working Group on Aquaculture Chemicals (WGAC). Dr. Woods appointed Mark Gaikowski (USGS's Upper Midwest Environmental Sciences Center) to be chair of the WGAC. Mr. Gaikowski will preside over the WGAC at all meetings, including the inaugural meeting being held at Aquaculture America 2009 (AA 09), scheduled for 15-18 February 2009 in Seattle, Washington. Any-and-all folks who are planning on attending AA 09 and may be interested in participating in WGAC are encouraged to attend!

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

Update on the 15th Annual Aquaculture Drug Approval Coordination Workshop (2009) planned for Little Rock, Arkansas: June 9th through 11th are the dates set for next year's Workshop. It will be held at the La Quinta Little Rock Downtown Conference Center. The meeting will be hosted by USDA/ARS's Stuttgart National Aquaculture Research Center, and will highlight regional aquaculture, including industry tours to a catfish

hatchery, a baitfish farm and a hybrid striped bass production facility. There will also be an opening mixer at a local brew-pub to get things started and allow everyone to become acquainted or reacquainted. Hope you'all can make this meeting and experience some Southern Hospitality! Keep checking the AADAP Workshop Webpage for updated information.

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.

Update on the planned 16th Annual Aquaculture Drug Approval Coordination Workshop to be held in Bozeman, Montana: Although it may be a long way off, for those of you who like to plan ahead the Workshop will be returning home to Bozeman, Montana in 2010. As has always been the case when it takes place in Bozeman, the 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) or just don't forget to check the AADAP website for news of upcoming workshops.

Update on the search for a new candidate zero-withdrawal anesthetic: Activities to select a new anesthetic (sedative) drug continues. At the request of the Association of Fish and Wildlife Agencies' Drug Approval Working Group (DAWG), AADAP requested a pre-submission conference/meeting with CVM to discuss all NADA technical section requirements for three drugs – benzocaine, eugenol and tricaine methanesulfonate. Representatives of the DAWG, as well as a number of interested drug sponsors, attended the meeting that was held on 20 August 2008 at CVM in Rockville, Maryland.

So where are we with respect to the sedative issue presently? The initial focus of the DAWG centers on a sedative use-pattern appropriate for short-term handling in natural resource management field activities. After an informative review of the technical section requirements at the pre-submission conference, the candidate list was narrowed to benzocaine and eugenol. It was also agreed by all parties to change the claim from "zero withdrawal" to "immediate-release", which better defines the time frame after a fish is sedated to the initial opportunity for capture. In the near future the official CVM Memorandum of Conference from the meeting will be publically available on the AADAP website.

At the annual Association of Fish and Wildlife Agencies (AFWA) Conference in September, the DAWG received approval to allocate remaining Multi-State Conservation Grant Program funding (originally dedicated to AQUI-S®) for additional assessment of both eugenol and benzocaine until the "best candidate" can be determined. DAWG members are now developing grant objectives for submission by 15 November 2008. These objectives tentatively include the following.

- Developing study criteria to establish a postsedation catchability timeframe for each drug. Such criteria are essential to determine if either drug will potentially meet requirements for an immediate release claim. Opportunities for academic-based and government studies exist, but study guidelines etc. need to be first developed and vetted.
- Finalize highest priority funding objectives needed to better define the label claim process for each drug, and work with potential sponsors.

The AFWA grant funding will definitely provide a "jump start" for a new immediate-release fisheries sedative. The overall approval process is just starting and will take considerable time, effort, commitment and funding. The DAWG is committed to work with sponsors to seek approval for this initial label claim. The possibility of having two anesthetic/sedative drugs available for the fisheries toolbox is an interesting opportunity to explore. Stay tuned...

Text provided by Steve Sharon; Chair, Association of Fish and Wildlife Agencies' Drug Approval Working Group; Wyoming Game & Fish; Casper, Wyoming

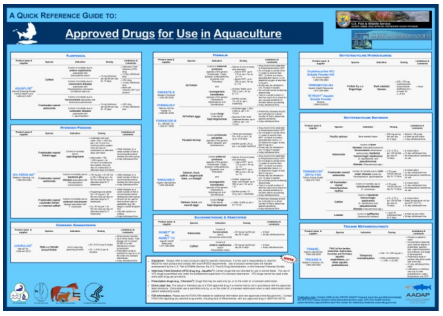
Good Laboratory Practices (GLP) inspection of AADAP facilities and studies: During the week of 18-22 August 2008, an Investigator from the FDA Seattle District Office - Investigations Branch paid the AADAP group a little visit. The purpose of the visit was to use a "fine-toothed comb" to inspect our facilities and one of the target animal safety (TAS) studies we had conducted since a previous inspection in 2006. The Investigator performed his inspection according to Compliance Program Guidance Manual 7348.808 for Bioresearch Monitoring (or BiMo for those familiar with the lingo). He spent the first 2.5 days inspecting our facilities and the various processes/protocols we use to launch and conduct TAS studies. He paid particular attention to: 1) development and revisions of study protocols and standard operating procedures, 2) maintenance of training records, 3) use of lab equipment and instrument logs, and 4) maintenance of active, archived, and historical records and documents.

The FDA Investigator then moved on to an in-depth inspection of a study conducted to evaluate the safety of AQUI-S® as a sedative for use on fingerling cutthroat trout. Following the inspection, a short debriefing meeting was held to discuss the Investigator's findings. Overall, the he concluded that "No Action was Indicated," and that: 1) he was happy that the data was as complete as it was; 2) he found no data errors, i.e., all data were transposed correctly; 3) data were attributable (signed) and contemporary (dated), 4) he was please with the data inspection and that data was 100% inspected; 5) all SOPs were signed and dated; 6) the historical SOP file (archive file) was complete; 7) training records were up to date; 8) he was impressed with the traceability of wet tissues, tissue blocks, and slides; and 9) the facility inspection was good, all equipment was in working order, log books were all signed and dated, all entry information was complete, and that training records indicated who was currently trained to operate each piece of equipment. Although the above-described inspection findings are technically unofficial, we are confident that the official inspection report will indicate GLP-compliance.

Although we had a pretty good feeling going into this inspection, due primarily to the efforts of Ms. Molly Bowman and Ms. Miranda Dotson, it was a huge relief to "pass" another inspection. An additional reason we felt pretty good going into the inspection was due to the help provided over the years by Dr. David Kennedy, QA

Officer at USGS's UMESC — many thanks David! Although we'll keep up our "GLP Attitude," it is quite a relief knowing that we won't be inspected for another two years.

AFS-AADAP "Aquaculture Drug-use Guidance" Poster: As many of you are aware, AADAP and the American Fisheries Society's Fish Health and Fish Culture Sections recently published, in limited quantities and for limited distribution, an "*Aquaculture Drug-use Guidance*" poster. The large-format laminated poster outlines the aquaculture drugs approved for use in the USA, and describes permitted aquatic species, diseases or conditions, and treatment regimens. Interest in the poster has been overwhelming and in very short order we've run out of copies to distribute.



But help is on the way; FDA's Center for Veterinary Medicine has committed to assisting us in publishing a new batch of posters, which should become available within a month or so. This new printing will be modified slightly to include very recent new approvals or label expansions, and will be printed with a different background color to allow one to quickly distinguish it from the earlier version. As soon as the new poster becomes available, we will announce it on our website, along with information on how to obtain copies.

FDA launches new public-access database of animal drug approvals: The FDA's Center for Veterinary Medicine (CVM) recently (1 October 2008) announced the availability of a new database of approved animal drugs. The database, called "Animal Drugs @ FDA," is a publicly-accessible web-based application available through the CVM home page.

"Animal Drugs @ FDA" replaces the "Database of Approved Animal Drug Products," or "*Green Book*," a database that was previously developed and managed by the Virginia-Maryland Regional College of Veterinary Medicine Drug Information Laboratory at Virginia Tech University.

This new application allows users to search for detailed descriptions of all FDA-approved new animal drugs. The search tool not only allows users to conduct simple word searches, but is also capable of more complex searches through the following eight specific search criteria: NADA/ANADA, Sponsor, Ingredients, Proprietary, Dose Form, Route, Species, and Indication.

Under the Generic Animal Drug and Patent Term Restoration Act, CVM will continue to make available electronic files of listed drugs previously provided through the *Green Book* on its web site.

Click here to access the FDA's searchable database.

AADAP establishes three Public Master Files — an update: A Public Master File, or PMF, is a type of master file recognized and held on file by FDA's Center for Veterinary Medicine (CVM) for the purpose of making data and information generated with public funds available to the public. The submission or establishment of a PMF is voluntary and not required by law or by FDA regulations. It is created to serve as a repository for information and data that supports the completion of a technical section(s) for a particular drug compound and associated claim. In AADAP's case, they serve primarily as a repository for efficacy and target animal safety data that are required to support a New Animal Drug Application (NADA). A PMF is not a substitute for an Investigational New Animal Drug (INAD) exemption or an NADA. The submission is not approved or disapproved by CVM, but rather simply found to be acceptable or not. CVM asks that all data submitted to a PMF be previously approved, i.e., the sponsor has received a "Technical Section complete" letter(s). The existence of a PMF is made known through a notice of availability published in the U.S. Federal Register. PMFs established by AADAP will also soon be announced/linked on the AADAP website. PMFs are retained in CVM's files and reviewed at the time it is referenced in support of a sponsor's NADA.

So now that you have a little background on the what, why and how a PMF comes to be, let's fill you in on what AADAP has done to date…

- On 24 May 2007 AADAP requested the establishment of a PMF for the use of chloramine-T as an immersion treatment to control mortality caused by external bacterial pathogens in a variety of fish species. CVM quickly acknowledged receipt of our submission and assigned it Public Master File # PMF 005-893. On 28 July 2008 CVM granted our request and concluded that the data was satisfactory to support the following label claim: "*...for use to control mortality caused by bacterial gill disease in freshwater-reared salmonids. Treat fish one time per day at 12 – 20 mg per L for 60 min in a static or flow-through bath on three alternate or consecutive days.*" On 21 August 2008 the availability of these data were announced in the Federal Register. Great news!
- To continue on the PMF trail, AADAP requested the establishment of a PMF for the use of oxytetracycline-medicated fish feed on 13 August 2008. All data submitted is anticipated to support the label claim: "*...for use to control mortality caused by coldwater disease associated with* Flavobacterium psychrophilum *in freshwater-*

reared salmonids and columnaris disease associated with Flavobacterium columnare *in freshwater-reared* Oncorhynchus mykiss. *Treat fish one time per day at 3.75g per 100 lbs of fish for 10 consecutive days."* Again, CVM quickly acknowledged receipt of our submission and assigned it Public Master File # PMF 005-927.

- On 1 October 2008 AADAP requested the establishment of a third PMF, this one for the use of florfenicol as a medicated feed treatment in a variety of fish species. All data submitted for this PMF is anticipated to support several label claims: "*...for use to control mortality caused by furunculosis associated with* Aeromonas salmonicida *in freshwater-reared salmonids; for use to control mortality caused by coldwater disease associated with* Flavobacterium psychrophilum *in freshwater-reared salmonids; and for use to control mortality caused by streptococcal septicemia associated with* Streptococcus iniae *in hybrid striped bass. Treat fish one time per day at 10 mg/kg of fish for 10 consecutive days*." CVM acknowledged receipt of our submission on October 3, 2008 and assigned it Public Master File # PMF 005-932.

To date, we are still awaiting word as to whether CVM will grant our request for these two new PMF submissions.

Update on the proposed temperature classification strategy for finfish: In the last edition of the Newsletter we described a document submitted to CVM by AADAP in which four temperature-based groups of finfish were proposed for consideration. This proposal was based on rearing water temperatures of millions of finfish, and represented data on over 100 species that are included in the 2005 Public Aquaculture Production Database. CVM responded on 19 August 2008 with a letter in which they discussed their perceived shortcomings relative to the proposal and their current thinking on finfish classification based on temperature. CVM noted that traditionally they have considered that there are three categories: coldwater, coolwater and warmwater finfish. As discussed in their letter, CVM identifies the following in the three categories:

Coldwater: Family Salmonidae (e.g., salmon, trout, char, grayling, whitefish).

Coolwater: Walleye, sauger, saugeye, yellow perch, northern pike, muskellunge, tiger muskellunge, June and razorback suckers, and shovelnose, pallid and white sturgeon.

Warmwater: Ictalurid catfish, tilapia, hybrid striped bass, tropical ornamental finfish and species commonly reared above 26°C that have been identified as coolwater species.

Although traditional-thinking is somewhat of a rarity in the data-oriented and ever-evolving drug approval regulatory process, it appears to work in this case. The submitted proposal and CVM's response letter can be found by clicking here.

Just the Stats, Man...Just the Stats; or The Statsman (with apologies to George Harrison): In aquaculture drug approval work, the term "pivotal" is often used to describe target animal safety or efficacy studies that are both biologically sound and statistically defensible. What constitutes "sound and defensible" is usually clarified and codified during study protocol writing and the CVM study protocol review-and-revision process. For AADAP, one of the biggest challenges in pivotal work has been, and continues to be, statistical data analysis. As such, during the past few years, we have worked closely with CVM, our contracted statistician, and the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) when designing and statistically analyzing pivotal studies (AADAP Newsletter Vol. 3-3, October 2007).

In this issue, we thank Mark Gaikowski (Acting Branch Manager and Research Physiologist, UMESC) for the pivotal efficacy data sets he has analyzed for us during the past year. Mark used a SAS PROC GLIMMIX-based model, co-developed by CVM (Dr. Todd Blessinger, Mathematical Statistician, Biometrics Team) and UMESC (Mark), to analyze mortality data generated in several pivotal efficacy studies conducted to evaluate the efficacy of 35% PEROX-AID® (hydrogen peroxide), Halamid® (chloramine-T), or AQUAFLOR® (florfenicol) to control mortality in a variety of fish species due to a variety of fish diseases associated with specific fish pathogens. Mark's efforts have helped us submit our 2008 pivotal efficacy final study reports to CVM in a timely manner and have helped facilitate CVM's reviews of those reports. Again, thanks much Mark, you're the Statsman!!

DRUG UPDATES:

General: AADAP is excited to report that we've been able to submit quite a few research study protocols and Final Study Reports to CVM since the last newsletter. After crunching some numbers through our mainframe, we reckon we've submitted three research study protocols (plus one resubmission) and seven Final Study Reports in the last few months!

Copper sulfate (Triangle Brand Copper Sulfate®) update:

Report on channel catfish egg studies: The following is an abbreviated write-up of recent studies conducted on channel catfish eggs provided by Dr. Dave Straus (USDA—Stuttgart National Aquaculture Research Center; Stuttgart, Arkansas).

The safety and effectiveness of $CuSO_4$ to control fungus on intact egg masses in channel catfish hatcheries

David L. Straus, Andrew J. Mitchell, Ray R. Carter, Matthew E. McEntire, Andrew A. Radomski and James A. Steeby.

Harry K. Dupree – Stuttgart National Aquaculture Research Center, Agricultural Research Service, U.S. Dept. of Agriculture, Stuttgart, Arkansas, USA.

Copper sulfate ($CuSO_4$) is widely used by the catfish industry as an economical treatment to control fungus (*Saprolegnia* spp.) on channel catfish eggs. This is an overview of our effectiveness and safety studies for the proposed indication *"...to control egg mortality associated with Saprolegniasis infecting channel catfish eggs.*"

Channel catfish were spawned on-site and spawns were moved to the hatching lab within 24 - 48 hrs. Similar portions of a single spawn were placed into mesh baskets of individual compartments of a customized hatching trough and acclimated for 1 hr in 23.5°C well water. Egg counts on smaller samples were also determined for each spawn to estimate number of eggs in each compartment. The effectiveness range-finding study consisted of five $CuSO_4$ concentrations (2.5, 5, 10, 20, and 40 ppm) and an untreated control. Eggs were treated daily until the embryos developed eyes. Chemistry of the well water was pH 7.5, 220 ppm alkalinity, and 90 ppm hardness. When hatching was complete for all viable eggs, fry were siphoned into individual jars containing 70% ethanol and counted within a few days to determine the percent of fry that hatched in each treatment. Fungus was severe in the untreated controls (2% survival) and the most effective treatment of 10 ppm $CuSO_4$ controlled fungus (63% survival). Very little fungus was present in treatments receiving 10 ppm $CuSO_4$ or higher except in 1 replication (1 spawn) that had numerous unfertilized eggs. Two dose-confirmation studies have been completed to verify the optimum dose of 10 ppm both in the lab and at a commercial hatchery.

The purpose of this second study was to access the safety of $CuSO_4$ to channel catfish eggs when treated at the therapeutic rate (10 ppm) determined in the above noted effectiveness study, and also at 30 and 50 ppm $CuSO_4$. Channel catfish were obtained as described above and eggs were treated daily until the embryos developed eyes; exchange rate of the 26ºC water was 90 minutes (3X the normal rate) during treatments. When hatching was complete, the percent hatch in each treatment was determined. Some fungus developed in the controls at this temperature and mean percent hatch was 40.8%. The percent hatch of the 10, 30, and 50 ppm $CuSO_4$ was 80.1, 64.2 and 80.2%, respectively. The difference between the 10 and 30 ppm $CuSO_4$ treatments was statistically significant, while the difference was not significant between the 10 and 50 ppm $CuSO_4$ treatments. The lower hatch-rate of the 30 ppm treatment is attributed to the random sampling within the original egg masses and the range of hatching rates that are common in the industry. A separate experiment looked at the hatching success when eggs were treated daily until the embryos developed eyes with 100 ppm $CuSO_4$. The water temperature was 24ºC and the exchange rate during the treatment was 30 minutes. The individual percent hatch of each replication was 62.7, 94.9, 59.7 and 64.8%.

Florfenicol (Aquaflor®) update:

Rainbow trout/systemic columnaris study: AADAP's long-awaited pivotal field efficacy trial to confirm that florfenicol is effective in controlling mortality due to systemic columnaris in a salmonid species, other than coho salmon, has finally been completed! With help from our good friends Dr. Jed Varney and Kevin Clark (Washington Department of Fish and Wildlife), along with on-site assistance from two students from the Bellingham Technical College (Jason Radany and Faith Sandretzky), a study was conducted at the Bellingham FH (Bellingham, WA) using rainbow trout as the test species. Moribund fish from each test tank were diagnosed with columnaris by Dr. Varney, the 10-d treatment period began, and after the 14-d posttreatment period mean percent cumulative mortality in treated tanks (18.4%) was lower than that in control tanks (30.4%).

We anticipate that CVM will accept this study. The Final Study Report was submitted to CVM in late October along with a letter requesting that the effectiveness technical section for the following claim be considered complete: *"...to administer Aquaflor® in feed at a concentration of 10 mg florfenicol per kg fish body weight for 10 consecutive days to control mortality due to columnaris disease in **all** freshwater-reared salmonids*."

If CVM agrees with this request, the expanded label claim for Aquaflor® will cover use to control mortality in freshwater-reared salmonids due to coldwater disease, furunculosis, and columnaris disease. Check the AADAP website for updates.

Largemouth bass/systemic columnaris study: In our world, too sick is not necessarily a bad thing. Mike Matthews (Richloam Fish Hatchery) experienced a columnaris outbreak in some largemouth bass that quickly became systemic. He called and asked us if we would mind if he tried to conduct a field effectiveness trial using

Aquaflor® to control mortality. How could we refuse?

With assistance from Dr. Roy Yanong (University of Florida's Tropical Aquaculture Lab; Ruskin, Florida, USA), the crew at Richloam successfully completed a study to demonstrate the effectiveness of Aquaflor® when administered at a dose of 10 mg florfenicol per kg fish body weight per day for 10 days to control mortality due to columnaris disease in largemouth bass.

At the end of the 14-d posttreatment period, mean percent cumulative mortality in treated tanks (6.6%) was significantly lower (P = 0.0171) than that in control tanks (14.3%). The Final Study Report was submitted to CVM in September requesting review, and we anticipate that it will be accepted (at a minimum) as providing supportive evidence for this effectiveness claim.

Target Animal Safety Research Study Protocols: AADAP recently submitted two research study protocols to CVM for review. The protocols were developed to describe procedures to evaluate the safety of Aquaflor® to 1) yellow perch, and 2) sunshine bass. Although the protocols are very similar, the study on yellow perch will be conducted in its entirety at AADAP's GLP lab in Bozeman, Montana, and the in-life phase of the sunshine bass study will be conducted at USDA-ARS's Stuttgart National Aquaculture Research Center (SNARC; Stuttgart, Arkansas).

AADAP staff will travel to SNARC to help launch the sunshine bass study, and return again to assist with study termination and the collection of fish tissues for histological evaluation. During AADAP's absence during the remainder of the in-life phase of the study, Dr. Dave Straus (SNARC) will be responsible for all day-to-day study activities. We anticipate that successful completion of these two studies, along with "data-mining" of existing TAS data on freshwater-reared salmonids, will satisfy all target animal safety data requirements to allow use of Aquaflor® at a concentration of 15 mg florfenicol per kg fish body weight in all freshwater-reared finfish.

Key researcher moving on: Dr. Vaughn Ostland, the current Director of Aquatic Pathology at Kent SeaTech Corp., has for many years played a key role in adding to the aquatic animal health knowledge base. In particular, Dr. Ostland has focused considerable attention and effort on the development of finfish biologics and effectiveness testing of prospective drugs. Although not an official member of AADAP, Dr. Ostland (OK….let's just call him Vaughn) is probably about as close as one could (or would choose to?) get. Vaughn is practically a founding principal of the FWS/AADAP's Annual Aquaculture Drug Approval Coordination Workshop, having attended 12 out of 14 Workshops. During the last 10 years or so, AADAP and Vaughn have "hooked-up" numerous times on a plethora of drug approval-related ventures (also known as pivotal studies). Some worked, and some didn't. None-the-less, Vaughn always gave the best he, and Kent SeaTech, had to offer…..and together we accomplished a lot! Most recently, AADAP and Vaughn were laying the groundwork necessary to conduct a pivotal Aquaflor® target animal safety study on hybrid striped bass. Unfortunately for all of us, Kent SeaTech Corp. is undergoing significant restructuring and Vaughn will soon be leaving their employ. Hence, part of the reason for the aforementioned sunshine base target animal safety study being now planned for SNARC. AADAP would like to take this opportunity to express our sincere gratitude to Vaughn for his valuable contributions to the field of aquatic animal drug approval research. We also thank him for his friendship. Although there has been no word as to where Vaughn will hang his hat next, we are certain our community of researchers will continue to gain from his contributions. Good luck Vaughn!

Halamid® (chloramine-T) update:

Largemouth bass/external columnaris efficacy study #2: With the help of the crew at Richloam Fish Hatchery (Webster, Florida, USA), a study was conducted in which chloramine-T was administered on three consecutive days to control mortality due to external columnaris in fingerling largemouth bass. After review by CVM, we were asked to revise the Final Study Report and address one issue that had not been adequately described in the original submission, and to reanalyze the data using a "worst-case scenario" approach. With the help of CVM's Biometrics Team, we were able to revise our fish mortality database and reanalyze the data. The revised FSR was submitted to CVM on 11 July 2008 with a request to consider the effectiveness technical section for the following claim to be complete *"...to administer chloramine-T at a concentration of 20 mg per L in a flow through or static bath for 60 min per day on three consecutive days to control mortality due to external columnaris in largemouth bass."* We await a response from CVM.

Bluegill/external columnaris study: Again, with the help of the crew at Richloam FH (thanks Mike

Matthews, Kathy Childress, Josh Sakmar, and Justin Elkins), another chloramine-T study was successfully completed. After sampling moribund bluegills from the reference population, columnaris was presumptively diagnosed as causing the mortality and morbidity. Fish were transferred to test tanks, and fish in three tanks received 20 mg per L chloramine-T for 60 min per day on three alternate days, and fish in three control tanks received a sham water treatment. At the end of the 14-d posttreatment period, mean percent cumulative mortality in treated tanks (12.9%) was significantly lower (P = 0.0304) than that in control tanks (26.9%). The ensuing Final Study Report was submitted to CVM in July 2008 summarizing the study conduct and results. We anticipate that CVM will agree that results from this study demonstrate the effectiveness of chloramine-T to control mortality due to external columnaris in bluegill when administered on three alternate days. However, official CVM review is still outstanding.

Largemouth bass/external columnaris study #3: With very little prodding, the gang at Richloam FH conducted one more study with chloramine-T to control mortality due to external columnaris in fingerling largemouth bass. This time, treatments were administered 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 = 0.0034) than that in control tanks (62.3%). All other study parameters were considered acceptable, and we anticipate that CVM will agree that results from this study demonstrate the effectiveness of chloramine-T to control mortality due to external columnaris in largemouth bass when administered on three alternate days.

The Final Study Report was submitted to CVM in early October along with a letter requesting that the effectiveness technical section be considered complete for the following claim be considered complete *"… to administer chloramine-T at a concentration of 20 mg per L in a flow through or static bath for 60 min per day on three alternate days to control mortality due to external columnaris in* ***all warmwater finfish****."*

If CVM agrees with this request, the initial label claim for chloramine-T will cover use to control mortality due to 1) bacterial gill disease in all freshwater-reared salmonids, and 2) external columnaris in walleye and all warmwater finfish. Stay tuned.

Oxytetracycline (OTC) update:

OTC medicated feed for marking pivotal study scheduled: In previous AADAP Newsletters, we've discussed our efforts, using INAD-generated data, to expand the current OTC skeletal-marking label claim from Pacific salmon to all freshwater-reared salmonids. CVM's response to our original submission basically stated that the INAD data were acceptable as supportive, but one pivotal effectiveness study on a representative freshwater-reared salmonid would be required to expand the label to all salmonids. AADAP, in coordination with the drug's sponsor Phibro Animal Health, has begun the work to complete the required study.

This fall, AADAP will submit to CVM for review a pivotal study protocol designed to evaluate the efficacy of Terramycin® 200 for Fish (oxytetracycline dihydrate) Type A Medicated Article (TM200; 200 g OTC per lb) for the following new indication *"… to administer TM200 orally in feed at 2.5 to 3.75 g OTC per 100 lbs fish per day for 10 consecutive days for the skeletal marking of all freshwater-reared salmonids for subsequent identification*."

If the review process goes smoothly (i.e., few substantive changes), AADAP will conduct a single study in which TM200-treated feed will be administered to test tanks of large fingerling/small juvenile rainbow trout (a representative salmonid) using the above-described treatment regimen. The in-life phase will comprise a 1-d acclimation period, 10-d treatment period, and 21-d posttreatment period. During the 10-d treatment period, TM200-treated feed will be administered to treated tanks and control feed (nontreated) will be administered to control tanks. During the 21-d posttreatment period, control feed will be administered to all tanks. On posttreatment day 21, test fish will be euthanized and vertebrae will be removed for OTC mark evaluation.

Evaluation of OTC marks (absence/presence and quality of a yellow fluorescent ring on a vertebral centrum) will be conducted in partnership with Montana Fish, Wildlife and Parks, and accomplished by viewing vertebrae under ultraviolet light and a dissecting scope. The null hypothesis to be tested is that the mean percentage of fish with marked vertebrae in Terramycin® 200-treated test tanks is equal to the mean percentage of fish with marked vertebrae in control test tanks. The alternative hypothesis to be tested is that the mean percentage of fish with marked vertebrae in Terramycin® 200-treated test tanks is not equal to the mean percentage of fish with marked vertebrae in control test tanks. The difference between treated and control tanks will be considered significant if $P < 0.05$.

35% PEROX-AID® (hydrogen peroxide) update:

Successful studies conducted on largemouth bass and bluegill: Not being able to keep the

Richloam FH gang from forging ahead, two pivotal field efficacy studies were successfully conducted that will assist to complete the data requirements needed to fulfill the following label claim *"...administer 35% PEROX-AID® at a concentration of 50–75 mg per L in a flow through or static bath for 60 min per day on three alternate days to control mortality due to external columnaris in all warmwater finfish fingerling and adults (50 mg per L for fry)."*

The first study was conducted on largemouth bass, and at the end of the 14-d posttreatment period mean percent cumulative mortality in treated tanks (49.0%) was significantly lower (P = 0.0085) than that in the control tanks (74.1%).

The second study was conducted on bluegill, and at the end of the 14-d posttreatment period mean percent cumulative mortality in treated tanks was lower (10.3%) than mortality in control tanks (20.0%). Preliminary analysis indicates that a significant difference will be detected.

If CVM agrees with this request, the expanded label claim for 35% PEROX-AID® will cover use to control mortality due to 1) bacterial gill disease in all freshwater-reared salmonids, and 2) external columnaris in all cool- and warmwater finfish. Stay tuned.

FINS & TAILS, BITS & BOBBERS

2009 INAD Sign-up Forms are now available: Once again it is that time of year for renewal of your facility's INADs for Calendar Year 2009. Please send in the completed sign-up forms to the AADAP Office by 31 December 2008. Invoices will be mailed out the end of February. All 2009 sign-up forms are available on our website at http://www.fws.gov/fisheries/aadap/SIGNUP.htm.

Examples of completed INAD forms are now available on the AADAP website: An completed example of every INAD Form is now available on the appropriate INAD drug fact sheet. These forms have been "mocked up" in order to aid Investigators in completing their INAD paperwork. Please use these forms as a guideline, and if you have any questions do not hesitate to contact Bonnie Johnson at 406-994-9905.

End of the Year INAD Forms due: If you have not already done so, please send in all Form 2's (Drug Inventory Form) and Form 3's (Results Report Form) for each of the INADs that were used at your facilities for INAD Year 2008. For the 17-α Methyltestosterone Medicated Feed participants, Form 6 (Year End Efficacy Report) will also need to be submitted.

AFS-AADAP "Aquaculture Drug-use Guidance" Poster statistics and its use in the field: Earlier this summer, the AADAP Program, in coordination with American Fisheries Society (AFS) Fish Culture and Fish Health Section, produced and distributed a quick reference guide poster "Approved Drugs for Use in Aquaculture".

David Oviedo; Hotchkiss National Fish Hatchery; Hotchkiss, Colorado, USA

The request for this outreach tool was deemed a huge success. A total of 485 posters were distributed nationwide. Provided below is a breakdown of distribution:

- 44 states
- 3 foreign countries (Brazil, England, Spain)
- 73% were requested by state/federal employees
- 15% were requested by private entities
- 12% were requested by private sector organizations, students, and retirees

As noted in the 'What's Shakin' section of this edition of the Newsletter, plans are already underway for the printing of an updated version of the Poster. Check AADAP's website for news of its publication and information on how to obtain copies.

Just a heads up to all of you participating in the National INAD Program: Bonnie Johnson will be on leave for 3 months starting around mid-October 2008. Please fax study worksheets to the AADAP Office instead of emailing or mailing them during this time. Please note if you have any pressing matters during this three month period to call Dave Erdahl at 406-994-9904.

FEATURE ARTICLE

AADAP's website: User statistics & what we've learned from them

Thomas Bell

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

The USFWS - Aquatic Animal Drug Approval Partnership (AADAP) Program website was designed, and has been evolving, with you in mind. When we began this project we had several goals and objectives

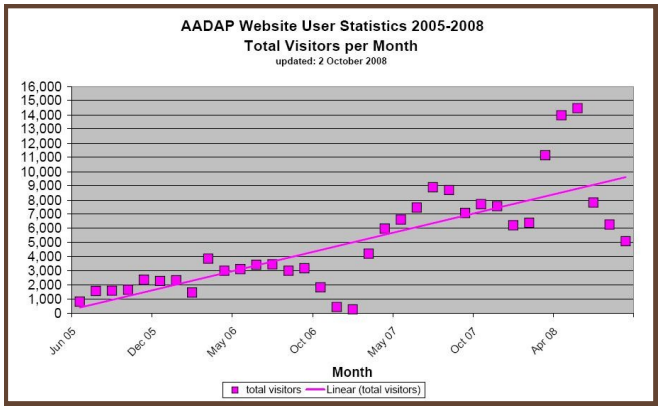
in mind, all of which we felt had your interests at the forefront.

1. Facilitate the approval of new drugs for aquaculture by allowing others to learn from AADAP's and others' experiences. That is, serving as an information- and experience-exchange forum for all those working in the aquatic animal drug approval arena.
2. Provide, to those participating in the FWS Investigational New Animal Drug (INAD) program, as much information as we can to make their involvement as easy and meaningful as possible.
3. Provide "one-stop-shopping" for information on aquatic animal drug approval activities, drug-use guidance, and drug approval status.
4. Be as dynamic, up-to-date, and user-friendly as possible.

With all due modesty, we believe that we have been reasonably successful at achieving most of our objectives. However, as none of our objectives have a defined endpoint, we will continue to work to improve upon the website through internal efforts and feedback from you'all, our partners.

Although it may be rather difficult, if not impossible, to measure the website's contribution to facilitating new drug approvals via information exchange, there has been several new aquaculture drug approvals or label claim additions within the last couple years (e.g., florfenicol, oxytetracycline and hydrogen peroxide). In fact, these recent new drug approvals (or new claims for existing approved drugs) are the first within more than a decade. The aquaculture drug approval "consortium" has always been a relatively small group, however, within the past few years it has certainly grown as a number of research entities, public and private hatchery facilities, and pharmaceutical sponsors have voluntarily thrown their hats into the ring. We would like to assume that part of the reason for their recent forays into aquaculture drug approval activities has been the ready availability of information (e.g., draft study protocols, notice of success stories, and contact information), much of which is available on the AADAP website. Not only has AADAP's information exchange grown during its four-year history, but that of most of our partners has as well.

The below "Total Visitors per Month" graph represents what might be interpreted as an overall increase in web-based activity associated with AADAP's website. There is no question that during the life of the AADAP website, the amount of drug approval-specific information being placed on the site has increased. In turn, we believe that the greater number of visitors to the site (as the graph indicates) may very well be attributed to this appreciable increase in the data and information available on the site. We are of the opinion that this apparent steady increase in activity has very likely had a positive impact on the quality and quantity of data being submitted in support of New Animal Drug Applications (NADAs) for aquaculture drugs.



As it relates to the U.S. federal, state, tribal and private aquaculture entities enrolled in any of the numerous U.S. Fish & Wildlife Service-administered INADs, AADAP's website has not only made their participation less difficult for AADAP to administer, but it has also functioned to improve the quality and quantity of data available to support expanded label claims for existing or new drugs. The latter in part due to the ease by which participants can obtain the required forms, and the volume of information available on AADAP's website that has direct applicability to the conduct of INAD studies in general, as well as specifically for any given investigational drug.

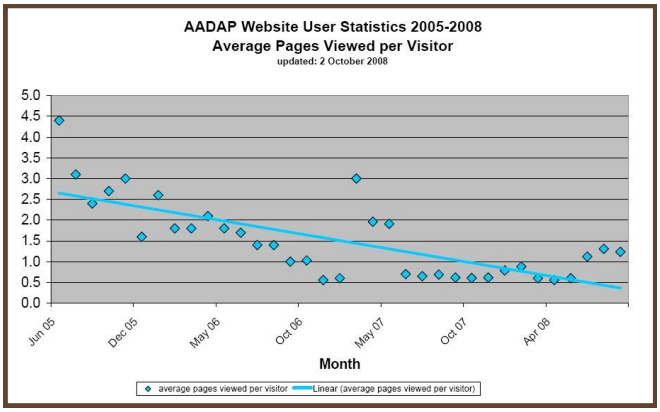
As previously reported in the Newsletter, the AADAP website will become, within the next year, even more integral to the management of FWS-administered INADs. Although not mandatory, participants in FWS-administered INADs will be initially provided the option that the entire enrollment and reporting process be totally web-based. Our hope is that web-based INAD participation will not only make the process easier, more straight-forward, and less time consuming for participants, but also increase the efficiency and accuracy of AADAP's mandatory reporting to FDA/CVM. Planned tutorials and other AADAP-organized training should assist in the latter.

Although we have our opinions, you are the true judges of whether or not AADAP has created, maintained and updated a website that offers, as claimed, "one-stop-shopping" for information pertaining to aquatic animal drug approvals in the USA. Notable additions to the AADAP website since its inception include, but are limited to: 1) downloadable INAD forms, 2) example study protocols and standard operating procedures (SOPs) from several laboratories, 3) a repository for the National Aquaculture Drug Research Forum's and the Association of Fish and Wildlife Agencies' Drug Approval Working Group's information and products, 4) dedicated pages for information on aquaculture biologics, and 5) presentations from all of the recent annual

Aquaculture Drug Approval Coordination Workshops. As noted earlier, and as depicted in the previous graph, there does appear to be a steady increase in the number of visitors to the AADAP website. If this increase in not due to more people having found it useful or that people have found more things on it that are useful and hence return more often, then we may be hard-pressed to come up with a reason for the apparent increase.

Our last, and certainly not least, goal/objective for the AADAP website has been to make it as dynamic, up-to-date, and user-friendly as possible. Although we have received numerous emails, etc. commending us on AADAP's website (not only as it relates to functionality, but on content as well) the number of comments pale in comparison to the number of visitors to the site. Hence, it has been somewhat difficult to gauge the actual response of users to: what is on the site, how useful is it, how difficult (or easy) is it to access, have changes and additions to features and information made it more or less difficult to use, is the information accurate and up-to-date, etc. Unfortunately, here is where we have to make decisions (regarding the website) on inferential information provided in the form of user statistics, such as that represented in the previous graph and that represented in the graph below.

Although the below graph may be perceived as depicting an unacceptable trend, we believe that it represents quite the opposite. We believe that a decreasing (over time) average number of pages visited by each visitor suggests that at least three positive things may be happening. First, many AADAP website visitors are becoming "educated" as to what is available on the site and how to get to where they want to go with the least amount of searching. Second, changes made to the site, including built-in redundancy, have made navigation less complicated. And third, we have to date been relatively successful in making the website as dynamic, up-to-date, and user-friendly as possible.



AADAP regularly obtains users statistics, provided by the U.S. Fish & Wildlife Service's information technology branch. The two incorporated graphs were generated from a portion of these user statistics. In addition, we internally generate several other graphs from the user-statistics package we receive. Although we believe that most of the graphs and their associated statistics can be useful, some can be misleading. Consequently, we have only included the two we believe to most accurately represent AADAP website use-patterns. As an interesting, and as yet unconfirmed aside, the "Total Visitors per Month" graph (previous page), as well as several other graphs generated from the user stats, appears to portray something of a seasonal pattern. As the AADAP website gets a few more seasons under its belt, we may be able to explain this cyclical behavior with some level of confidence.

In summary, the AADAP website (and our complementary Newsletter) will continue to play a major role in AADAP's mission "*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 (FDA) approval for drugs, chemicals, and therapeutants needed in aquaculture and fisheries management programs,"* and we will strive to fulfill the goals and objectives of our website. As always, if you have any comments or suggestions, please do not hesitate to contact us.

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 from 2008. Please note that this list does not include those provided in previous issues of the AADAP Newsletter.

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 technique to any particular situation, (2) concurrence with a treatment procedure/drug, (3) acceptance by 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.

Adebayo, OT, and Popoola, OM. 2008. Comparative evaluation of efficacy and cost of synthetic and non-synthetic hormones for artificial breeding of African catfish, *Clarias gariepinus* (Burchell, 1822). *Journal of Fisheries and Aquatic Science* **3(1):**66-71.

Aly, SM, et al. 2008. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of *Tilapia nilotica* (*Oreochromis niloticus*) to challenge infections. *Fish & Shellfish Immunology* **25(1-2):**128-136.

Avendano-Herrera, R, et al. 2008. Evolution of drug resistance and minimum inhibitory concentration to enrofloxacin in *Tenacibaculum maritimum* strains isolated in fish farms. *Aquaculture International* **16(1):**1-11.

Banavreh, A, et al. 2008. Effects of hydrogen peroxide on fungal disinfection, hatch rate and larval deformities of rainbow trout (*Oncorhynchus mykiss*). *Iranian Scientific Fisheries Journal* **16(4):**163-168.

Dougherty, AB. 2008. Daily and sub-daily otolith increments of larval and juvenile walleye pollock, *Theragra chalcogramma* (Pallas), as validated by alizarin complexone experiments. *Fisheries Research* **90(1-3):**271-278.

Durham, BW, and Wilde, GR. 2008. Validation of daily growth increment formation in the otoliths of juvenile cyprinid fishes from the Brazos River, Texas. *North American Journal of Fisheries Management* **28(2):**442-446.

Feng, JB, et al. 2008. Tissue distribution and elimination of florfenicol in tilapia (*Oreochromis niloticus* × *O. aureus*) after a single oral administration in freshwater and seawater at 28ºC. *Aquaculture* **276(1-4):**29-35.

Gomez-Jimenez, S, et al. 2008. Oxytetracycline (OTC) accumulation and elimination in hemolymph, muscle, and hepatopancreas of white shrimp *Litopenaeus vannamei* following an OTC-feed therapeutic treatment. *Aquaculture* **274(1):**24-29.

Hargrave, BT, et al. 2008. A micro-dilution method for detecting oxytetracycline-resistant bacteria in marine sediments from salmon and mussel aquaculture sites and an urbanized harbour in Atlantic Canada. *Marine Pollution Bulletin* **56(8):**1439-1445.

Kang, IJ, et al. 2008. The effects of methyltestosterone on the sexual development and reproduction of adult medaka (*Oryzias latipes*). *Aquatic Toxicology* **87(1):**37-46.

Meinertz, JR, et al. 2008. Chronic toxicity of hydrogen peroxide to *Daphnia magna* in a continuous exposure, flow-through test system. *Science of the Total Environment* **392(2-3):**225-232.

Nakaya, M, et al. 2008. Validation of otolith daily increments for larval and juvenile Japanese halfbeak *Hyporhamphus sajori*. *Fisheries Research* **93(1-2):**186-189.

Poapolathep, A, et al. 2008. Distribution and residue depletion of oxytetracycline in giant freshwater prawn (*Macrobrachium rosenbergii*). *Journal of Food Protection* **71 (4):**870-873.

Rhodes, LD, et al. 2008. Characterization of *Renibacterium salmoninarum* with reduced susceptibility to macrolide antibiotics by a standardized antibiotic susceptibility test. *Diseases of Aquatic Organisms* **80(3):**173-180.

Rosenblum, ES, et al. 2008. Efficacy, tissue distribution, and residue depletion of oxytetracycline in WS-RLP infected California red abalone *Haliotis rufescens*. *Aquaculture* **277(3-4):**138-148.

Sharif Rohani, M, et al. 2008. A study of the anesthetic effect of *Zataria multiflora* Boiss (Labiatae) essence on *Oncorhynchus mykiss* and cultured *Salmo trutta caspius*. *Iranian Scientific Fisheries Journal* **16(4):**99-106.

Treble, MA, et al. 2008. Growth analysis and age validation of a deepwater Arctic fish, the Greenland halibut (*Reinhardtius hippoglossoides*). *Canadian Journal of Fisheries and Aquatic Sciences* **65(6):**1047-1059.

Vendrell, D, et al. 2008. Minimum inhibitory concentrations of erythromycin in *Lactococcus garvieae* strains isolated from cultured rainbow trout (*Oncorhynchus mykiss*) in Spain. *Bulletin of the European Association of Fish Pathologists* **28(3):**125-128.

Vincent, M, and Thomas, KJ. 2008. Nuptial colouration and courtship behaviour during induced breeding of the swamp barb *Puntius chola*, a freshwater fish. *Current Science* **94 (7):**922-925.

Woods, LC, et al. 2008. Efficacy of Aqui-S® as an anesthetic for market-sized striped bass. *North American Journal of Aquaculture* **70(2):**219-222.

Wu, T, et al. 2008. Medication of the tremor disease in Chinese mitten crab *Eriocheir sinensis*. *Fisheries Science* **27(7):**325-329.

USGS's CORNER

UMESC expands environmental safety assessment capabilities: As a follow up to the posting in the previous newsletter, UMESC conducted another mussel survival and growth study. The previous posting stated that an effort was underway to expand our environmental safety capabilities by investigating methods to chronically expose juvenile mussels to pharmaceutical agents, including those used in aquaculture. According to published reports, of

the nearly 300 taxa of freshwater mussel (unionids) populations in North America, 70 species (23%) are listed as endangered or threatened and another 40 species (14%) are candidates for listing as endangered or threatened. The causes for the gradual loss of unionid abundance and diversity have not been well characterized. As part of an effort to characterize potential risk associated with human and veterinary (including aquaculture) drug use for mussels in the St. Croix National Scenic Riverway, we are developing methodology to assess the chronic toxicity of drugs with juvenile mussels, an organism sparingly used as a test organism. This methodology may provide researchers the ability to enhance future environmental assessment submissions with unique data, supplying environmental assessment reviewers the pertinent data needed to make informed decisions.

It is generally understood that the earliest life stages of aquatic organisms are regarded as the most sensitive life stage. Our studies were designed to assess the survival and growth of newly transformed mussels in a flow through test system where the mussels could be continuously exposed to drugs for extended periods of time. In this latest study, largemouth bass (Micropterus salmoides) were infested with glochidia from plain pocketbook (*Lampsilis cardium*) and fatmucket (*L. siliquoidea*) mussels. One day after juvenile mussels (termed transformers) dropped off the fish, transformers were siphoned from the bottom of aquarium and transferred to test chambers (40 of one species per chamber) containing 200 mL of well water and 4 mm of silica sand. The test system contained 60 chambers, 30 chambers with plain pocketbook and 30 with fatmucket mussels. For each species, the 30 chambers were separated into 5 blocks of 6 chambers (2 x 3 configuration) with each chamber in a block receiving 1 of the following 6 food types prepared with Reed Mariculture concentrated algal products: (1) *Nannochloropsis* sp., (2) *Nannochloropsis* sp. and *Tetraselmis* sp., (3) *Nannochloropsis* sp., *Tetraselmis* sp., and *Chlorella* sp., (4) *Nannochloropsis* sp. and *Thalassiorsira weissflogii*, (5) *Nannochloropsis* sp. and *Pavlova* sp. and (6) *Nannochloropsis* sp., *Thalassiorsira weissflogii*, and *Pavlova* sp. The nominal water temperature was 21°C. After 28 days of continuous feeding in the flow through system, chambers were surveyed for live and dead mussels; live mussels were retained for growth measurements.

For each species, the overall mean recovery of live and dead mussels from the test chambers exceeded 83%. For the plain pocketbook, mean survival for each food type ranged from 12% (food type 5) to 66% (food type 1). For the fatmucket, mean survival for each food type ranged from 35% (food type 6) to 81% (food type 3). The plain pocketbook mean valve length ranged from 437 µm (food type 2) to 612 µm (food type 3). The fatmucket mean valve length ranged from 464 µm (food type 2) to 643 µm (food type 3). The plain pocketbook mean growth rate ranged from 5.5 µm per day to 11.8 µm per day. The fatmucket mean growth rate ranged from 6.3 µm per day to 12.7 µm per day.

This study is one of the first attempts to develop methods to culture juvenile mussels for a sufficient time period post-transformation to conduct chronic toxicity assays. Because of the relatively good survival and growth data with the fatmucket mussels, we are confident that this species and the techniques we developed can be successfully used to conduct chronic toxicity studies of pharmaceutical compounds. These impending studies will enhance our ability to appropriately assess the risk of both human and veterinary pharmaceutical compounds to native mussel populations. (*Contact: Jeff Meinertz, UMESC; jmeinertz@usgs.gov*)

UMESC completes erythromycin thiocyanate toxicity study: As part of an effort to characterize potential risk associated with human and veterinary (including aquaculture) drug use for mussels in the St. Croix National Scenic Riverway, UMESC conducted a chronic toxicity study exposing Daphnia to two pharmaceutical compounds, diphenhydramine hydrochloride (DH) and erythromycin thiocyanate (ET). Both DH and ET were identified in surface water samples collected in Wisconsin and have the potential to impact native mussels in the St. Croix National Scenic Riverway. Erythromycin thiocyanate is also presently under development as an aquaculture drug to control bacterial kidney disease. The objective of the study was to determine if DH or ET concentrations of approximately 2X, 1000X, and 10,000X of nominal environmental concentrations have an effect on *Daphnia magna* survival and production during a continuous, chronic exposure period of 21 days. In this study, there were 8 treatment groups, a control group, nominal DH concentration groups of 0.12, 71, and 850 µg per L, nominal ET concentration groups of 0.45, 250,

and 3000 µg per L, and a nominal DH:ET concentration group of 84:320 µg per L. Each group consisted of 7 test chambers (volume, about 200 mL) with one Daphnia per chamber. The flow rates through the chambers were continuous at about 2.5 mL per min. The study was initiated when one <24 h old *Daphnia magna* was distributed to each chamber. Daphnia were fed a food designed for aquatic invertebrates twice per day during the week and once per day during the weekends. Samples of water from each treatment group were acquired each day throughout the trial. Chemical concentrations were determined within 4 days of sampling. Survival of first generation Daphnia and young production were monitored daily. The probabilities of death, times to death, times to first brood, numbers of broods, total numbers of young, and length of Daphnia surviving to the end of the trial were compared among treatment groups. Continuous exposure of Daphnia to DH concentrations of ≤ 0.120 µg per L for 21 days did not increase the probability of death or the time to death and did not affect time to first brood, the number of broods, the total number of young produced, and growth. Continuous exposure of Daphnia to ET concentrations of ≤ 248 µg per L for 21 days did not increase the probability of death or the time to death and did not affect time to first brood, the number of broods, the total number of young produced, and growth. Continuous exposure of Daphnia to DH concentrations of 84 µg/ per L and ET concentrations of 318 µg per L increased the probability of death and time to first brood and decreased the number of broods and total number of young produced. The chronic toxicity data generated for *Daphnia magna* are supportive of data for *Ceriodaphnia* sp. which were previously used in the initial ET environmental assessment. The Daphnia data reported here will be incorporated into the ET environmental assessment and should help address the concerns of the U.S. Food and Drug Administration's Center for Veterinary Medicine Environmental Safety Team regarding potential effects of ET on aquatic invertebrates. (*Contact: Jeff Meinertz, UMESC; jmeinertz@usgs.gov*)

Determinative method for p-TSA: Recently, UMESC was notified that the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM) had made a small change in a human food safety issue. Although the change may seem relatively minor to many of our colleagues, the change has a rippling effect on the data previously generated by UMESC. UMESC was notified that the previously proposed tolerance limit of 1000 ppb for the chloramine-T marker residue (p-toluenesulfonamide, p-TSA) was not acceptable and a much lower tolerance was likely to be established. UMESC had conducted a plethora of human food safety studies that had been reviewed and accepted by CVM with the understanding that the marker residue tolerance concentration would likely be 1000 ppb including studies that resulted in the development of a determinative method for p-TSA in fish fillet tissue. Additional work must now be completed to lower the determinative method quantitation limit because the p-TSA tolerance limit will likely be set below the present determinative method quantitation limit (~30 ppb depending on species). UMESC recently initiated discussions with CVM to outline the proposed work required to accomplish this task including identification of the fish species to be used, the proposed method modifications, and the proposed method validation procedures. The development of these data are critical to the completion of the human food safety technical section and the ultimate approval of a new animal drug approval for chloramine-T. (*Contact: Jeff Meinertz, UMESC; jmeinertz@usgs.gov*)

Manuscript accepted: The following manuscript was recently accepted for publication in the journal *Aquaculture*: "*Histopathology of Repeated, Intermittent Exposure of Chloramine-T to Walleye* (Sander vitreum) *and* (Ictalurus punctalus) Channel Catfish." by M.P. Gaikowski, C.L. Densmore and V.S. Blazer. The manuscript will appear in an upcoming issue of the journal.

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

The spawning season for our catfish egg research ended in mid-July and after completing our copper sulfate studies, we finished with a few other fungus control treatments on intact egg masses. We have a great system to conduct egg fungus studies and there are more compounds we plan to look at in the future. Since the end of our busy spawning season, we have been working hard setting up other studies, analyzing data, and you-name-it.

Aquaculture America 2009: Preparations are well underway for the upcoming Therapeutic Drug Research Special Session at Aquaculture America 2009 in Seattle, WA. The session is being organized by Jim Bowker, Mark Gaikowski and Dave Straus, and this will be the 7th consecutive year we have held this session focused on research necessary for aquaculture drug approvals.

Copper Sulfate - Label for Ich: SNARC has partnered with Phelps Dodge (now Freeport-McMoRan) to draft a label for their product. FDA/CVM has concurred with the draft label that we have prepared, and the sponsor will begin the process of moving it through their channels to create a mock-label that they will formally submit to FDA/CVM.

Copper Sulfate - Human Food Safety technical section for Ich: A hazard characterization has been submitted to complete the Human Food Safety technical section for Triangle Brand® Copper Sulfate. This document is in response to FDA/CVM limiting the Human Food Safety technical section complete statement to only channel catfish as opposed to previous technical section complete statements for all finfish. We hope to hear from them soon.

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.

Upcoming meetings

9th International Symposium on Aquatic Nutrition; 24-26 November 2008, Ensenada, B.C. México: This year's symposium is being held at the Hotel Coral in Ensenada (web: http://www.hotelcoral.com; phone: 800-862-9020). The Symposium's format comprises oral presentations given by invited speakers with international recognition in specific nutrition areas, as well as a poster session. Prospective presenters have been requested to focus there presentations on one of the following topics: 1) digestibility and evaluation of ingredients and feeds; 2) nutritional requirements and nutrition of aquatic organisms in different life stages; 3) nutritional metabolism and physiology of aquatic organisms; 4) live feeds for aquaculture; 5) nutrition and feeding of emerging aquaculture species; 6) process and quality control of aquatic products; 7) alternative feed management and feeding strategies; 8) environmentally friendly feeds for sustainable aquaculture; 9) alternative protein sources for aquaculture feeds; 10) additives, attractants and immunostimulants; 11) standardization of chemical and biological analysis for the evaluation of aquaculture ingredients and feeds and 12) other topics related to aquaculture nutrition will be considered and their acceptance will be determined by the scientific committee. For more information refer to their website, by clicking here.

Aquaculture America 2009; 15-19 February 2009; Seattle, Washington, USA: Next year's meeting of the U.S. Aquaculture Society is being held at the Washington State Convention Center in Seattle. AQUACULTURE AMERICA 2009 returns to one of the favorite tourist spots in the world for the only major national aquaculture conference and exposition held in the U.S. The U.S. Aquaculture Society (formerly U.S. Chapter of WAS) joins with National Aquaculture Association and the U.S. Aquaculture Suppliers Association to produce the annual Aquaculture America meetings. These sponsors are joined by the annual meetings of Aquacultural Engineering Society, American Tilapia Association, Striped Bass Growers Association, US Trout Farmers Association, US Shrimp Farming Association and many more associations to make Aquaculture America 2009 the one meeting in the U.S. that you don't want to miss! Refer to the conference website for more information.

Catfish Farmers of America Annual Convention; 5-7 March 2009; Natchez, Mississippi, USA: The 2009 annual meeting will be held at the Eola Hotel in Natchez. During the annual meeting, the 2009 Catfish Culture Research Symposium is being held. The Symposium is intended to be a forum for exchange of scientific and technical information among researchers, extension personnel, catfish farmers and graduate students of aquaculture. The organizers are encouraging contributions on results of specific research

projects as well as reviews of recent advances in technology. Due to the current financial difficulties faced by the U.S. farm-raised catfish industry, preference will be given to those authors whose findings demonstrate a positive economic impact on commercial facilities. For more information on the Symposium contact Jimmy Avery (phone: 662-686-3273; email: javery@drec.msstate.edu; fax: 662-686-3320) For general details on the convention (email: catfishjournalth@bellsouth.net; phone: 601-206-1600; fax: 601-977-9632).

World Aquaculture 2009; 25-29 May 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.

AQUAVET® I & II Courses; 17 May—13 June 2009 and 17-30 May 2009, respectively; Woods Hole, Massachusetts, USA: The detailed announcements for the 2009 AQUAVET® I and AQUAVET® II courses are now available. Both courses are designed for veterinary students and practicing veterinarians who have an interest in applying their veterinary training to aquatic animals. Enrollment is limited and applications are due no later than 15 January 2009. For more information visit their website by clicking here.

Recent meetings

2008 American Fisheries - Fish Health Section Annual Meeting; 9-12 July 2008; Charlottetown, Prince Edward Island, Canada: The Local Organizing Committee Chair, Dr. Dave Groman (University of Prince Edward Island) has assembled (for those for which he has permission) the full presentation or poster and/or the abstract from the poster or presentation. These can be accessed by clicking here or going to the following webpage: http://ocs.vre.upei.ca/index.php/FHS/FHS2008/schedConf/presentations.

ROZ's CORNER

Supplemental Approval for Oxytetracycline Dihydrate (Terramycin® 200 for Fish) received on 6 July 2008: The approval of Terramycin® 200 for Fish (oxytetracycline dihydrate) for controlling mortality due to coldwater disease in all freshwater-reared salmonids and columnaris disease in all *Oncorhynchus mykiss* and for use below 9°C is the result of a cooperative effort among the sponsor, Phibro Animal Health (PAH), federal researchers, and the National Coordinator for Aquaculture New Animal Drug Applications.

The Upper Midwest Environmental Sciences Center (UMESC, U.S. Geological Survey, La Crosse, Wisconsin) 1) supported effectiveness studies by providing feed analyses, 2) developed the environmental assessment based in part on its effluent survey on use in continuous-flow systems, 3) developed the robust analytical methods to detect oxytetracycline in fish tissue, 4) conducted the bridging studies between the official microbial inhibition assay and the HPLC method, and 5) conducted the marker residue depletion studies in salmonids below 9°C. UMESC developed the data with financial support through base funds and the Federal-State Aquaculture Drug Approval Partnership Project that was under the auspices of the Association of Fish and Wildlife Agencies.

The Aquatic Animal Drug Approval Partnership Program (AADAP, U.S. Fish and Wildlife Service, Bozeman, Montana) 1) developed a document to evaluate the microbiological effects on bacteria of human health concern and 2) conducted and coordinated the pivotal and supportive efficacy studies. The U.S. Fish and Wildlife Service's Coleman National Fish Hatchery (NFH), Quilcene NFH, Olympia Fish Health Center, and California/Nevada Fish Health Laboratory aided AADAP in conducting the effectiveness studies.

The National Coordinator for Aquaculture New Animal Drug Applications provided 1) coordination of the approval-oriented activities with all involved partners including the Center for Veterinary Medicine, 2) provided input to PAH's document that assessed the effect of residues in the human intestinal flora, 3) helped PAH in developing its labeling, All Other Information, and Administrative

NADA submission, and 4) helped PAH gain MUMS designation for the newly approved label claims.

The sponsor, Phibro Animal Health (Ridgefield Park, New Jersey), is to be congratulated as well for this success and for investing in this approval. This is a very important approval because it is:

- the first new therapeutic label claims approved for oxytetracycline for finfish in almost four decades,
- the first antimicrobial approved (not counting conditional approvals) for controlling mortality due to columnaris disease in an aquatic species
- the second antimicrobial approved for controlling mortality due to coldwater disease in freshwater-reared salmonids, and
- the first label claim for Terramycin® 200 for Fish to gain designation under the Minor Use and Minor Species Animal Health Act which entitles PAH to seven years of exclusivity for marketing rights.

Progress on Chloramine-T (Halamid® Aqua+): Two initial label claims are close to completion: control of mortality due to 1) bacterial gill disease on all freshwater-reared salmonids and 2) external columnaris disease on walleye and possibly largemouth bass.

- On August 6, 2008, CVM offered two options to complete the Human Food Safety (HFS) Technical Section: 1) provide human intestinal flora data including minimum inhibitory concentration studies on 10 bacterial intestinal flora isolates using the marker residue or 2) improve the determinative method performance so the marker residue can be reliably quantitated to a lower level than currently possible. If this is accomplished, then CVM would assign an 11-day withdrawal time. In addition, CVM may have other options available to complete this technical section. UMESC is in the process of improving the determinative method.
- On 9 and 11 July 2008, AADAP resubmitted efficacy data on the control of mortality in bluegill due to external columnaris disease and in largemouth bass due to external columnaris disease and requested an Effectiveness Technical Section Complete

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

CVM's NOTES

Indexing: it's not about what's for dinner! In enacting the Minor Use and Minor Species Animal Health Act of 2004 (MUMS Act), Congress sought to encourage the development of animal drugs for use in minor species (species other than cattle, swine, chickens, turkeys, dogs, cats, and horses). One of the incentives of the MUMS Act is to provide an entirely new means of legally marketing new animal drugs that have not gone through either the new animal drug application (NADA) approval process or the conditional approval process. This new process is called Indexing. The Index will consist of a list of legally marketed unapproved new animal drugs that have met the requirements of section 572 of the Federal Food, Drug and Cosmetics Act. **Only drugs for use in non-food-producing minor species or in very early life-stages of food-producing minor species will be eligible for Indexing**.

Indexing differs from the regular approval process in that the decision to Index a product for a given intended use will largely be based on the opinion of a panel of experts outside of FDA. In a nutshell, the Indexing process includes three major steps. Each of the three steps involves a review and decision by our Office of Minor Use and Minor Species (OMUMS).

The first step is initiated by a requestor asking that OMUMS determine whether a particular drug for a specific intended use would be eligible for Indexing. This step involves the review of all aspects of the drug's safety other than target animal safety. The first step also involves a review of a comprehensive summary of the manufacturing process to determine whether the requestor has an understanding of current good manufacturing practices (cGMPs), and also whether the requestor has established specifications for the manufacture and control of the new animal drug.

In the second step, the sponsor will ask us to concur with their proposed expert panel members. In this step, OMUMS must determine if the panel, as a whole, is qualified to assess all relevant drug target animal safety and effectiveness information to determine whether

the benefits of using the new animal drug for the proposed use in a minor species outweigh its risks to the target animal, taking into account the harm being caused by the absence of an approved or conditionally-approved new animal drug for the proposed use in that specific minor species.

The third and final step involves the requestor submitting the findings of the qualified expert panel, proposed labeling, etc., along with a request that the drug be added to the Index. If OMUMS concludes that the outside expert panel's findings were sufficiently comprehensive, and the labeling proposed for the product adequately reflects the panel's report, etc., OMUMS will add the product to the Index maintained on the CVM website.

Our Agency published the Index of Legally Marketed Unapproved New Animal Drugs for Minor Species Final Rule in the Federal Register on December 6, 2007. CVM started accepting Indexing submissions February 19, 2008, which was the date on which the rule became effective. Multiple products are now in the process of completing the steps necessary for addition to the Index.

Anyone wishing to Index a drug for use in non-food-producing minor species or in a very early life-stage of a food-producing minor species should carefully read the paragraphs contained in 21 CFR Part 516 Subpart C. This Subpart contains a detailed description of the steps involved to get a drug added to the Index. Anyone with questions regarding Indexing should contact me at 240-276-9331.

Text provided by Dr. Joan Gotthardt, Office of Minor Use and Minor Species Animal Drug Development, Center for Veterinary Medicine, Food and Drug Administration

Who's on first? Dr. Cindy Burnsteel has been selected as the Director of the Division of Therapeutic Drugs for Food Animals in the Office of New Animal Drug Evaluation. This is the position previously held by Dr. Joan Gotthardt before she moved to the Office of Minor Use and Minor Species.

While Dr. Don Prater is Acting Director of the Division of Scientific Support, which includes the Environmental Safety and Biometrics Teams, Dr. Jennifer Matysczak will be the Acting Leader of the Aquaculture Drugs Team (through December 20, 2008). Jen can be reached at 240-276-8338 or jennifer.matysczak@fda.hhs.gov.

The Aquaculture Drugs Team has a new reviewer, Dr. Eric Anderson. A graduate of the Virginia-Maryland Regional College of Veterinary Medicine, Eric is a long-time fish hobbyist and sought out a number of aquaculture-related externships during vet school.

Dr. Matt Lucia has moved on to the Antiparasitic and Physiologic Drugs Team in the same division.

Text provided by Dr. Jennifer Matysczak, Aquaculture Drugs Team, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration.