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 3-2

AADAP NEWSLETTER

July 2007

WHAT'S SHAKIN'

13th Annual Drug Approval Coordination Workshop is just around the corner: If you haven't registered for the Workshop yet, please do so now, as it helps us immensely with the preparation of materials. It's easy to do online and the registration fee is not due until you arrive. This year's Workshop will be held 31 July through 1 August 2007 at the Holiday Inn, Bozeman, Montana. Check-out the link below for information about other activities associated with the Workshop, including two special sessions that will be held in the morning on Thursday, 2 August. First is a meeting of the JSA's National Aquaculture Drug Research Forum, and second is a "Partner Meeting" hosted by the USGS Upper Midwest Environmental Sciences Center (UMESC) that will provide an opportunity for interested folks to provide to UMESC input as to UMESC's future research direction(s). Please see USGS Corner for more information on the Partner Meeting. Co-hosting with AADAP will be USDA's Stuttgart National Aquaculture Research Center. We have a great lineup of distinguished keynote speakers, in addition to an excellent group of frontline aquaculture-drug scientists. For more information, please visit our website. We hope to see you in Bozeman! More information...

Erratum: In the last edition of the AADAP Newsletter (which has since been corrected), we erroneously listed the causative agent of furunculosis as *Aeromonas psychrophilum* instead of the correct bug *Aeromonas salmonicida*. Sorry for the confusion and thanks to the many folks that contacted us regarding the error.

New Website navigation tools: As is the case with most websites, the more information that becomes available, the more difficult it can be to find it; everything doesn't just fall into a couple of neat categories. The AADAP website is no different. In an attempt to make it easier to find information on AADAP's site, we are in the process of modifying the navigation buttons at the top of the homepage. Instead of a single row of buttons, there will be 2 rows. And, instead of a limited number of submenu items that open horizontally, each button will have a vertical drop-down list of submenu items. Each drop-down menu can comprise numerous submenu items. Please, don't be bashful about telling us if you like it or not. The AADAP website will be modified soon and can be found at http://www.fws.gov/fisheries/aadap/home.htm.

Hydrogen peroxide removed from FDA's LRP Drug List: The Food and Drug Administration (FDA) announced recently (2 May 2007) the removal of hydrogen peroxide from the list of Low Regulatory Priority Aquaculture Drugs identified in the Program Policy and Procedures Manual Guide 1240.4200. As a consequence, the only approved hydrogen peroxide product available is Eka Chemicals 35% Perox-Aid®, and its use is limited to only those indications on the approved product label. For more information, refer to CVM's Update dated 2 May 2007.

CVM reviewers visited AADAP: This past May Matt Lucia and Dave Petullo (left to right, below) from FDA's Center for Veterinary Medicine spent a week at the

Bozeman AADAP facilities viewing the aquaculture drug approval process from the "other end of the pipe." Matt is a reviewer with the CVM's Aquaculture Drugs Team, while Dave is reviewer with the Biometrics Team at CVM. Both Matt and Dave got a lot of hands-on fish-handling/squeezing experience, not to mention getting to see the who's, how's and why's of submission packages sent to CVM for review and ultimate inclusion within New Animal Drug Applications. They also had the opportunity visit and experience day-to-day operations at a couple of production hatcheries in MT. Speaking for us here at AADAP, we thought it was an exceptionally valuable experience for all involved. We've also heard that the CVMer's were possibly even "gushing" about the value of their trip when they got back to the DC area.

Designation of cool-, cold- and warmwater fish proposed to CVM: Based on a request from CVM's Aquaculture Drugs Team through the Association of Fish and Wildlife Agencies' Drug Approval Working Group (DAWG), an effort is underway to develop a classification system that will allow fish produced in the public sector to be more clearly defined as either cold-, cool-, or warmwater finfish. When established, such a classification system will facilitate the simplification of testing and product-labeling for representative fish species. From the Public-Sector Aquaculture Production Database, AADAP has determined that there were approximately 100 different fish species produced by public-sector resource agencies during calendar year 2005. AADAP-staff are currently scouring the literature looking for spawning and rearing temperature data for all fish listed in the Fish Production Database. As suggested by the DAWG group, the first attempt at classifying each of the ~100 fish species will be based on spawning temperature. However, issues related to spawning temperature versus rearing temperature have already popped up, and such issues will make this exercise a little more complicated that originally thought. Keep your dial set right where it's at for new information, as it becomes available.

National Aquaculture Drug Research Forum (NADRF) Products: The Effectiveness and Target Animal Safety (TAS) Technical Project Team of the NADRF has generated a few products that will soon be posted on the AADAP website. Look for them under the NADRF navigation button and the "Products" menu item.

1. AADAP developed and submitted to CVM a paper argument requesting that efficacy and/or TAS conducted on either rainbow or steelhead trout be sufficient to satisfy data requirements for all *Oncorhynchus mykiss* (i.e., both rainbow and steelhead trout).
2. A hydrogen peroxide pivotal efficacy research protocol has been developed by AADAP in which a short section was devoted to dealing with concomitant diseases. For those of us struggling to conduct efficacy studies in the field, we know all too well about the difficulties concomitant diseases present. We anticipate that the procedures described in this section will provide us with a little wriggle-room when conducting field efficacy studies in which concomitant pathogens pop-up.
3. Lastly, a document was prepared by AADAP that describes the pathogenesis of a concomitant pathogen that did pop-up during a field effectiveness study (i.e., *Trichodina* spp.). The study was accepted by CVM, in part, because (a) the pathogenesis of this parasite was described and (b) we made a strong case that the prevalence and severity of the concomitant infection did not affect the outcome of the study.

Adios amigo: On 25 June 2007 Jim Peterson, Fish Health Coordinator for Montana Fish, Wildlife, and Parks (MTFWP), hung up his spurs and hit the Retirement Trail. For the past 35 years (all spent with MTFWP) Jim has been a positive and constructive force in our mutual pursuit of enhancing the natural resources we all cherish. Throughout the Northwest part of the country Jim is well known for his steadfast and proactive approach to fish health management. Jim has also been a strong (and willing!) advocate of the INAD/NADA process, and under his direction MTFWP hatcheries have contributed significantly to partnership drug approval efforts. Although Jim will be sorely missed for all of his many talents, it will be his positive personality and "we can do it together" attitude that we all will miss the most. Best o' luck Jim!

Chloramine-T publications: Some of the chloramine-T research that was conducted a few years back is finally being published. The following two manuscripts have been accepted for publication in the *North American Journal of Aquaculture*:

Bowker, JD, L Telles, B David, D Oviedo, and D Carty; "Efficacy of chloramine-T to control mortality in freshwater-reared salmonids diagnosed with bacterial gill disease"

Bowker, JD, D Carty, and MP Bowman; "Inexpensive apparatus to rapidly collect water samples from a linear-design, plug-flow hatchery raceway."

Good news on distribution of VFD drugs: The recent aquaculture drug approvals for Aquaflor® resulted in much needed anti-microbial treatments for salmonid and catfish producers. However, the Veterinary Feed Directive (VFD) drug classification for Aquaflor® has provided some challenges to growers compared to the over-the-counter status of other aquaculture antibiotics. The requirement for a feed mill to receive a VFD directly from a veterinarian prior to shipment in a specific prescribed amount can be very problematic if the infected fish lot is small in number, or if the feed poundage prescribed does not fit within the forty to fifty-pound increments that a feed company typically ships. Fortunately, the vast majority of prescribed VFDs typically deal in tons of feed shipped and can easily be filled directly by the feed company.

The first VFD "prescription" for the Wyoming Game and Fish Department involved bacterial coldwater disease in a very small, but extremely valuable, brood recruitment lot of cutthroat trout. The entire ten-day treatment called for 6 pounds of medicated-feed…total! Since the prescribed amount was well below fifty pounds, the department could not receive feed directly from the mill. In consultation with the Food and Drug Administration and the feed company, an alternative process was settled upon and is now a reality – a feed distributorship. A feed distributorship is an agreement with a feed company to receive medicated-feed directly from the mill to a site where feed is then distributed to fish culture facilities. Under this arrangement, a company or wildlife agency, as a feed distributorship, can receive VFD-medicated fish feed in forty or fifty-pound increments without the requirement of a VFD "prescription."

In turn, the feed distributorship can maintain a feed inventory and distribute the exact prescribed amount to fish culture facilities upon receiving a VFD. This enables a feed distributorship to:

- send small feed prescriptions under a VFD,
- maintain VFD feeds on-hand for anticipated or cyclic fish health issues, and
- deliver feed to a fish culture facility with a short turnaround time.

To become a feed distributorship, a company or wildlife agency:

- must notify the Food and Drug Administration of their intent to become a feed distributorship for VFD feeds,
- must develop an agreement with a feed company to become a VFD feed distributorship as regulated by federal law,
- must established the feed distribution point at a location where fish are **NOT** reared,
- must distribute to a fish culture facility **ONLY** after receiving a VFD,
- may ship VFD-medicated feed to another feed distributorship without a VFD prescription,
- must maintain inventory control, original VFDs and supporting documentation at the feed distributorship site for a minimum of two years.

As with any VFD drug, it is extremely important to have a strong veterinarian-client-patient relationship (VCP) to maintain an effective treatment protocol. The development of a feed distributorship provides the opportunity to allocate specific feed amounts in a short time frame that cannot always be accomplished by a feed mill. Also, the opportunity to network with other feed distributorships to consolidate or utilize inventories can shorten delivery time if medicated feed is not readily available from a feed mill. For the Wyoming Game and Fish Department, a feed distributorship has enhanced our capability to treat any given fish lot, regardless of its number, and has shortened response time on several treatments over the summer.

For further information on developing a VFD feed distributorship, contact Steve Sharon at 307-473-3407 or Steve.Sharon@wgf.state.wy.us. Text and information provided by Steve Sharon, Wyoming Game and Fish Department.

Oxytetracycline (OTC) updates:

New Oxytetracycline approval for skeletal marking: The Food and Drug Administration (FDA) announced on 9 May 2007, via the Federal Register, that Cross Vetpharm Group Ltd (Dublin, Ireland) had received approval for their generic version of an oxytetracycline soluble powder (immersion product) to be used for skeletal marking in finfish fry and fingerlings. Refer to the Federal Register notice for more information.

Submission of a new label: Phibro Animal Health (PAH) is revising their OTC medicated feed label and plans to have a new draft into CVM by August 2007. It will include two new salmonid claims (coldwater disease in all FW-reared salmonids and systemic columnaris in *Oncorhynchus mykiss*) and the old skeletal marking claim. *Text and information provided by Roz Schnick*.

Human Food Safety Technical Section: PAH will request a Human Food Safety Technical Section complete letter soon. PAH has provided to CVM data that supports the use of OTC medicated feed below 9°C, an HPLC method for detection of OTC in tissue, a bridging study for the official microbial inhibition assay with HPLC, a residue depletion study in salmonids, and microbial food safety data (i.e., documentation in response to CVM's Guidance to Industry Documents #152 and #159). *Text and information provided by Roz Schnick*.

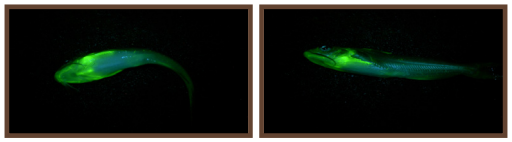
Environmental Assessment: UMESC is currently working on completing the final revision for the environmental assessment for OTC medicated feed and plans on submitting it soon to CVM. CVM has promised a quick turnaround time. *Text and information provided by Roz Schnick*.

All Other Information (AOI) Technical Section: When the aforementioned items are submitted and accepted, all that will be left to be completed before the submission of the Administrative NADA is the AOI. *Text and information provided by Roz Schnick*.

Skeletal marking claim for all salmonids proposed to CVM: AADAP recently (late June 2007) submitted a proposal to CVM, with supporting documentation, to expand the skeletal marking claim for OTC medicated feed. The current label claim for marking only includes treatment of Pacific salmon. Data collected under AADAP's OTC medicated feed INAD (#9332) over the past 8 years demonstrates that three other species of freshwater salmonids can be successfully marked (over 99% of fish treated were successfully marked). It is being proposed that these data should be considered representative for all salmonids. The hope is that if CVM accepts our proposal, PAH will also be able to include this claim on their new label (see above).

Calcein (SE-MARK®) updates:

More pilot immersion marking studies completed: AADAP has been conducting a few "quick and clean"



studies to get some sense of the breadth (species- and life stage-wise) of SE-MARK®'s effectiveness. In two separate study sets, we've evaluated SE-MARK® treatment of channel catfish fingerlings and pallid

sturgeon fry. In the case of the former, 2-3" fingerlings marked exceptionally well with or without a pre-treatment salt bath (see pictures above). However, treatment of 2 to 5-day old pallid sturgeon fry with SE-MARK® (without a pre-treatment salt bath) failed to produce an easily detectable mark. A pre-treatment salt bath was not tested as it was felt that it would be much too stressful on this very early life-stage. As opportunities present themselves, AADAP will continue to test other species and life-stages for their "markability."

Copper sulfate update:

One major technical section left: With the exception of Environmental Safety, all major technical sections for the treatment of Ich in channel catfish (i.e., Product Chemistry, Mammalian Toxicology, Effectiveness, Human Food Safety and Target Animal Safety) have been completed and accepted. An Environmental Assessment (EA) was submitted to FDA/CVM in December 2006, and a response letter was received in June 2007, in which CVM provided comments and requested revisions. These comments will be addressed in the coming months and the EA will be re-submitted. *Text and information provided by Dr. David Straus; USDA, Stuttgart National Aquaculture Research Center.*

Aquaflor® (florfenicol) updates:

Conditional Approval for columnaris in channel catfish: More great news from SPAH!! On 18 April 2007 Schering Plough Animal Health (SPAH) issued a press release, stating that they had received notice from FDA's Center for Veterinary Medicine that their Aquaflor® product had been conditionally approved for use in controlling columnaris in channel catfish. Apart from the difference in disease claim, the labeled-use of Aquaflor® for columnaris is precisely the same as that for treating ESC in catfish (not to mention CWD in freshwater-reared salmonids). However, it is important to note that currently the only product that can be used for the treatment of columnaris in catfish is Aquaflor® - CA1, which is exactly the same formulation as Aquaflor®, but requires special labeling because it does not yet have a full approval.

Conditional approvals (this is the first conditional approval CVM has ever granted for any animal) have exactly the same data requirements as a "standard" approval, with the notable exception that the effectiveness technical section has not yet been completed. The drug's sponsor has 5 years to complete those data, and must show progress on an annual basis. If after the 5 years the efficacy data have not been provided to CVM and accepted, the conditional approval will be withdrawn.

Furunculosis studies complete for all freshwater reared salmonids: AADAP recently received a technical section complete letter from CVM stating that all data required to demonstrate the effectiveness of florfenicol to control mortality caused by *Aeromonas salmonicida* (causative agent for furunculosis) in all freshwater reared salmonids have been accepted. Yeehaw! AADAP has forwarded a copy of the letter and the Final Study Report to Dick Endris at SPAH, and Dick is currently shepherding through CVM the request for approval of Aquaflor® for this disease claim! Stayed tuned.

Systemic Columnaris – We welcome Dr. Scott Foott (Project Leader, USFWS, California/Nevada Fish Health Center) into the inner circle of frustrated and plumb-wore-out researchers trying to conduct field effectiveness studies. Scott launched two studies in recent months to demonstrate the effectiveness of florfenicol to control mortality in steelhead trout caused by *Flavobacterium columnare*. Before Scott began his studies, he received some serious encouragement that "…just ONE more study was needed to complete the work for this claim to include all freshwater-reared salmonids" – and unfortunately, it appears that statement will remain unchanged for the time being…rats!! Without going into any of the gory details, suffice it to say that despite the best intentions and much hard work, both studies ended up without demonstrating meaningful results. Nonetheless, it is extremely important to note that we wouldn't be where we're at today without folks like Scott stepping up to the plate and giving it a try. Many thanks to Scott and his crew for their efforts...and to Scott Hamelberg (Project Leader; Coleman National Fish Hatchery) for kindly providing test fish.

17-α methyltestosterone updates:

Environmental Assessment (EA) mini-meeting: A short meeting to discuss the next steps in the EA process is scheduled to occur in conjunction with the upcoming 13th Annual Aquaculture Drug Coordination Workshop. The EA meeting is scheduled to take place at the Bozeman Fish Technology Center at 2:00 pm on Monday, 30 July (the day before the Workshop begins).

Target Animal Safety studies: A target animal safety study for the use of 17-α methyltestosterone (MT) in feeds for use in sex inversion in tilapia is well under way. The study includes feeding fish diets that contained 0, 60, 180 or 300 mg/kg of MT. The in-life feeding portion of the study is complete, and tissue samples have been collected for histological evaluation by a Certified Fish Pathologist for evaluation. This study is being conducted by Dr. Anita Kelly, Southern Illinois University, and funded by the North Central Regional Aquaculture Center. *Text and information provided by Dr. Anita Kelly.*

MT research conducted at the University of Wisconsin-Madison: To date, UW-Madison researchers have accomplished four (4!!!) major

research objectives in support of the 17-α methyltestosterone approval package. First, they have developed a robust high performance liquid chromatography (HPLC) method to measure MT in fish feed. The method has been published (Marwah et al., 2005, *J. Chromatography* 824:107-115), and accepted by CVM for use in the MT feed stability studies. Second, they have completed a series of stability studies on the fate of MT in fish feed. A key finding is that MT is stable in fish feed stored in the refrigerator or freezer, but degrades by approximately 5% for each 10 days when stored at room temperature. A paper on these results has been accepted for publication in the journal *Aquaculture*. Third, they developed a highly sensitive LC-Mass Spectroscopy (LC-MS) method for detecting extremely low levels (i.e., ppb) of MT in water. Lastly, they have conducted a biodegradation study to determine the fate of MT in water/sediment systems. Two sediment types (clay and sand) were tested under both aerobic and anaerobic conditions. The results showed that MT is rapidly converted into a MT metabolite(s) that becomes strongly associated with the sediment. The half-life of MT in the water column was 2-3 days in the presence of oxygen, and 5-9 days in the absence of oxygen, depending on the sediment type. Final reports of the feed stability and environmental biodegradation studies will be submitted to CVM in July 2007. *Text and information provided by Dr. Terry Barry; University of Wisconsin – Madison.*

AADAP Efficacy studies: As reported previously, AADAP has conducted three studies to demonstrate the effectiveness of 17-α MT (60 mg/kg feed) to produce predominantly male populations of tilapia. Below is an update for each study.

Study 1: In the AADAP Newsletter (Vol. 3 -1), we reported that the Final Study Report (FSR) for the first study (conducted at SeaPac of Idaho) to demonstrate the effectiveness of 17-α methyltestosterone (MT) had been submitted to CVM. Although we have not heard back from CVM in the form of an official response letter, based on discussions with CVM's Aquaculture Drugs Team reviewers we are cautiously optimistic that the FSR will be accepted. As always, we're keeping our fingers crossed.

Study 2: Preliminary results from Study 2, which was conducted at Simaron Freshwater Fish (Waller County, Texas), indicate that treatment of larval tilapia produced >90% males and that the analysis of the medicated feed confirmed that the actual concentration of MT in the feed was within ± 10% of the target concentration. We are currently summarizing the rest of the data and drafting the FSR. We hope this second piece of the 3-piece puzzle fits snuggly. We'll keep you posted.

Study 3: The third and (hopefully) final study (conducted at SeaPac of Idaho) to demonstrate the effectiveness of MT to produce male populations of tilapia has been completed and we're in the process of analyzing the data. One challenge we are again faced with (i.e., here and in the above studies) is explaining the potential impact of scattered immature and/or degenerative oocytes observed in testicular tissue of some MT-treated fish. To assist us (yes…once again hopefully!) in supporting our contention that these scattered oocytes are physiologically inconsequential, we have solicited input from a number of fish histologists, physiologists, and endocrinologists. Thanks to Dr. Terry Barry (Univ. of Wisc – Madison), Dr. Ron Phelps (Auburn Univ.), John Morrison (USFWS – retired), Beth McConnell (USFWS, Bozeman Fish Health Center), and Charlie E. Smith (USFWS-retired) for providing information to help resolve this issue. Once again, stayed tuned.

AQUI-S® updates:

Target Animal Safety studies: The Final Study Report (FSR) summarizing results from a study conducted to demonstrate the safety of 40 and 80 mg/L AQUI-S® to small fingerling cutthroat trout has been reviewed and passed muster by AADAP's Quality Assurance Officer. All QA and GLP compliance documentation has been established and AADAP is in the process of killing a few trees to copy this report in triplicate and package it up for its journey to CVM. Assuming that this study is accepted, it will complete the target animal safety technical section for all freshwater salmonids. Check back in about 6 months for an update.

Updated status of the U.S. Fish & Wildlife Service's AQUI-S® INAD #10-541: Isoeugenol (the active ingredient in AQUI-S®) has been under evaluation by the National Toxicology Program (NTP), a Federal interagency program whose mission is to evaluate chemical agents for potential public health risks. Recently, the NTP was forced to delay (until February 2008) the review of their nearly completed two-year toxicology studies on isoeugenol because of higher priorities. Although the study data have not been fully analyzed, an independent preliminary assessment of the data does not eliminate the possibility that isoeugenol residues in treated fish could pose a human health risk.

Because we need to be absolutely certain that there are no human food safety issues that would preclude the use or approval of AQUI-S®, effective 2 May 2007 the U.S. Fish & Wildlife Service temporarily suspended all field activities under its Investigational New Animal Drug exemption (INAD #10-541) until the NTP review is complete. Although the decision to take such measures was not an easy one to make, it is the most prudent course of action. We look forward to reinstating all AQUI-S® INAD field activities in February 2008.

We appreciate your patience and understanding, and we remain committed to obtaining approval for a zero-withdrawal fish anesthetic for field operations. If you have any questions or comments, please do not hesitate to contact Dr. Dave Erdahl (email: dave_erdahl@fws.gov; phone: 406-994-9904).

Halamid® (chloramine-T) updates:

Efficacy & Target Animal Safety: AADAP recently established a Public Master File or PMF (#005-893) for our Chloramine-T effectiveness and target animal safety data packages. For more information about PMFs please refer to the CVM's NOTES at the end of this Newsletter and CVM's Guidance to Industry Document #57 (*Master Files: Guidance for Industry for the Preparation and Submission of Veterinary Master Files*).

Columnaris in largemouth bass Efficacy studies: HEADLINES…"New Player Involved in Conducting Pivotal Field Effectiveness Trials in Cool and Warmwater Species…" and after talking to Michael Matthews (Richloam Hatchery, Florida Bass Conservation Center; Florida Fish and Wildlife Conservation Commission), we don't know who's more excited about his involvement…AADAP or Michael. At press time Michael is in the final stages of wrapping up a study valuating the effectiveness of chloramine-T to control mortality caused by external columnaris in largemouth bass. Preliminary results look good and we're optimistic that the data will support a broader initial approval (for warmwater finfish) for chloramine-T. Michael also has plans to evaluate the effectiveness of chloramine-T to control mortality due to external columnaris in channel catfish. If results from these two studies are adequate (e.g., significant difference in mortality between treated and untreated groups, dose verification results within ± 25% of the target dose, etc.) and accepted by CVM, then we should be able to complete the effectiveness technical section for the use of chloramine-T to control mortality caused by external columnaris in all warmwater finfish. Although it's a lot to hope for, all we can say is "Go Michael and crew" and a hearty welcome to The Richloam Team!

Revised pivotal Efficacy study protocol submitted: Recently AADAP submitted a revised (i.e., seems few can get their first submission right) chloramine-T efficacy research protocol entitled "A Research Protocol to Determine the Effectiveness of Chloramine-T to Control Mortality Due to Bacterial Gill Disease or External Columnaris in Cool- and Warmwater Finfish" to CVM for review. The protocol was developed so AADAP and its partners can work cooperatively conducting studies needed to complete the effectiveness technical sections for coolwater (one more pivotal study required) and warmwater (one pivotal and one supportive study required) finfish. We were very fortunate to be able to spend time with Dr. Matt Lucia during his visit to Bozeman to go through the CVM original protocol response letter issue-by-issue. It was a great opportunity for AADAP research staff to pick Matt's brain on very specific protocol-related issues and work collaboratively drafting language that will be suitable for protocol concurrence. We hope to hear the good word (protocol concurrence!) later this summer.

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

Columnaris Efficacy study planned: Michael Matthews (Richloam Hatchery, Florida) just can't seem to get enough! In addition to his ongoing and planned chloramine-T activities, Michael also has plans (provided that there are enough test fish) to evaluate the effectiveness of 35% PEROX-AID® to control mortality caused by external columnaris in largemouth bass. One successful study is all that is needed to complete the effectiveness technical section for all warmwater finfish. Dave Lovetro (Eka Chemicals, Inc.) has generously donated several liters of 35% PEROX-AID® for use. Thanks to all…check back later for a progress report.

Pivotal Efficacy protocol ready for submission: The AADAP research staff developed, for CVM's ultimate review, "A Research Study Protocol to Determine the Efficacy of 35% PEROX-AID® (35% hydrogen peroxide) to Control Mortality Due to Bacterial Gill Disease or External Columnaris in Cool and Warmwater Finfish". We are hopeful that by implementing language drafted in response to CVM's review comments from the chloramine-T research protocol (see above), we can get CVM protocol concurrence the first time around on this 35% PEROX-AID® protocol (and yes…that would be a milestone!). The protocol will be submitted to CVM as soon as a FWS INAD number is established. Results from studies conducted under this research protocol will be used to complete the effectiveness technical sections for cool- and warmwater finfish.

FINS & TAILS, BITS & BOBBERS

National INAD participants - Did you know that all INAD forms are available on the AADAP Website? The forms are available in either MS Word or PDF formats and are located at http://www.fws.gov/fisheries/aadap/signup.htm (under the INAD participation forms section). **Please be sure to check this site regularly to ensure you are using the most current version of a specific INAD form**. We are currently submitting several INAD study protocols to CVM for re-authorization, and once approved *newer versions* of forms will be posted on the website. One very good request that an INAD Monitor recently asked all of his Investigators to do was "….please delete/toss/burn all older versions of INAD forms so outdated forms would not be submitted." If you are in doubt as to the status of any INAD form you are using, please check the website!

Heads-up on expiration date for drugs: We're getting reports from some of our INAD participants that product they have ordered and received has an expiration date that extends only 6 to 12 months post receipt. While this

is not an issue with certain drugs, it can be problematic if it is a product that is oftentimes stored for future, periodic use. Upshot...if in doubt, be sure to inquire about the expiration date of a specific product when purchasing to help ensure that you will be able to use it when the need arises.

Size of fish to be treated with 17MT: Although most in the industry know this already, we thought we'd share some information we garnered while researching and conducting studies to demonstrate the effectiveness of MT to produce male populations of tilapia. For best results (and perhaps to minimize the number of intersex fish), treat your tilapia fry sooner, rather than later. AADAP's studies have reaffirmed the work of others showing that the gender of tilapia treated with 17MT at <1.0 cm in length will be more effectively manipulated (i.e., sex-reversed) than those fish treated at >1.0 cm.

FEATURE ARTICLE

Treatment of Microbiologically Polluted Aquaculture Waters by a Novel Photochemical Technique of Potentially Low Environmental Impact

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Porphyrins as Antimicrobial Agents

Our laboratory's current general research plan objective is the development of specific protocols for the decontamination of water that contains potentially dangerous concentrations of pathogenic agents, as well as the prevention of water pollution by undesired microbial strains. The decontamination is accomplished by an innovative technique, already experimented with positive results at a laboratory level and in aquaria. The proposed procedure is based on the combined action of a phototoxic compound (photosensitizer) that brings about a selective action against microbial cells and visible light. The technique is of low environmental impact, because the selected photosensitizers belong to the class of porphyrins, i.e., compounds of natural origin that exhibit no appreciable biotoxicity in absence of light at the doses that are photochemically active.

The necessity to develop innovative methodologies in order to fight or prevent the colonization of various types of water by pathogenic agents of microbial origin (Gram-positive and Gram-negatives bacteria, fungi, mycoplasmas, parasites) becomes more urgent for various reasons:

- the general deficiency of water makes re-utilization and recycling of waters necessary;
- the recent climatic variations favor an important growth of microbial strains, often with partially modified properties as compared with the existing microbial flora;
- the appearance of resistant strains to different antimicrobial treatments currently in use, in particular to the antibiotic treatment, which become established owing to the rapid evolutionary changes of these cells;
- the primary role of water in the preservation, delivery and spread of these pathogenic agents, also as a consequence of the ubiquity of water and its essential role in almost all the vital processes; and
- the dangerousness of microbial infections, above all in underdeveloped countries, with effects on different ecosystems and indirect social costs that presently reach heavy levels.

Such problems concern drinking water or water for irrigation, but also water utilized in fields such as fish-farming, that currently are in a phase of remarkable expansion because of the continuous decrease in the wild fish fauna.

The cytotoxic action on various types of microorganisms performed by the combination of visible light and suitable photosensitizing agents is known since the beginning of the 20th century. In the last decade, the intensification of the investigations on this subject, and the consequent achievement of a better understanding as regards the characteristic features of the photosensitized inactivation of the main classes of microorganisms opened new challenging scenarios for medical and environmental applications: thus, this technique has been proposed for the treatment of localized microbial infections and the sterilization of water. In particular, the molecule of specific photosensitizers has been engineered to induce a high affinity for a broad spectrum of microbial cells; this is a necessary premise to guarantee their selective photo-inactivation.

Several photosensitizing agents have been checked for their phototoxic action toward microbial cells. However, porphyrins appear to possess antimicrobial properties which are superior to those typical of other photosensitizers, especially in the environmental field, where their photo-activation can be often achieved by using sunlight. The main photosensitizing properties of porphyrins can be schematized as follows.

- Possibility to modulate the chemical structure of the porphyrin molecule through a variety of approaches, such as the extension of the tetrapyrrole macrocycle, the insertion of metal ions at the center of the macrocycle, or the attachment of substituents in the peripheral positions of the macrocycle; thus, the possibility exists to fine tune the physicochemical properties of porphyrins, hence to determine their selective or preferential orientation toward specific cells or subcellular targets.
- Broad diffusion of porphyrins in several natural systems (e.g., chlorophyll in plants; the prosthetic group of hemoglobin and cytochromes), so that these molecules are devoid of any significant toxicity against

the vast majority of biological systems at least at photochemically active doses.

- Porphyrins exhibit a very high efficiency in the absorption of essentially all near-UV and visible wavelengths of the solar spectrum. Thus, phototoxic effects are achieved in the presence of porphyrin doses appreciably smaller than those which are active in the case of other photosensitizers that absorb only a fraction of the visible sun spectrum. When porphyrins are used for the photo-sterilization of water, it is appropriate to underline the circumstance that the penetration of visible light into water is far greater than that typical of UV radiations, especially if one has to deal with poorly transparent waters.
- The high photosensitizing efficiency which is typical of many porphyrins and their analogues: this parameter is commonly expressed in terms of quantum yields and it generally ranges between 0.6 and 0.8 for porphyrins, which means that 60-80% of the absorbed photons is actually used to cause a biotoxic action.
- Porphyrin-photosensitized processes involve the generation of hyperactive intermediate species whose lifetime is in the microsecond range; hence, the overall photo-process is characterized by a high time- and space-selectivity: only those sites which are in the close microenvironment of cell-bound porphyrins are actually damaged.
- Porphyrins are typically hydrophobic molecules; hence they predominantly associate with cell membranes: the cell death is consequent to alterations photo-induced at the level of membrane components with no involvement of DNA or other nuclear constituents. This minimizes the risk of inducing the onset of mutagenic effects or selecting photo-resistant microbial strains.

Out of the porphyrins which have been so far tested as antimicrobial photosensitizers, the maximal efficiency has been displayed by those bearing peripheral substituents of cationic nature, e.g. quaternary ammonium salts, pyridinium or morpholinium derivatives which are N-substituted by hydrocarbon chains. The positive charge immediately induces the formation of an electrostatic bond of the porphyrin with the numerous negative charges which are present on the external wall of bacterial and fungal cells; therefore, irradiations can be carried out within very short time intervals after the addition of the porphyrin, i.e. before the porphyrin can appreciably interact with other cells broadly diffused in higher organisms, such as fibroblasts and keratinocytes. This circumstance represents a determining factor for the selectivity of the photo-sterilization process. In general, the possibility exists to cause a 5-6 log decrease of the microbial population in a fluid system by using porphyrin-cell incubation times not exceeding 5 min and irradiation times shorter than 10 min, while the fluence-rate can be kept in the 10-20 mW/cm^2 range and the porphyrin concentrations are in the micromolar range.

The photocidal action of porphyrins is characterized by a broad spectrum of action. Laboratory studies pointed out that an extensive decrease in cell survival can be obtained by photosensitization of both antibiotic-sensitive and antibiotic-resistant bacterial strains (including the meticillin-resistant *Staphylococcus aureus*), fungi such as *Candida albicans* (again including antibiotic-resistant strains) and parasites in the state of both cysts and vegetative cells

Photo-disinfection of Aquaculture Waters: Application to Trout-farming

It is well known that mycotic infections caused by members of Saprolegnia species, as well as by their close water mould relatives, represent a major challenge to a number of freshwater fishes and their eggs. Prevention and control of saprolegniasis are especially difficult, even under fish-farming conditions, owing to the broad diffusion and the rapid spreading of such fungi. Many fungicides that are effective against higher fungi exhibit a low activity against oomycetes, while spores of the pathogen can successfully locate in the fish epidermis and, whilst the majority are removed or inactivated during quarantine in clear water, a significant number of viable fungal units persist on the body surface.

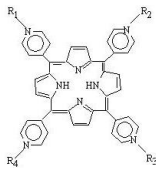
So far, mycotic infections arising in fisheries from *Saprolegnia* spp. have been most frequently treated by the application of malachite green. However, recent concerns about the safety of this derivative led to the banning of its use as a fisheries chemotherapeutant in many parts of the world [editor's note: malachite green is an FDA-prohibited drug in the USA]. At present, formalin, a 24% [editor's note: 37% in the USA] aqueous solution of formaldehyde, represents the most frequently used disinfectant for prophylaxis of finfish eggs and fry, and has been recently proposed as an effective treatment to reduce mortality in infected adult fish. Intensive investigations are being developed in order to find alternative strategies that allow a reasonably cheap and environmentally acceptable control of Saprolegnia infections in hatcheries.

Due to the high phototoxic action of porphyrins against microbial pathogens, it appeared of interest to investigate the efficacy and safety of this technique for the control of Saprolegnia infections in fish-farming pools. In particular, two porphyrins which proved to be very photoactive in laboratory experiments were used: tetra-meso(N-methyl-pyridyl)porphine (C1) and its analogue tri-meso(N-methyl-pyridyl), meso (N-tetradecyl-pyridyl)porphine (C14) (see below).

$R_1=R_2=R_3= CH_3$

$R_4= CH_3$ C_1

$R_4= C_{14}H_{29}$ C_{14}



The experiments with adult rainbow trout, *Oncorhynchus mykiss*, were performed in the pilot plants of the IASMA research facility (Italy). The skin infection on the trout epidermis was obtained by scraping scales from about

2 cm^2 of the dorsal region, between the adipose tissue and the caudal fin, and by direct contact of the lesion with mycelium wads. Presence or absence of the infection was evaluated by the occurrence of cottony-white patches on the lesion of the fish after one and two weeks from the artificial infection procedure.

The treatment of fish with porphyrins and visible light irradiation in pilot plants was performed with a two-fold aim: a) to prevent the onset of fish infections; and b) to treat infections developed by fish that had been naturally exposed to Saprolegnia.

a) Preventive protocol

Four experimental groups of 30 individuals were randomly created starting from the same stock and maintained in separate 1000 l tanks. Through the entire experiment no food was supplied and a daily load of about $4x10^{11}$ zoospores was added to each tank.

In the first group the individuals were not scraped and were maintained as a general control (uninfected control) of the health status of the stock. In the second group the individuals received no other treatment than artificial infection (infected control). In the third group the artificially infected individuals were dark incubated for 10 min with 0.44 µM (0.6 mg/l) C1 doses. The tank was then irradiated for 1 h, with two 500 W quartz-halogen lamps emitting white light (400-800 nm) which were operated at a constant fluence-rate of 50 mW/cm^2. During the whole incubation/irradiation period, the water (total volume 1000 l) was kept in a closed circuit and recirculated by a motor-driven pump, and its temperature was monitored and maintained at 13°C. At the end, the normal flow of circulating water was restarted. The incubation/irradiation treatment was repeated at daily intervals for ten consecutive days, starting from the first day after the infection.

In the fourth group the artificially infected individuals were incubated for 10 min with 0.2 mM (0.3 mg/l) C14 using the above described closed circuit, after which the standard water flow was restored. The procedure was repeated for ten consecutive days, using the same irradiation protocol detailed for C1.

b) Curative protocol

Three groups of 10 naturally infected trout, showing cottony-white patches of mycelium, were used.

The first group was maintained as an untreated control in a 1000 l tank. The second group was dark incubated with 0.6 mg/l C1 for 10 min and irradiated for 1 h using the same water-recirculating procedure as described above. The irradiation was performed by using the 400-800 nm wavelength interval emitted from two 100 W incandescent filament lamps and the water temperature was kept at 13°C throughout the light exposure. The treatment was daily repeated for six consecutive days. At predetermined intervals, water samples were taken for analysis of the microbial charge. The third group was incubated with 0.6 mg/l C14 for 24 h and then treated as specified for C1.

In actual fact, about 27% of inoculated control fish, where lesions had been induced in the dorsal region by scraping, underwent the expected infection by Saprolegnia (Fig. 1a, a more detailed view of the infection is shown in Fig 1a'). The experimental treatment with C1 and light (Fig 1b and 1b') or C14 (Fig. 1c and 1c') following the preventive protocol section determined a reduction of the infected percentage to 10% and 13%, respectively, after one week. These results indicate (a) the feasibility of our experimental approach, and (b) a positive trend as regards the effect of C1 and C14 on the control of saprolegniasis.

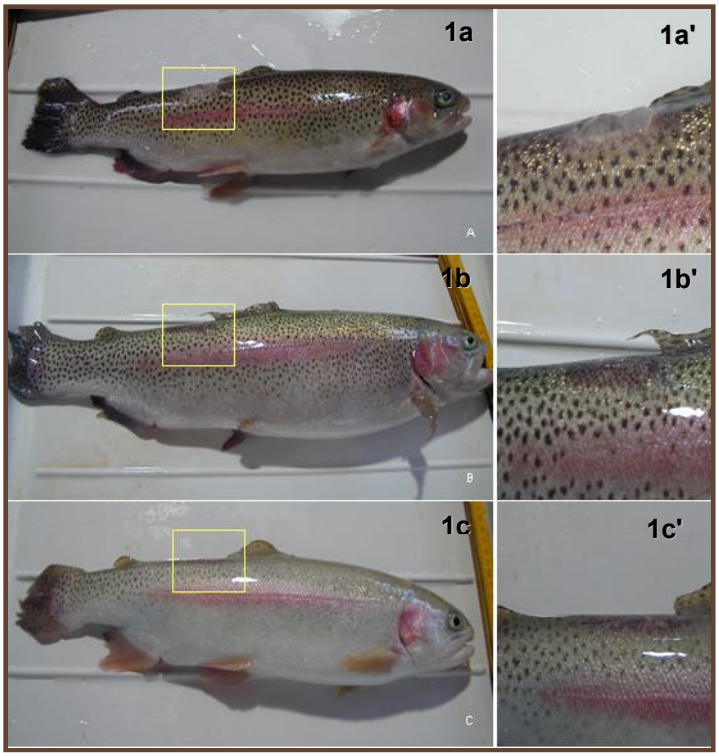


Figure 1. (a) Examples of trout which was infected by inoculation of Saprolegnia into an artificially induced lesion in the dorsal region. The infection appears as a cotton-type mycelial mass as shown in Figure 1a'. (b) Example of a Saprolegnia-infected trout that has been treated by 0.6 mg/l C1 according to the preventive protocol. (c) Example of a Saprolegnia-infected trout that has been treated with 0.3 mg/l C14 according to the preventive protocol. Figures 1b' and 1c' show no detectable appearance of fungal infection.

When trout with spontaneous Saprolegnia infections (Fig. 2a), are exposed to C1 or C14 and visible light according to the curative protocol, a complete remission of the infection was induced within one week. This was followed by the complete healing of the ulcerated lesion, which had been formed after elimination of the mycelial mass (Fig. 2b). Analysis of the water samples obtained from such plants before and after the treatment showed that the treatment with C1 and C14 induced an about 3 log decrease in the population of the overall microbial population.

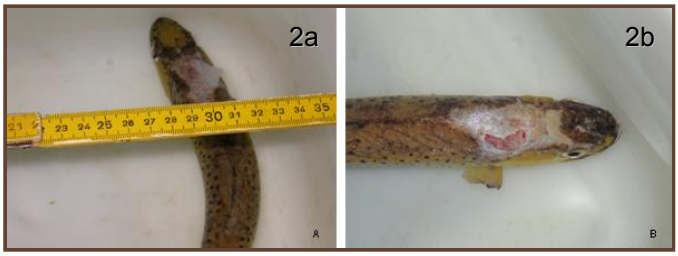


Figure. 2 (a) Example of Saprolegnia infection spontaneously developed in farmed trouts. (b) The same infected trout after 1 week treatment with 0.6 mg/l C1 + white light according to the curative protocol, showing the total disappearance of the mycelium, which was then followed by a complete healing of the initially formed ulceration.

Remarkably, this result was achieved by using mild experimental conditions and was not accompanied by important photo-induced damage at the level of the perilesional tissues, as shown by the ready and complete healing process. Either an incubation time of 1 h per day or a single 24 h incubation are sufficient to generate satisfactory levels of either preventive or curative effects.

The porphyrins studied by us display no significant toxicity toward trout at photochemically active doses that induce a marked mortality of microbial pathogens. This finding is in agreement with previous observations dealing with the effect of porphyrins on a variety of higher organisms, which justifies their increasing utilization as food additives or phototherapeutic agents. Several porphyrins are widespread in numerous ecosystems and their toxicity to cells and tissues becomes important only at millimolar concentrations, i.e., dosages which are 2-3 orders of magnitude larger than those yielding a satisfactory antimicrobial effect.

It appears that the water disinfection can be successfully obtained by using a simple and inexpensive technology, and in particular low light intensities, of the order of 50 mW/cm^2, which can be easily reached by irradiation with halogen or incandescent filament lamps, that is light sources of low cost, which have a long life span (a few thousand hours) and require no protective measures for the operators, the fishes and the consumers. Moreover, the low fluence-rate causes no detectable thermal effect, thus avoiding any problems related with the possible increase in water temperature.

Lastly, the proposed methodology appears to be environmentally friendly, since it is based on the use of sunlight or sunlight simulators in combination with antimicrobial agents of natural origin. The accumulation of porphyrins in the various ecosystems is unlikely owing to their gradual photo-bleaching to non-toxic products under the action of ambient light, as repeatedly reported in the literature.

In conclusion, our results clearly suggest that the combination of cationic porphyrins and visible light could represent a viable alternative for the control of the population of potential pathogens in waters from fish farming ponds. The approach should be also considered in connection with findings by previous investigators, showing that visible light-excited cationic porphyrins can efficiently induce the destruction of helminth eggs and fecal bacteria in waste waters. Thus, porphyrin photosensitization appears to represent a very useful and flexible tool for the decontamination of microbiologically polluted waters.

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USGS's CORNER

Chloramine-T Environmental Assessment submission: A revised version of the draft Environmental Assessment for the broad use of chloramine-T in U.S. freshwater aquaculture was submitted by the Upper Midwest Environmental Sciences Center (UMESC) to the Center for Veterinary Medicine for review on 11 April 2007. (Bill Gingerich; La Crosse, WI; phone 608.781.6225; email bgingerich@usgs.gov).

UMESC to hold Partner Meeting in conjunction with Bozeman Workshop: The UMESC's Fisheries Management Chemicals and Aquaculture Drugs Team (Team) will hold a partner meeting in conjunction with the 13th Annual Aquaculture Drug Approval Coordination Workshop in Bozeman, MT. The purpose of the meeting is to gain customer feedback to access program needs and support among partners/clients as part of the Center's strategic planning process. It is expected that the results of the workshop will help USGS to refine and prioritize current and future directions of USGS in the project area of fishery management chemicals and aquaculture drugs. The meeting will be held at the Bozeman Fish Technology Center from 9:00 a.m. to 12:00 on Thursday, 2 August 2007 following the National Aquaculture Drug Research Forum meeting. All interested Team partners and customers are invited to attend. The meeting will feature short presentations followed by focused discussions concerning the direction of current and future research of the Team at UMESC. (Bill Gingerich; La Crosse, WI; phone 608.781.6225; email bgingerich@usgs.gov).

USGS scientist recognized by the Food and Drug Administration: USGS scientist William H. Gingerich was presented the Food and Drug Administration's

Commissioner's Special Citation at the Center for Veterinary Medicine 2007 Honors Awards Ceremony on 11 June 2007 in Gaithersburg, Maryland. The prestigious award recognizes Dr. Gingerich "For exceptional leadership, outstanding coordination of resources, and sustained efforts in the development of data for the approval of new animal drugs for aquaculture." (Bill Gingerich; La Crosse, WI; phone 608.781.6225; email bgingerich@usgs.gov).

MEETINGS, ETC.

Recently held meetings

Western Fish Disease Workshop; 4-6 June 2007; Grand Teton National Park, Wyoming, USA: Beautiful Jackson Lake Lodge and the surrounding Grand Teton National Park was the venue for the 48th Western Fish Disease Workshop and 2007 American Fisheries Society Fish Health Section Annual Meeting. By all accounts, the meeting was very successful and well attended. Fortunately, for those not in attendance the complete agenda and abstracts have been made available and can be obtained at: http://www.fws.gov/fisheries/aadap/PDF/wfdw_fhs_2007.pdf.

Upcoming meetings

13th Annual Drug Approval Coordination Workshop; 31 July-1 August 2007; Bozeman, Montana, USA: This year's Workshop will be held 31 July through 1 August 2007 at the Holiday Inn, Bozeman, Montana, USA. Co-hosts will be the USFWS's AADAP Program and USDA's Stuttgart National Aquaculture Research Center. One workshop highpoint will undoubtedly be a celebration of a couple of new FDA aquaculture drug approvals. The workshop is a great opportunity to get "up-to-speed" on recent aquaculture drug research activities and the status of initial or expanded approvals. In addition, a short session is scheduled to discuss current research and commercial status of fish vaccines. For more information, please visit our website. We hope to see you in Bozeman. More information...

Asian-Pacific Aquaculture 2007; 5-8 August 2007; Hanoi, Vietnam: This is the first major aquaculture conference to take place in Vietnam, and the theme of this year's conference is "Prospering from Dynamic Growth." Conference organizers have plans for more than 20 sessions, including an aquatic animal disease session. The deadline for submission of abstracts has already passed (15 March). Early registration fees are available if you register before 25 May or 12 July. See conference brochure for more details.

137th Annual Meeting of the American Fisheries Society; 2-6 September 2007; San Francisco, California, USA: This year's meeting is being held at the Marriott Hotel (downtown San Francisco). "Thinking Downstream and Downcurrent: Addressing Uncertainty and Unintended Consequences in Fish and Fisheries" is the theme for the conference. Detailed information can be obtained at the conference website.

Mollusc Health and Disease Management Course; 13-19 September 2007; Atlantic Veterinary College University Of Prince Edward Island, Canada: This advanced five-day training targets diagnosticians, scientists, students and professionals of mollusc health management. The session will address major issues and challenges surrounding the most important mollusc species in wild and farmed situations. Topics will include significant infectious diseases, disease causation, techniques for sampling for the presence/prevalence of disease, diagnostic techniques and test interpretation, and outbreak investigation. Laboratory sessions will involve the whole range of technical procedures for diagnosis and demonstrations of significant diseases and conditions, including field exercise. This is a 5-day course, the formal classes of which run on September 13, 14, 17, 18 &19 (participants can attend the International Shellfish Festival on 15-16 September). More information on registration, accommodations, etc. is available on their website at http://www.upei.ca/cai/molluschealth.htm.

13th International EAFP Conference on Diseases of Fish and Shellfish; 17-21 September 2007; Grado, Italy: This year the European Association of Fish Pathologists (EAFP) will hold their 13th annual conference at the Conference Centre in Grado, Italy. 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 party, a civic reception and the traditional conference banquet. The conference is being organized by the council of the EAFP and the local organizing committee. Additional information can be found on the conference webpage.

7th International Symposium on Fish Parasites and the 3rd Myxozoan Workshop; 24-28 September 2007; Viterbo, Italy: This year's symposium is being held at the Palazzo dei Papi on the 24th and Domus la Quercia on the 25th through the 28th. There are 20 planned sessions on a diversity of topics, including "Helminth systematics: from faunistics to DNA barcoding," "Parasites as biological tags of fish stocks and biology," and "Fish parasites control." The deadline for Abstract submissions is 15 May 2007. Additional information can be found at their website: http://www.7isfp.com. For additional information on the Myxozoan Workshop, click here.

Health Management of Laboratory Fish Course; 17-22 September 2007; Salisbury Cove, Maine, USA: This one week course is being offered by the Mount Desert Island Biological Laboratory of Salisbury Cove. Topics to be discussed include general system design and water quality management, anatomy and histology of fish, general fish diseases and disease management strategies. Faculty from Oregon

State and Cornell Universities are scheduled to conduct the classes. Registration deadline is 1 June 2007. For more information refer to the course website.

Aquaculture Canada[OM] 2007; 23-26 September 2007; Edmonton, Alberta, Canada: The 24th annual meeting of the Aquaculture Association of Canada will be held at the Shaw Conference Centre and the Westin Edmonton. This is Canada's national forum on business, science and technology of aquaculture. For more information contact Christopher M. Pearce at PearceC@pac.dfo-mpo.gc.ca or refer to the conference website.

Aquaculture Europe 07; 24-27 October 2007; Istanbul, Turkey: This year's annual meeting of the European Aquaculture Society will be held at the World of Wonders City Hotel (conference portion) and the Istanbul Expo Centre (exhibits portion) both in Yesilkoy, Istanbul and opposite Istanbul's Ataturk Airport. The conference theme "Competing Claims" addresses the various levels of competition that aquaculture faces at present, but upon which its future development will depend. For more information, refer to their website. The conference comprises 5 thematic sessions, as well as 12 subject-specific sessions, including a "Health Management and Welfare" session. The deadline for submission of abstracts has passed (1 April). Early registration fees are available if you register before 15 May or 10 September. See conference webpage for more details, including conference brochure, on-line registration, accommodations, etc.

Caribbean and Latin American Aquaculture 2007; 6-9 November 2007; San Juan, Puerto Rico: The Latin American and Caribbean Chapter of the World Aquaculture Society are organizing this year's conference and have more than 20 sessions planned with one for "fish and shrimp health management" and one on "aquatic animal-derived therapeutic drugs for humans." The conference is being held at the Condado Plaza Hotel in San Juan. Submission of abstracts must be made online by 15 April 2007. Please refer to the conference website for more details.

Aquaculture America 2008; 9-12 February 2008; Lake Buena Vista, Florida, USA: The 2008 conference will be held at Walt Disney World's Coronado Springs Resort. This year's conference is being held in conjunction with Marine Ornamentals '08. The deadline for submission of abstracts is 3 August 2008. For more information refer to the conference website.

Seventh Symposium on Diseases in Asian Aquaculture; 22-26 June 2008; Taipei, Taiwan: The Fish Health Section (FHS) of the Asian Fisheries Society (AFS) is holding its Seventh Symposium on Diseases in Asian Aquaculture (DAA VII). It is being held in Taipei, Taiwan from the 22nd through the 26th of June 2008. The DAA Symposia are a series of triennial meetings of the world's leading scientists and students working in aquatic animal health, where all participants share their latest research findings, exchange ideas and establish new collaborations. To meet the current interest in disease control, DAA VII will also offer two "extra curricular" seminar/workshops on risk analysis and on recent advances on fish and shellfish immunology. More detailed information will be provided in the second official announcement. In the interim, a brochure and other Conference information can be found at: http://homepage.ntu.edu.tw/~daaseven/ or at: http://www.fhs-afs.org/.

ROZ's CORNER

The North Central Regional Aquaculture Center (NCRAC) Board of Directors approved and set a maximum funding level for projects based on a prioritized list that had been presented by the NCRAC Industry Advisory Council. The projects included $150,000 for efficacy studies on Terramycin 200® for Fish (oral tetracycline) and Aquaflor® (florfenicol) to control *Aeromonas* sp. in NCR warmwater and coolwater fish species. The National Coordinator for Aquaculture New Animal Drug Applications (NADAs) will spearhead the development of the project(s) to undertake these studies. The request for proposals will go out this summer and the funding will be available summer 2008.

The designation provision of the Minor Use and Minor Species Animal Health Act of 2004 (MUMS) gives sponsors seven years of marketing exclusivity. There have been NADA approvals for two MUMS designations for Schering-Plough Animal Health's Aquaflor® and three MUMS designations for Eka Chemicals, Inc.'s 35% PEROX-AID®. So far, the MUMS Office has granted 44 designations, 40 of those were for aquaculture uses to aquaculture drug sponsors who received extensive help from the National Coordinator for Aquaculture NADAs.

The Center for Veterinary Medicine (CVM) issued several rulings this quarter that affect the use and approval of aquaculture drugs:

1. On 24 April 2007, CVM revised the Guidance for Industry #150 dealing with concerns related to the use of clove oil (eugenol) as an anesthetic for fish by correcting information on its ingredients and safety.
2. On 2 May 2007, CVM removed hydrogen peroxide from the list of Low Regulatory Priority aquaculture drugs because the drug is now the subject of an approved NADA for 35% PEROX-AID®. This

means that 35% PEROX-AID® is the only hydrogen peroxide product that is legal to use.

3. On 4 May 2007, CVM clarified the extra-label use of medicated feeds in minor species under the Compliance Policy Guide #615.115 to include (1) veterinarian involvement, (2) treatment use only, (3) no production use, and (4) no feed reformulation or re-labeling.

There were several highlights concerning aquaculture drug submissions, acceptances, and approvals this quarter:

1. On 13 April 2007, the Upper Midwest Environmental Sciences Center (UMESC) submitted the final environmental assessment (EA) on chloramine-T to CVM.
2. In May 2007, CVM accepted the microbial food safety for HALAMID® (chloramine-T) for all finfish (Guidance for Industry document #152) from Axcentive SARL.
3. In May 2007, the University of Idaho submitted the final EA on erythromycin to CVM.
4. Cross Vetpharm Group Ltd. gained an abbreviated (generic) NADA approval for TETROXY Aquatic® (immersion oxytetracycline) for use as a skeletal marking aid in finfish fry and fingerlings (*Federal Register*; 9 May 2007).
5. In May 2007, UMESC submitted to CVM pivotal efficacy studies on immersion oxytetracycline for the control of mortality in coolwater and warmwater finfish due to external columnaris disease.

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

CVM's NOTES

What is a Public Master File?

Master Files are generally created to allow a party other than the holder of the master file to reference material that supports a New Animal Drug Application (NADA). Public Master Files (PMFs) usually contain safety and effectiveness data that have been generated with public funds or compiled from published literature. The contents of a PMF can be made available to the public because it does not contain trade secrets or confidential business information, such as might be found in other types of master files.

At the request of a PMF's sponsor, CVM announces the availability of the PMF and the information it contains (usually completed technical sections) in the Federal Register. After the announcement in the Federal Register, any pharmaceutical company or other drug sponsor can reference the file to support an NADA or supplemental NADA.

The PMF is the vehicle through which the USDA's National Research Project Seven (NRSP-7) shares its data with manufacturing sponsors; they have had thirty-one PMF publications in the Federal Register for drugs for a variety of species. Four companies have garnered approvals for oxytetracycline hydrochloride for skeletal marking of fry and fingerlings using NRSP-7's PMF 5667; this is quite a testament to the usefulness of a PMF!

Also noteworthy is PMF 5369, sponsored by the Upper Midwest Environmental Sciences Center (UMESC) of the U.S. Geological Survey. The data in this PMF provided the effectiveness, target animal safety, and environmental safety technical sections for the NADA for hydrogen peroxide (35% PEROX-AID®), which CVM approved earlier this year.

For those readers familiar with Investigational New Animal Drug (INAD) files, it is important to note that PMFs are not the same as INADs. One important difference between these file types is that CVM does not conduct data reviews in PMFs.

Of final mention, there is a new page on the CVM Aquaculture website on which we will identify PMF packages for aquaculture drugs. You may have noticed other additions to the CVM Aquaculture website over the past several months.

For more information regarding PMFs or to communicate suggestions regarding the CVM Aquaculture website, please contact Dr. Donald Prater, Leader, Aquaculture Drugs Team, at 301-827-7567 or donald.prater@fda.hhs.gov, or the author of this communiqué, Dr. Jennifer Matysczak, Aquaculture Drugs Team, at 301-827-4359 or jennifer.matysczak@fda.hhs.gov.

IN MEMORIAM

With sadness the staff of AADAP notes the recent passing of Randy Rickert, a seasoned U.S. Fish and Wildlife Service biologist who for several years (beginning in 2004) was the assistant hatchery manager at the Makah National Fish Hatchery, Neah Bay, Washington. Randy was the on-site investigator for several pivotal efficacy studies that we conducted at Makah. We found Randy to be quiet and professional —and someone who could be counted on to do research well. We and the Service will miss him.