



# The Aquatic Animal Drug Approval Partnership Program

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



Volume 2-1

## AADAP NEWSLETTER

October 2005

### WHAT’S SHAKIN’

#### NEWS FLASH !!!!

**CVM Approves Florfenicol for ESC:** We have all been waiting a long time for this, and it’s finally a reality!!! FDA’s Center for Veterinary Medicine announced on Tuesday, 25 October 2005, that they had approved the NADA submitted by Schering-Plough Animal Health (SPA) for Aquaflor® (florfenicol). SPA’s NADA (#141-246) is an antimicrobial Type-A Medicated Article and, at this time, is limited for the control of mortality due to enteric septicemia of catfish. For more details visit CVM’s website (<http://www.fda.gov/cvm/catfishapp.htm>).

There are a tremendous number of folks out there deserving of credit for making this happen, and they will be recognized. However, at the moment, suffice to say that none of this would have come about if it had not been for SPA’s dedication and commitment to the aquaculture industry – we salute you !!

#### NEWS FLASH !!!!

**Newsletter via snail-mail:** Starting with our next edition of the AADAP Newsletter (Volume 2-2, January 2006), we will provide a paper copy ONLY to those individuals that specifically request their name be placed on the paper-copy mail-list. We are sorry for any inconvenience that this may cause, but the time and expense to print and mail paper copies has become too high. To have your name placed on the paper-copy mail-list, contact us at <http://www.fws.gov/fisheries/aadap/contact.html>, and merely type “paper copy” in the subject line.

**The 2006 INAD Sign-up Forms are available:** Once again it is that time of year for renewal of your facility’s INADs for calendar year 2006. Please send in the completed sign-up sheets to the Bozeman INAD/AADAP Office by 31 December 2005. Invoices will then be mailed out by the end of January. Also, 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 2005. All 2006 sign-up forms are available for downloading on our website at: (<http://www.fws.gov/fisheries/aadap/signup.htm>).

**Quarterly INAD Investigator/Monitor Award:** A tip-of-the-hat and a pat-on-the-back to Robert Piper of Bozeman, Montana and Joseph Hinton of the Makah Fisheries Tribal Hatchery, Washington. “Piper,” Bozeman Fish Technology Center Director emeritus, has played a key role, as the Study Monitor for all Montana Department of Fish, Wildlife and Parks facilities participating in the INAD process. Joe, as a study-site investigator, consistently provides, to AADAP, study results of the highest quality.

**Drug Approval Coordination Workshop:** The 11<sup>th</sup> Annual Drug Approval Coordination Workshop (formerly known as the INAD Workshop) was held this past August 2<sup>nd</sup> & 3<sup>rd</sup> in

Bozeman, Montana, and by all accounts it was a great success. This year’s Workshop rewrote the record book, with approximately 80 participants in attendance. As in the past, there was broad representation from pharmaceutical companies, academia, state and federal resource/research agencies, private aquaculture companies and federal regulatory agencies. Good fun was had by all at the Workshop BBQ (a small collage of pictures from the BBQ can be found on our website at ([http://www.fws.gov/fisheries/aadap/inad\\_workshop\\_presentations.html](http://www.fws.gov/fisheries/aadap/inad_workshop_presentations.html))).

In an effort to make as much of the information exchanged at the Workshop available to as many interested parties as possible, most of the presentations from the Workshop have been made available on our website at the aforementioned address.

**JSA Biologics Focus Group meeting:** The Federal Joint Subcommittee on Aquaculture’s Working Group on Quality Assurance in Aquaculture Production (JSA-QAAP) recently sponsored a special meeting on biologics. The meeting was held in Bozeman, Montana on 1 August 2005, in conjunction with this year’s Drug Approval Coordination Workshop. The meeting was attended by over 20 scientists and government regulators. A few of the presentations from the meeting are likewise available on our website at the previously noted address. Incidentally, several presentations given at the Drug Approval Coordination Workshop are of particular relevance to the topic of biologics. Dr. Melisse Schilling’s presentation (Tuesday, August 2<sup>nd</sup> at 10:45 am) provided an overview of the biologics registration process. Dr. David Scarfe’s presentation (Wednesday, August 3<sup>rd</sup> at 1:45 pm) provided a synopsis of the Biologics Focus Group meeting from two days previous. Two other presentations at the Drug Approval Coordination Workshop (Wednesday, August 3<sup>rd</sup> at 1:45 pm) also focused on biologics and are available for viewing.

**Requested SLICE® INAD not authorized:** CVM recently responded to AADAP’s earlier request for a SLICE® (emamectin benzoate) INAD to be used as a freshwater ectoparasiticide. CVM responded by not authorizing the INAD, citing insufficient information regarding the environmental safety of effluents. AADAP is working with Schering Plough Animal Health to generate the necessary information/data to resolve these issues.

#### Aquaflor® (florfenicol) update:

**NADA approved for ESC:** see above.

#### Request for “Technical Section Complete” letter:

Following acceptance of an AADAP study to demonstrate the efficacy of florfenicol to control mortality in chinook salmon caused by furunculosis, AADAP requested that CVM consider all pivotal studies complete to support the following claim: “use of florfenicol-medicated feed administered at a dosage of 10 mg active drug per kg of fish per day administered for 10 consecutive days to control mortality in all freshwater-reared salmonids caused by

furunculosis." If CVM accepts this request, then the first amended label claim (i.e., for salmonids) should include language allowing the use of Aquaflor<sup>®</sup> to control mortalities in all freshwater reared salmonids, caused by bacterial coldwater disease (for which all required studies had been previously submitted and accepted) and furunculosis.

#### Chloramine-T update:

**New source of chloramine-T:** Axcentive SARL/International Specialty Chemicals, Inc. remains as one of the two sources of the investigational drug; however, the Axcentive SARL authorized distributor has changed to Mr. Larry Holzman (email [lbh@ischem.com](mailto:lbh@ischem.com), phone 914-333-0606, FAX 914-333-0333). For further details on chloramine-T sources refer to AADAP website (<http://www.fws.gov/fisheries/aadap/chloramine.htm>).

#### 17- $\alpha$ methyltestosterone update:

**Pivotal Study Protocol submitted:** An AADAP pivotal research protocol entitled "The Efficacy of 17- $\alpha$  Methyltestosterone Medicated Feed to Induce Gender Manipulation in Tilapia to Produce Populations Comprising  $\leq$  20% Reproductively Capable Females" was submitted to CVM in May 2005. Following CVM review, changes were made to reach protocol concurrence. The revised protocol is now entitled "The Efficacy of 17- $\alpha$  Methyltestosterone Medicated Feed to Produce Predominately Male Populations of Tilapia." We hope to begin field efficacy trials under this revised protocol in the next few months.

#### AQUI-S<sup>®</sup> update:

**Target Animal Safety** – An AADAP TAS study was conducted in March 2005 on small fingerling rainbow trout to evaluate whether 40 mg/L (the highest proposed efficacious dose to sedate salmonids to the handleable stage) meets CVM's target animal safety criteria. Mortality and pathology associated with exposure to AQUI-S<sup>®</sup> were the two primary response variables measured. Histological evaluation to select tissues has been completed, and the data are currently being analyzed. Our goal is to complete the Final Study Report by the end of the calendar year. At that time, we will begin making preparations to evaluate the safety of AQUI-S<sup>®</sup> to representative cool- and warmwater fishes.

**Efficacy Studies** - AQUI-S<sup>®</sup> efficacy studies were recently completed on fingerling and juvenile Kokanee salmon and fingerling June suckers; Final Study Reports were submitted to CVM in September 2005.

In addition, a study was completed and report submitted to CVM that compared the efficacy of AQUI-S<sup>®</sup> on individually sedated and group-sedated fingerling walleye. Results from this study (1) showed that there was no significant difference between times to sedate fish to handleable when sedated either individually or in groups, and (2) were consistent with results from similar studies conducted on rainbow trout and channel catfish. Details of this study can be found in the second article of our Feature Articles Section of this newsletter. Many thanks to Montana Fish, Wildlife and Parks, Fish Breeders of Idaho, and the USFWS Bozeman Fish Tech Center for providing test fish.

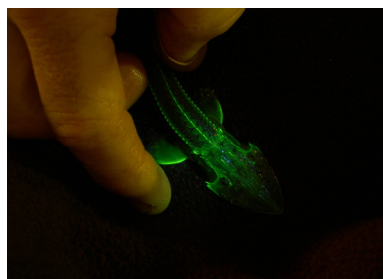
**Data to support a claim for all freshwater fish** - Based on discussions with CVM, we believe that a sufficient number of studies have been completed to demonstrate the following efficacy claim: "AQUI-S<sup>®</sup> as an anesthetic to sedate all freshwater fish to the handleable stage of

anesthesia." Such a request was made to CVM, and we are awaiting their reply.

#### SE-MARK<sup>®</sup> (calcein) update:

**Feed studies conducted:** A USGS-funded Science Support Project (SSP) recently began at Bozeman Fish Technology Center (BFTC) to evaluate the effectiveness of calcein-medicated feed in marking calcified tissue of shovelnose sturgeon and Snake River cutthroat trout (two of six species being tested under the SSP project). Studies with the other species are scheduled to begin soon at the Lamar Fish Technology Center (LFTC) and the Appalachian Research Lab (NARL). Test fish will receive a standard dose of calcein mixed with feed in either an encapsulated or straight pre-mix form for one of several durations (5, 10, or 15 d). Marks resulting from administration of medicated feed will be compared with marks made when calcein is administered in the water as a static bath.

**Pilot Studies Conducted:** Preliminary results conducted at BFTC showed that shovelnose sturgeon readily ate calcein-medicated feed when calcein was incorporated in either the encapsulated form or as straight premix. The fins, scutes and other bony tissues "lit up like Times Square" when



Calcein marks on shovelnose sturgeon after 15 days of calcein-medicated feed

viewed with a SE-MARK<sup>®</sup> Detector in fish consuming the calcein medicated feed for 10-15 days. Preliminary studies with rainbow trout and Snake River cutthroats indicated that these species found certain levels of non-encapsulated calcein to be

unpalatable. Related pilot work at LFTC indicated that there may be a threshold level of non-encapsulated calcein below which some freshwater *Oncorhynchus* spp. find calcein palatable. Special thanks to Dale Honeyfield, NARL; Jerre Mohler and Tom Kehler, LFTC; and Greg Kindschi, BFTC.

**Work under the INAD:** If you are interested in mass-marking larval fish via immersion to produce a non-lethally detectable mark, SE-MARK<sup>®</sup> (calcein) may provide you with a useful management tool. For more information, please contact AADAP or Western Chemical, Inc. (phone 800-283-5292).

## FEATURE ARTICLES

### COMPARISON OF THE EFFICACY OF HYDROGEN PEROXIDE AND FORMALIN TO CONTROL FUNGUS ON LAKE TROUT EGGS

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#### Introduction

Aquatic fungi (Saprolegniales) are ubiquitous in natural water supplies of fish hatcheries and can cause serious disease



problems. Formalin is currently used to control mortality associated with fungal infections on lake trout (*Salvelinus namaycush*) eggs. However, increasing concerns over user safety and environmental discharge following formalin use at hatcheries are causing fish culturists to consider alternative drugs, like hydrogen peroxide (a CVM-designated Low Regulatory Priority Drug), to treat fungal infections on fish. It is believed that many hatchery personnel would prefer to use the more environmentally friendly hydrogen peroxide in their hatchery production facilities. However, little information is available on the safety and efficacy of hydrogen peroxide to control fungal infections on lake trout eggs under actual hatchery production conditions. The U.S. Geological Survey and the U.S. Fish & Wildlife Service conducted a joint study to compare the efficacy of hydrogen peroxide versus formalin treatments to control fungal infections on four strains lake trout egg incubated at the Iron River National Fish Hatchery (Iron River, Wisconsin).

## Methods

**Test chemicals:** Hydrogen peroxide used in this study was Perox-aid™, 35% active ingredient. All test concentrations were based on active ingredient. Hydrogen peroxide treatment concentrations were verified by a potassium permanganate titration method. Formalin used in this study was 37% formaldehyde gas (by weight) dissolved in water with 10-15% methanol added to retard polymerization. All treatment calculations were based on formalin concentration. Formalin concentration was verified using a spectrophotometric method.

**Test eggs:** Eggs were obtained from cultured Lake Superior or Lake Michigan broodstock. The strains tested were Superior Apostle Island (SAW; year class 1995 and 1997), Superior Traverse Island (STW; year class 1995 and 1997), Superior Isle Royal (SIW; year class 1994), and Michigan Green Lake (GLW; year class 1992).

**Test system:** Each chemical was tested in three double-stack Heath incubators with each tray contained approximately 25,000 to 30,000 eggs (2 million eggs treated). Eggs of similar strains and spawning dates were positioned in similar locations in either the hydrogen peroxide or formalin incubators. There were three to nine replicates of each strain. Each incubator was supplied with a water flow of approximately 19 L/min.

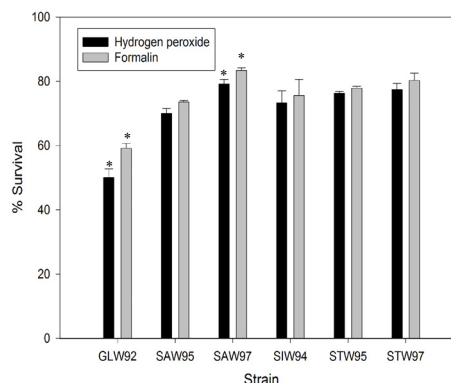
**Chemical treatments:** Eggs received either 1,667 mg/L formalin or 1,000 mg/L hydrogen peroxide once daily for 15 min until eggs reach the eyed stage (mean of 481 Daily Temperature Units). No controls were tested because past historical hatchery data indicated all untreated eggs become infected with fungus and die. Each egg group received approximately 36 treatments. When eggs reached the eyed-egg stage, live eggs and dead eggs were then separated using a photoelectric egg sorter and the number of each egg type was determined volumetrically. The test water temperature during the study ranged from 6.2 to 8.9°C. Total hardness and alkalinity (as CaCO<sub>3</sub>) of the test waters were 67 and 60 mg/L, respectively. The probability of survival was modeled using the mixed-effects logistic model. Adjusted least-square means (Tukeys-Kramer adjustment) were used to evaluate differences among the treatment levels. Treatment levels were judged statistically different if  $P \leq 0.05$ .

## Results

Hydrogen peroxide concentrations were analyzed on five occasions, and the mean concentration was approximately 29% higher than the target concentration. Formalin concentrations were analyzed on one occasion, and the mean concentration was approximately 9% higher than the target concentration.

Hydrogen peroxide treated eggs in the upper nine trays had a minimal number of fungal egg clumps. However, fungal infections were more severe in the bottom trays containing the GLW 92 egg strain (this strain had approximately 17% lower egg fertility and the lowest percent egg eye-up for the strains tested with either chemical). The eggs treated with formalin had no visible fungus on the eggs. The combined survival to egg eye-up of all four strains treated with hydrogen peroxide (70%) was significantly less than the eggs treated with formalin (75%). Percent eye-up for individual strains tested was not significantly different between hydrogen peroxide or formalin treatments for SAW 95, STW 95, STW 97, and SIW 94 strains, however, percent eye-up was significantly different between hydrogen peroxide or formalin treatments for SAW 97 (4% lower eye-up) and GLW 92 (9% lower eye-up; see Figure 1).

**Figure 1:** Comparison of percent of eyed eggs of four lake trout (*Salvelinus namaycush*) strains (two year classes) treated daily for 15 min with either 1000 mg/L hydrogen peroxide or 1,667 mg/L formalin. Comparisons of individual strains that were statistically different ( $P \leq 0.05$ ) between hydrogen peroxide and formalin are designated with an asterisk (\*).



## Conclusion

The higher verified treatment concentrations appeared to be related to variable water flows and variable pump flow rates to the individual incubators. Hatchery personnel identified several problems that occurred during the study that affected the flow rates and treatment regimens. By periodically monitoring the treatment concentrations, fish culturists can identify potential problems and correct them before eggs are negatively impacted.

Hydrogen peroxide was not as effective as formalin in controlling fungus and producing eyed lake trout eggs. However, the overall percent of eyed eggs in an incubator varied by only 5% between the formalin and hydrogen peroxide treatments and eggs of one strain accounted for much of the difference in egg survival to eye-up.

Hydrogen peroxide treatment is a viable option for the control of fungus on lake trout eggs, especially considering its environmental friendly breakdown products of water and oxygen. In species requiring lengthy incubation periods at cold water temperatures, higher hydrogen peroxide concentration may be required than the 500 mg/L concentration currently allowed under the Low Regulatory Priority statues of hydrogen peroxide.



## Reference

Rach J.J., S. Redman, D. Bast, and M.P. Gaikowski. 2005. A comparison of the efficacy of hydrogen peroxide versus formalin treatments to control mortality associated with saprolegniasis on lake trout eggs (*Salvelinus namaycush*). *Journal of North American Aquaculture*. 67:148-154.

### EFFICACY OF AQUI-S®: GROUP SEDATION VS. INDIVIDUAL SEDATION

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## Introduction

The U.S. Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership (AADAP) program has conducted several pivotal efficacy studies to help obtain U.S. Food and Drug Administration approval of AQUI-S® (active ingredient, isoeugenol) for use to sedate fish to "handleable" (herein defined as a state of sedation where a fish has lost equilibrium, stopped swimming, lost reactivity to external stimuli except strong pressure, and is easily hand-captured, held above the water's surface, and measured for length). In these pivotal studies, fish were individually sedated and timed to handleable; however, in many fisheries operations, fish will be sedated in small groups (5-20 fish per group). Therefore, AADAP recently conducted three AQUI-S® efficacy studies to evaluate whether "time to handleable" is similar between group-sedated and individually sedated fish.

## Methods

All three "group sedation vs. individual sedation" efficacy studies were conducted at a nominal AQUI-S® concentration of 20 mg/L (currently, the lowest proposed efficacious dose for the label claim). Test fish used in these studies were rainbow trout *Oncorhynchus mykiss*, walleye *Sander vitreus*, and channel catfish *Ictalurus punctatus*. Rainbow trout (mean total length, 1.7 in) were tested at 54°F; walleye (mean total length, 2.4 in) were tested at 72°F; and channel catfish (mean total length, 8.8 in) were tested at 77°F.

In each study, test fish were either sedated to handleable as part of a group (Treatment 1) or individually sedated to handleable (Treatment 2), and each treatment was replicated three or four times. Each replicate of group sedation comprised 10 new (previously unused) fish — where all 10 fish were simultaneously placed into and sedated in a single container of a fresh (previously unused) AQUI-S® solution. Each replicate of individual sedation also comprised 10 new fish — where each fish was sedated in a separate container of fresh AQUI-S® solution. For each replicate, time to handleable was recorded for each of the 10 fish tested, and median time to handleable was calculated. Subsequently, "average median time to handleable" was the response variable used to make comparisons between treatments within studies.

## Results and Discussion

In all three studies, there was little practical difference in average median time to handleable between group-sedated and individually sedated test fish (Tables 1 - 3). For example, differences in average median time to handleable were only 0.1 min in the walleye study, 0.3 min in the rainbow trout study, and 0.5 min in the channel catfish study. In two of the studies (rainbow trout and channel catfish), average median time to handleable for group-sedated test fish was slightly longer than that for individually sedated test fish; however, additional species and life stages of fish would need to be tested at additional AQUI-S® concentrations and water temperatures to determine if such a "two-out-of-three" result is a real trend or merely a random outcome. Overall, the results of all three "group sedation vs. individual sedation" studies suggested that there is no "group effect" that substantially alters the time required to sedate a fish to handleable. As such — given a sufficient amount of AQUI-S® solution relative to fish number and size — we conclude that time to handleable will be similar for group-sedated or individually sedated fish.

**Table 1.** Median time to handleable for rainbow trout (mean total length, 1.7 in) group-sedated or individually sedated at 20 mg/L AQUI-S® and a water temperature of 54°F.

Replicate	Median time to handleable (minutes)	
	Group sedation	Individual sedation
1	5.2	4.8
2	4.7	4.9
3	4.9	4.8
4	5.9	5.2
Average median time	5.2	4.9

**Table 2.** Median time to handleable for walleye (mean total length, 2.4 in) group-sedated or individually sedated at 20 mg/L AQUI-S® and a water temperature of 72°F.

Replicate	Median time to handleable (minutes)	
	Group sedation	Individual sedation
1	5.9	5.3
2	5.1	6.2
3	6.1	5.9
Average median time	5.7	5.8

**Table 3.** Median time to handleable for channel catfish (mean total length, 8.8 in) group-sedated or individually sedated at 20 mg/L AQUI-S® and a water temperature of 77°F.

Replicate	Median time to handleable (minutes)	
	Group sedation	Individual sedation
1	7.1	6.4
2	6.7	5.5
3	5.1	5.6
Average median time	6.3	5.8

## INAD INFORMATION & STATUS

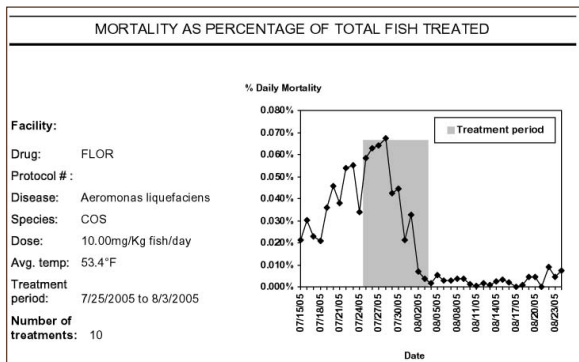
A question commonly asked by many Investigators and Monitors is "What happens to our paperwork once it is submitted to the AADAP Office?" Well, contrary to popular belief that the paperwork simply ends up in a "black hole" and is never seen again, all paperwork is reviewed to ensure adherence to the INAD study protocol and that data are complete and accurate. Furthermore, the data are then entered into a customized Microsoft Access database where it is compiled and analyzed.



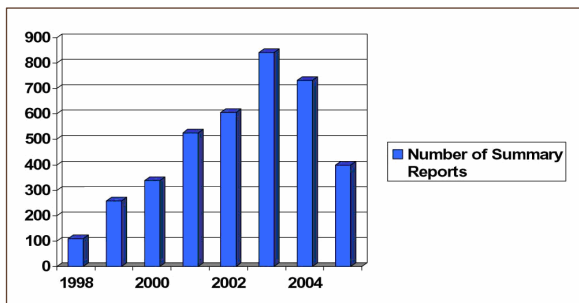
The following information demonstrates how the AADAP office uses some of the requested data:

1. Study Worksheets are reviewed to ensure that the study protocols are being followed and that all calculations relative to planned treatments are correct.
2. Sign-up and Drug Receipt Forms are used for quarterly reports that are sent to FDA's Center for Veterinary Medicine (CVM) for each INAD. These reports include information on the number of fish treated; drug shipments received by facilities; and updated lists of facilities and fish species.
3. Result Report Forms are used to create a two page "Summary Report" for each treatment. These reports contain the following information: facility, investigator, and study information; summary of study results; disposition of treated fish; a mortality graph (see Figure 1 below) if applicable, and a brief conclusion relative to apparent treatment efficacy. The annual number of summary reports we (and you!) have generated has grown steadily over the years, resulting in an increase from 109 Summary Reports in 1998 to 841 Summary Reports in 2003 (see Figure 2 below; total Summary Reports totals for 2004 and 2005 are incomplete due to outstanding Reports).

**Figure 1.** Example of mortality graph for an individual data summary report as submitted to CVM



**Figure 2.** Annual Number of Summary Reports provided to AADAP by INAD investigators.



4. At the end of the year the data are compiled for each INAD to create the "Annual Report" that is submitted to CVM. This report contains the following information: facilities, materials, and treatment procedures; fish species and fish disease; data collected; discussion of study results, and all Summary Reports. Annual Reports, through year 2004 may be viewed from the AADAP website at: <http://www.fws.gov/fisheries/aadap/annual.htm>.

The collection and submission to CVM of supportive data generated from INAD studies is a critical component in our pursuit of new aquatic species drug approvals. These data sets

demonstrate treatment efficacy over a broad range of fish species, life-stages, disease conditions, and water quality parameters. These data also provide important evidence that treatments are not (hopefully!!) resulting in any unexpected or adverse effects under a similar broad range of treatment parameters. Such data, when submitted in combination with smaller datasets of higher quality ("pivotal" data), play a key role in our efforts to obtain broad (i.e., "all fish") drug labels. SO PLEASE, rest assured that your efforts and hard work are NOT going unnoticed and are indeed a valuable component of our collaborative efforts. Keep up the good work everyone! Now get back out there and send us some more data!

## FEATURED INAD DRUG

**Calcein for skeletal marking:** The calcein story began in 1993 when biologists from the USFWS-Northeast Fishery Center in Lamar, PA (NEFC) canvassed northeast federal fish hatchery managers to determine their needs in the way of new technology for evaluating their particular hatchery products. Responses from the managers revealed a common need for a new, low-cost tagging or marking technology which would allow a non-lethally detectable mark to be applied to large numbers of small fish simultaneously with some degree of mark longevity. In addition, this mark would need to be easily detectable without the need for complicated equipment and procedures. As a result, the quest for discovery of such technology began with a literature search. This effort led NEFC biologists to hypothesize that calcein dye may have the ability to produce brilliant, long-lasting marks in calcified fish tissues in addition to otoliths.

The first experiment with calcein was initiated at NEFC in 1995 by exposing Atlantic salmon fry to 24 and 48-hour immersions in calcein concentrations similar to those used with oxytetracycline (i.e., 125-250 mg/L). Biologists were excited to find brilliant, bright green marks on fin rays and other calcified tissues when viewed under an epifluorescence microscope. Subsequent experiments resulted in improvements in calcein mark application such that the mark can now be applied to thousands of fry simultaneously via "osmotic induction" in only 7 minutes. Further work at NEFC included a preliminary tissue residue study along with development of a pure grade of calcein by Western Chemical of Ferndale, WA. Work by the AADAP program at Bozeman, led to an Aquaculture Investigational New Animal Drug exemption (INAD #10-987) for immersion of fish in calcein (SE-MARK<sup>®</sup>; for details on INAD #10-987 go to <http://www.fws.gov/fisheries/aadap/calcein.htm>). As a result, studies are being conducted from coast to coast with a variety of fish species to determine the capabilities and limitations of using calcein as another "tool" in the fish management toolbox. Early in 2005, the USFWS was officially awarded a patent for a hand-held calcein detection device which was developed at NEFC and licensed to Western Chemical, Inc. of Ferndale, WA.

We're happy to say that the calcein-marking story does not end here, but continues with improvements in calcein mark application, and has led to a new round of experimentation with sturgeon, salmonids and striped bass in a project funded with USGS Science Support Program funds. The project is being conducted jointly at the Bozeman FTC/AADAP, Northern Appalachian Research Lab in Wellsboro, PA and NEFC, with much appreciated input and assistance from Ron Secor of Western Chemical, Inc. Western Chemical is the sponsor and manufacturer of calcein (SE-MARK<sup>®</sup>). The project involves inducing and evaluating calcein marks administered via fish feed. Once perfected, this technique will hopefully simplify calcein mark application by allowing the mark to be applied to any size fish by offering calcein medicated feed to the target animals for a few days. We are hopeful that this work will lead

to another INAD exemption for inducing calcein marks in fish via feed. Preliminary results from these studies are very exciting!

Additional information on calcein and calcein studies can be found at <http://www.wchemical.com> and <http://www.btc.ctc.edu/Departments/Fisheries/hatchery.html>.  
*Jerre Mohler; Fishery Biologist; U.S. Fish & Wildlife Service; Northeast Fishery Center; Lamar, Pennsylvania and Ron Secor; Western Chemical Inc.; Ferndale, Washington.*

## FINS & TAILS, BITS & BOBBERS

This Newsletter section provides readers with specific INAD informational tidbits. Hopefully, this information will assist co-investigators under Service INADs (in particular) and others in the effective and efficient use of investigational drugs.

- **Extralabel drug use:** See "CVM's Notes Section."
- **New 2006 INAD forms now available:** see "What's Shakin' Section."
- **New features on AADAP's Website:** a search function and an animated "hot-off-the-press topic."
- **New source of chloramine-T:** see "What's Shakin' Section."
- **A new oxytetracycline product approved for skeletal marking:** Pfizer, Inc. (New York, NY) was recently granted an approval to their filed request to supplement their Terramycin-343 (oxytetracycline HCl) Soluble Powder NADA (#8-622) with a finfish skeletal marking claim. See: <http://www.fda.gov/cvm/aqualibtoc.htm>, CVM's website, for more information. There are now **three** oxytetracycline products approved for skeletal marking in finfish.

## PARTNERS' CORNER

**Utah Division of Wildlife Resources - Fisheries Experiment Station:** The Utah Division of Wildlife Resources, Fisheries Experiment Station (FES) has a mission of aquaculture, in addition to disease diagnostics and research. Among the species raised here are June suckers, least chub, leatherside chub, and Bonneville and Colorado River cutthroat trout. All of these species are either federally listed as threatened or designated as a state sensitive species. Currently we are utilizing INADs for the use of calcein as a marker for June suckers and florfenicol for the treatment of coldwater disease at other state production hatcheries. In the past, rainbow and brook trout were sterilized by the use of an INAD (now inactive) for 17- $\alpha$  methyltestosterone. Wild cyprinids that are introduced into fish culture recovery facilities are often extra-label treated with praziquantel to prevent introduction of cestode pathogens, such as the Asian tapeworm.

The state of Utah historically has attempted to stress quality control in hatchery production. Springs and wells are utilized as water sources, and fish densities are held at low levels to reduce the need for chemotherapeutants. Increasing urbanization around hatchery sites has occasionally compromised water quality, resulting in gill diseases that have been successfully treated with low regulatory priority compounds such as salt and hydrogen peroxide.

The emergence of the pathogen *Flavobacterium psychrophilum*, agent of bacterial coldwater disease, has resulted in the increased use of antimicrobials in recent years. While most bacterial isolates are still sensitive to florfenicol and oxytetracycline, researchers at FES are experimenting with proactive strategies such as vaccines and egg disinfection protocols to reduce or eliminate the need for antibiotic treatments.

While public aquaculture has suffered for the lack of approved drugs and chemicals, it is appropriate to acknowledge the assistance of folks at the AADAP office in Bozeman, Roz Schnick, and individuals at FDA/CVM like Dr. Don Prater for their help in this difficult transition time. *Chris Wilson, DVM, Fish Pathologist; Fisheries Experiment Station, Utah Division of Wildlife Resources; Logan, UT 84321.*

## USGS's CORNER

**What's happening at the Upper Midwest Environmental Sciences Center?**

- **Mark Gaikowski's departure to support "Operation Enduring Freedom:"** Staff members working on development of data sets to support the approval of several aquaculture drugs at the Upper Midwest Environmental Sciences Center (UMESC) and close DOI partner agencies are stepping up to fill in for Mark Gaikowski. Mark's National Guard Military Intelligence Unit was activated in mid-October and is not expected to return to the States until March, 2007. Jeff Meinertz will replace Mark to represent USGS on the Aquaculture Drug Research Forum. Jim Bowker (AADAP) has been asked to consider replacing Mark as a Study Monitor for field efficacy trials for florfenicol conducted under a Cooperative Research and Development Agreement with Schering-Plough Animal Health. Bill Gingerich will supervise work to develop an external columnaris disease model acceptable to the Center for Veterinary Medicine staff. Jeff Rach and Jeff Bernardy will oversee efficacy trials of external columnaris disease with waterborne oxytetracycline. Godspeed Mark in your efforts for our country. Contact: Bill Gingerich, phone 608-781-6225, email [bjingerich@usgs.gov](mailto:bjingerich@usgs.gov).
- **Environmental Assessment (EA) progress:** Final Environmental Assessments for hydrogen peroxide, chloramine-T, and oral oxytetracycline medicated feed are nearing completion. Submission of the amended hydrogen peroxide EA should be made by the first of November 2005; that for chloramine-T by December 2005; and for oxytetracycline medicated feed by March 2005. Contact: Larry Schmidt; email [lschmidt@usgs.gov](mailto:lschmidt@usgs.gov); phone 608-781-6272.

## MEETINGS, ETC.

### Upcoming meetings

**Bacterial Kidney Disease - Challenge for the 21<sup>st</sup> Century; 15-17 November 2005; Seattle, Washington, USA:** DOC National Oceanic and Atmospheric Administration Fisheries; USDA Cooperative States Research, Education and Extension Service and USDA Agriculture Research Service are sponsoring this conference to be held at Seattle's Museum of History and Industry. The purpose of the conference is to bring together experts to report on and examine the latest research and management techniques used to deal with this important pathogen and recommend the next steps that need to be taken. For more information see: <http://www.nwfsc.noaa.gov/research/divisions/reutd/fhm/bkd-conference/index.html>.

**56<sup>th</sup> Annual Northwest Fish Culture Conference; 6-8 December 2005; Boise, Idaho, USA:** The theme for this year conference is "Native Fish, a Challenge for Fish Culture." This year's conference is being hosted by the Idaho Department of Fish and Game. Inquiries can be made to Lynette Moran; phone 208-334-3791, email [lmoran@idfg.idaho.gov](mailto:lmoran@idfg.idaho.gov). For conference details see: <http://fishandgame.idaho.gov/nwfcc/>.





**American Fisheries Society 14<sup>th</sup> Annual Southern Division Meeting; 8-12 February 2006; San Antonio, Texas, USA:** The Texas Chapter of AFS is hosting the AFS Southern Division meeting. In addition to numerous thematic plenary sessions, the meeting will include half-day and full-day workshops, including one on largemouth bass virus and another on the INAD/NADA process. For more information, see the meeting website: <http://www.sdafs.org/meetings/2006/>.

**Aquaculture America 2006; 13-16 February 2006; Las Vegas, Nevada, USA:** The next annual meeting of the U.S. Chapter of the World Aquaculture Society will be held in Las Vegas, Nevada from the 13<sup>th</sup> through 16<sup>th</sup> of February 2006. Further information on the conference can be obtained from the Director of Conferences at (phone) 760-432-4270, (FAX) 760-432-4275 or by visiting their website at: <http://www.was.org/meetings/ConferenceInfo.asp?MeetingCode=AA2006>.

**17- $\alpha$  Methyltestosterone Special Session, 13-16 February 2006 (exact date to be determined); Las Vegas, Nevada, USA (Aquaculture America 2006):** AADAP will facilitate a special session, the object of which is to re-examine the status of 17MT, i.e., to see how well we are all doing compared to where we were at the time of the last 17MT mini-session at Aquaculture America 2005. Materials to be presented include a review and revision of our "activities timeline;" a series of brief presentation/status reports from involved researchers; input from CVM, the American Tilapia Association, the drug sponsor and others. For details contact Tom Bell, email [thomas\\_a\\_bell@fws.gov](mailto:thomas_a_bell@fws.gov), phone 406-994-9911.

**Therapeutic Drug Research Special Session; 13-16 February 2006 (exact date to be determined); Las Vegas, Nevada, USA (Aquaculture America 2006):** USDA-ARS Stuttgart National Aquaculture Research Center and AADAP will facilitate a special session to showcase the work of researchers conducting aquatic animal drug studies. The program will include presentations on: disease models, columnaris therapy comparisons, JSA-QAAP Research Forum update and calcein feed studies. For details contact Jim Bowker, email [jim\\_bowker@fws.gov](mailto:jim_bowker@fws.gov), phone 406-994-9910; or Dave Straus, email [dstraus@spa.ars.usda.gov](mailto:dstraus@spa.ars.usda.gov), phone 870-673-4483.

**Tentative meeting of the JSA-QAAP National Aquaculture Drug Research Forum; 13-16 February 2006 (exact date to be determined, to immediately follow Therapeutic Drug Research Special Session); Las Vegas, Nevada, USA (Aquaculture America 2006):** The Drug Research Forum will focus their attention on the activities of several "technical project teams," which include: efficacy, environmental safety, antimicrobial resistance and analytical methods validation. For more information contact: Renate Reimschuessel, email [rreimsch@cvm.fda.gov](mailto:rreimsch@cvm.fda.gov), phone 301-210-4024, FAX 301-827-8250 or Jim Bowker, email [jim\\_bowker@fws.gov](mailto:jim_bowker@fws.gov), phone 406-994-9910, FAX 406-582-0242.

**Eastern Fish Health Workshop; 27-31 March 2006; Charleston, South Carolina, USA:** USGS's National Fish Health Research Laboratory (Leetown, West Virginia) will be hosting the 31<sup>st</sup> Annual Eastern Fish Health Workshop. The workshop will include special sessions focused on several themes, including: imminent threats affecting our coral reefs, advances in crustacean health and disease, clinical approaches in pet fish medicine, practical vaccination in aquatic animal health management, health management and clinical conundrums in public aquaria, case diagnostics, and the INAD/NADA process. For further information contact Rocco

Cipriano, email [rcipriano@usgs.gov](mailto:rcipriano@usgs.gov), phone 304-724-4432, FAX 304-724-4435.

**Aqua 2006; 9-13 May 2006; Florence, Italy:** The next annual meeting of the World Aquaculture Society and the European Aquaculture Society is scheduled for the 9<sup>th</sup> through the 13<sup>th</sup> of May 2006 in Florence, Italy. The themes for the conference are "Linking Tradition and Technology" and "Highest Quality for the Consumer." Planned topics for the conference include: Sustainable Environment, Society & Aquaculture, Consumer Issues, Economics & Business, Shrimp, Juvenile Production - Fish, Juvenile Production - Invertebrates, Nutrition, Health & Welfare and Production Systems. Information available on their website (<http://www.was.org/meetings/WasMeetings.asp>) or by contacting the Director of Conferences at (phone) 760-432-4270, (FAX) 760-432-4275.

**International Symposium on Veterinary Epidemiology and Economics; 6-11 August 2006; Cairns, Australia:** This 11<sup>th</sup> triennial symposium is being sponsored by: Biosecurity Australia, Department of Agriculture, Fisheries and Forestry; Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease; New Zealand Food Safety Authority; and Murdoch University School of Veterinary and Biomedical Sciences (Australia). Epidemiology and animal health economics are disciplines which depend on integrating expertise from a wide range of people, from virologists to sociologists and many in between. For more information refer to their website: <http://www.isveexi.org/content.php?page=home>.

**5<sup>th</sup> International Symposium on Aquatic Animal Health; 2-6 September 2006; San Francisco, California, USA:** The 5<sup>th</sup> ISAHA is being sponsored by the Fish Health Section of the American Fisheries Society and will be held at the San Francisco Marriott Hotel. The first official announcement of the symposium is available on the AFS-FHS website at [http://www.fisheries.org/fhs/isaah\\_2006.htm](http://www.fisheries.org/fhs/isaah_2006.htm). Although details regarding registration, abstracts, etc. are not yet available, that information will be posted on the previously noted AFS-FHS website as it becomes available.

#### Recently held meetings

**Western Fish Disease Workshop; 28-29 June 2005; Boise, Idaho:** The meeting was well attended and the organizers have made a copy of the proceedings available on-line at: <http://www.fws.gov/fisheries/aadap/recentlyheldmeetings.html>.

**Antibiotic Resistance meeting with FDA's Center for Veterinary Medicine; 5 October 2005; Rockville, Maryland:** CVM's Microbial Food Safety Team and Division of Therapeutic Drugs for Food Animals met with the National Coordinator for Aquaculture New Animal Drug Applications and representatives from the University of Arizona, USGS (Upper Midwest Environmental Sciences Center), Phibro Animal Health and USFWS (AADAP). The purpose of the meeting was two-fold. First, the Microbial Food Safety Team was interesting in learning more about the aquaculture "industry" to help project impacts of new drug approvals from a microbial food safety perspective. Secondly, the non-CVM attendees were there to learn how best to address the antibiotic resistance component of a New Animal Drug Application (NADA), in particular as it relates to impending NADAs for oxytetracycline as a feed additive and chloramine-T. The meeting went exceptionally well and should be of great value to both the completion (i.e., by the aquaculture community) and review (i.e., by CVM) of the human food safety components of these and all other new drugs.



## ROZ's CORNER

The North Central Regional Aquaculture Center and the Multi-State Conservation Grant Program will fund studies on AQUI-S<sup>®</sup>, a zero withdrawal anesthetic. These studies should help keep us on track for final study reports for all data requirements to be submitted in 2008 and potential approval for 2008 or early 2009.

The company sponsors remained active in pursuing approvals for their aquaculture drug products and also received some good news: (1) Schering-Plough Animal Health submitted its Administrative New Animal Drug Application for Aquaflor<sup>®</sup> (florfenicol) for control of mortality associated with enteric septicemia in catfish to the Center for Veterinary Medicine (CVM); an approval should be coming shortly for this most important antibacterial [Editor's note: ESC claim actually approved 24 October 2005]; (2) Eka Chemicals, Inc. received acceptance letters from CVM on 6 June and 16 September regarding its hydrogen peroxide product (PEROX-AID<sup>®</sup>) microbial food safety submissions; this completes the Human Food Safety Technical Section for hydrogen peroxide; (3) Phibro Animal Health submitted a change on 21 September 2005 in its formulation to a dihydrate salt for their oxytetracycline product, Terramycin for Fish<sup>®</sup>; (4) Axcentive SARL called a meeting with CVM on 1 August 2005 to resolve the remaining issues related to their proprietary environmental assessment for their chloramine-T product, Halamid<sup>®</sup>; and (5) Bimeda, Inc. met with CVM on 23 May 2005 to clarify the remaining product chemistry data requirements for their erythromycin product, Aquamycin<sup>®</sup>.

I organized a meeting with CVM on October 5, 2005 to determine the microbial food safety requirements for oral oxytetracycline and chloramine-T. Clear paths for completing these tasks were identified. I developed a major survey to determine unmet label claim needs for aquaculture drug approvals; it went out 21 September 2005 to the 38 states that supported the Federal-State Aquaculture Drug Approval Partnership Project. Responses are due October 28, 2005. *Rosalie (Roz) Schnick, National Coordinator for Aquaculture New Animal Drug Applications, Michigan State University, La Crosse, Wisconsin.*

## CVM's NOTES

### Extralabel Use of Drugs for Aquaculture - Past and Present:

The Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA) allows veterinarians to prescribe extralabel uses of certain approved animal drugs and approved human drugs for animals under certain conditions. Extralabel use (ELU) refers to the use of an approved drug in a manner that is not in accordance with the approved label directions. The key constraints of AMDUCA are that any extralabel use must be by or on the order of a licensed veterinarian within the context of a veterinarian-client-patient relationship, must not result in violative residues in food-producing animals, and the use must be in conformance with the implementing regulations published at 21 CFR Part 530. A list of drugs specifically prohibited from extralabel use appears in the Code of Federal Regulations and can be found at <http://www.fda.gov/cvm/amducatoc.htm>.

Significantly, extralabel use of medicated feeds was excluded from the original ELU provisions in AMDUCA. In 2001, FDA recognized that extralabel use of medicated feed for treatment of minor species could be considered when the health of animals is threatened and suffering or death would result from failure to treat the affected animals. As a directive to its field staff, FDA wrote the Compliance Policy Guide (CPG) 615.115: Extralabel Use of Medicated Feeds for Minor Species, found at: [http://www.fda.gov/ora/compliance\\_ref/cpg/cpgvet/cpg615-115.html](http://www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg615-115.html), which described conditions under which veterinarians

could prescribe medicated feeds in an extralabel manner to treat minor species. In addition to the original conditions of ELU, the CPG imposed further constraints when the minor species are food producing animals. For aquaculture, extralabel use of medicated feed is limited to medicated feed products approved for use in aquatic species as formulated into the diet intended for the approved species.

This means that a veterinarian could order one of the approved over-the-counter (OTC) products to treat a different fish species than that described on the label, or could use the product to treat a different disease condition.

As a result of the Animal Drug Availability Act of 1996 (ADAA), a new option for regulating the distribution of new animal drugs was added to the traditional over-the-counter and prescription options. Regulations implementing the Veterinary Feed Directive (VFD) became effective in 2001; for information see <http://www.fda.gov/cvm/vfd.html>. This new VFD option was developed to provide a more tailored way to regulate what might have been called "prescription medicated feeds".

Specifically, extralabel use of VFD products is prohibited according to the regulations. Recently, FDA/CVM approved Aquaflor<sup>®</sup> (florfenicol) for the control of mortality due to enteric septicemia of catfish. For details on this new approval see [http://www.fda.gov/cvm/CVM\\_Updates/catfishapp.htm](http://www.fda.gov/cvm/CVM_Updates/catfishapp.htm). Aquaflor<sup>®</sup> was approved as a VFD product, which means it can only be distributed on the order of a veterinarian.

A few questions regarding Aquaflor<sup>®</sup> and other drugs:

**Q:** Can Aquaflor<sup>®</sup> be used in an extralabel manner under the CPG? **A:** *No, as a VFD product its use is limited to the species and indication on the approved label.*

**Q:** Can veterinarians continue to use the other approved over-the-counter products in an ELU manner under the CPG? **A:** *Yes, the provisions of the CPG as they pertain to non-VFD products are not changed.*

**Q:** Will all new products for aquaculture be limited to use only by veterinarians? **A:** *It depends. New products are evaluated by FDA/CVM on the basis of safety and effectiveness, and a decision is made on whether adequate directions for use can be written for non-veterinarians. If appropriate label directions can be written and other safety issues do not preclude the condition of use, the product can be marketed OTC.*

*For further information contact Dr. Donald A. Prater, Leader, Aquaculture Drugs Team at 301-827-7567 or [dprater@cvm.fda.gov](mailto:dprater@cvm.fda.gov).*