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 1 - 3

AADAP NEWSLETTER

May 2005

WHAT'S SHAKIN'

We've moved and have new direct-dial phone numbers: The AADAP Office and our entire crew have relocated across campus in the Bozeman Fish Technology Center's new Piper Building. Although our mailing address, FAX number and our email addresses have not changed, all of our phone numbers are different: Dave Erdahl 406-994-9904, Bonnie Johnson 406-994-9905, Miranda Dotson 406-994-9906, Jim Bowker 406-994-9910, Tom Bell 406-994-9911, Dan Carty 406-994-9912 and Molly Bowman 406-994-9916.

Tommie Crawford – one of our best: On April 22nd we lost a dear friend and colleague, Tommie Crawford, from the Missouri Department of Conservation. See page 8 for a special tribute.

Quarterly INAD Investigator Award: A tip-of-the-hat and a pat-on-the-back to Edmund Washuta and Craig Lemon of the New Jersey Division of Fish and Wildlife for their continuing involvement in efficacy studies and timely submissions of detailed data forms and reports.

Drug Approval Coordination Workshop: Mark your calendars; make your plans for the U.S. Fish & Wildlife Service's 11th Annual Drug Approval Coordination Workshop**!!!** As in the past, it is being held in Bozeman, Montana at the Holiday Inn (5 Baxter Lane). The dates this year are August 2-3, 2005. Like last year, other related meetings and activities will be held immediately before or after the Workshop. For more details, including the most recent announcement and draft agenda, see our website (http://fisheries.fws.gov/aadap/inadworkshop.html).

SLICE® INAD submitted: AADAP has submitted a request for a SLICE® (emamectin benzoate) INAD to be used as a freshwater ectoparasiticide. CVM has not yet approved our request. Within our letter to CVM, we requested authorization (i.e., release of animals into public waters or permission to slaughter for public consumption) for approximately 15 million fish, comprising 22 species from 16 named facilities.

Oxytetracycline for skeletal marking: AADAP conducted a pilot study investigating pH shifts in various source waters with incremental additions of Phoenix Scientific's newly approved oxytetracycline hydrochloride (OTC). Additionally, the buffering necessary to maintain OTC-treated water in the safe range for fish was investigated. See the "OTC Feature Article" on page 3 for a detailed description.

17-α methyltestosterone update:

New INAD: AADAP has submitted a request for a 17-α methyltestosterone (MT) INAD for gender manipulation in tilapia, which CVM has formally accepted (July 2004). Within our letter to CVM, we requested authorization (i.e., permission to slaughter for public consumption) for approximately 46 million fish from 24 potential facilities.

AADAP activities: AADAP will be responsible for the conduct of pivotal effectiveness studies and has submitted to CVM the protocol for these studies. We are awaiting a response to our protocol submission.

Aquaflor® (florfenicol) update:

Catfish studies: The drug sponsor, Schering-Plough Animal Health, has curtailed all new catfish studies under any florfenicol INAD. This action is referred to as "pharmacovigilance," typically occurs during the 11th hour of the approval process, and is being undertaken to ensure that no potential obstacles hinder their imminent approval for enteric septicemia in catfish. They're really, really close**!!!**

Talking about obstacles !!!

Pivotal efficacy study: Only one more FDA-approved pivotal efficacy study is required on a salmonid species (other than steelhead trout) to add (once accepted by CVM) the following claim to the label: "Control mortality in all freshwater-reared salmonids caused by columnaris." Florfenicol final study reports can be found on our website at http://fisheries.fws.gov/aadap/ssflorfenicol.htm.

We still need your help: Cooperators are needed to help conduct pivotal efficacy studies on coolwater fish species for florfenicol (any fish species diagnosed with columnaris), oxytetracycline (any fish species diagnosed with columnaris), and chloramine-T (any fish species other than walleye diagnosed with external columnaris or BGD). If we can conduct studies at your facilities, please give us a call.

AQUI-S® update:

Pivotal efficacy studies - Pivotal AQUI-S® efficacy studies have recently been conducted on chinook salmon (*Oncorhynchus tshawytscha*) and kokanee salmon (*Oncorhynchus nerka*). These studies will be submitted to CVM in May 2005. A big "thank you" goes out to Montana Fish, Wildlife and Parks and Idaho Fish and Game for their assistance.

AQUI-S® sedated fish with tag for post-treatment evaluation

Supportive efficacy studies – Four supportive studies were recently completed and/or submitted. Two of these have been submitted to CVM comparing the efficacy of AQUI-S® on individually sedated and group-sedated rainbow trout *Oncorhynchus mykiss* and channel catfish *Ictalurus punctatus*. There was no significant difference between times to induce fish to the handleable stage of anesthesia when sedated either individually or as a group.

Thanks to the Bozeman Fish Technology Center and Fish Breeders of Idaho for their assistance on these studies. The third study was conducted on chinook salmon *Oncorhynchus tshawytscha* at the Rapid River Fish Hatchery, Riggins, ID, and will be submitted to CVM in May 2005. The fourth is considered a "high-quality" supportive study and was conducted by the Missouri Dept. of Conservation (Lost Valley Fish Hatchery) on several groups of walleye broodstock. The Final Study Report is undergoing in-house review and will support the efficacy technical section for all coolwater fish. Thanks to the Missouri Dept. of Conservation for their help.

Target animal safety - CVM accepted our Target Animal Safety (TAS) research study protocol entitled "The Safety of AQUI-S® as an Anesthetic on Rainbow Trout *Oncorhynchus mykiss*." Completion and acceptance of this study should satisfy the TAS technical section for all freshwater-reared salmonids. The TAS study was conducted March 2005 to evaluate whether 40 mg/L meets the safety criteria as the highest proposed concentration for salmonids (preliminary testing has indicated that it will!); data are currently in the review, data entry and analysis process.

Nearing completion - We are almost finished with AQUI-S® pivotal and supportive studies required to complete the efficacy technical section for all freshwater-reared fish. To complete this technical section, CVM's Aquaculture Team requires acceptance of 2-3 pivotal studies and 3-6 supportive studies (per group) on representative cold-, cool-, and warmwater fish species.

Other information - for further information on the status of AQUI-S® efficacy studies please refer to http://fisheries.fws.gov/aadap/studiesAquis.htm.

SE-MARK® update:

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

Field trial report: In field studies recently completed by the Canadian Department of Fisheries and Oceans, calcein marks on out-migrating sockeye salmon were "vividly visible" after a 1 year residence in a lake. For more information, contact Western Chemical, Inc. (phone 800-283-5292).

Photo courtesy of Ron Secor

Feed studies to be conducted: The USGS Northern Appalachian Research Laboratory in Wellsboro, PA, will be funding research there and at the Service's Lamar and Bozeman Fish Technology Centers to investigate the feasibility of administering SE-MARK® via feed. The 3-year study will investigate the effectiveness of marking brook trout, rainbow trout, Atlantic salmon, striped bass, cutthroat trout and shovelnose sturgeon with calcein-medicated feed. The mark quality will also be assessed as a function of feed ingredients and long-term exposure of marked fish to natural light.

New spawning hormone approved in Norway and the European Union: In July 2003, the European Agency for the Evaluation of Medicinal Products (EMEA) approved a new spawning hormone, Gonazon™. As stated in EMEA's *European Public Assessment Report (EMEA/CVMP/259/03)* "The indication approved for Gonazon™ is for the induction and synchronisation of ovulation for the production of eyed-eggs or fry [of salmonids]." For more detailed information on Gonazon™, see *World Aquaculture* (March 2005, Vol. 36, No. 1) or see: www.emea.eu.int/vetdocs/PDFs/EPAR/gonazon/025903en1.pdf.

FEATURE ARTICLES

An Introduction to the U.S. Department of Agriculture's Center for Veterinary Biologics (CVB)

Melisse Schilling, DVM; Senior Staff Veterinarian
USDA, Animal and Plant Health Inspection Service
Center for Veterinary Biologics; 510 South 17th Street, Suite 104; Ames, Iowa 50010

The CVB regulates veterinary biologics (vaccines, bacterins, antisera, diagnostic test kits, and other products of biological origin) to ensure that those available for the diagnosis, prevention, and treatment of animal diseases are pure, safe, potent, and effective. The use of veterinary biologics in aquaculture is becoming more prevalent as vaccination is increasingly being used as a means for prevention and control of fish pathogens. Vaccination has enabled the reduction in the use of antibiotics in aquaculture in several countries. Reliable diagnostic test kits have been used to aid in disease control. The use of veterinary biologics may contribute to the sustainability of aquaculture.

The CVB operates under the authority of the Virus-Serum-Toxin Act (VSTA) of 1913, as amended in 1985. The CVB has two units: Policy, Evaluation, and Licensing (PEL); and Inspection and Compliance (IC). The CVB-PEL reviews license applications for production facilities and biological products; reviews applications for permits for importation of products; establishes licensing, testing, and permit requirements and procedures; reviews production methods, and supporting data involved in the licensing and permit process; and conducts laboratory testing as necessary. Dr. Melisse Schilling is the CVB-PEL Senior Staff Veterinarian assigned to aquaculture products.

The CVB-IC is responsible for developing and implementing programs to ensure that biologics are prepared and distributed in compliance with the VSTA. The VSTA requires that both products and facilities be licensed, and that products distributed in the United States are not worthless, dangerous, contaminated, or harmful. Dr. Lawrence Elsken is the CVB-IC Section Leader assigned to aquaculture products. Please feel free to contact either Dr. Schilling or Dr. Elsken by telephone at 515-232-5785 with questions related to the regulation of aquaculture biologics.

The CVB posts a list of currently licensed fish biologics on our website: http://www.aphis.usda.gov/vs/cvb/aqualicenses.htm. Biologics available for use in the United States that have an active license status are included on the list. Biologics available include products produced and licensed in the United States, as well as products manufactured in foreign countries that have a permit for distribution and sale in the United States. This list was last updated on April 18, 2005. The U.S. Code of Federal Regulations used to regulate biologics is also posted on this

website, as well as guidance documents such as Veterinary Services Memoranda and CVB Public Notices. The CVB is currently developing guidance documents specific to the aquaculture industry, which will be posted on this website.

Buffering Oxytetracycline Hydrochloride Immersion-marking Solutions with Sodium Phosphate Dibasic

Daniel Carty, Jim Bowker, Molly Bowman, and Bonnie Johnson; USFWS AADAP Program; 4050 Bridger Canyon Road, Bozeman, Montana 59715, USA

Introduction: Oxytetracycline hydrochloride (OTC-HCL) water soluble powder is approved in the U.S. for use in the skeletal marking of finfish fry and fingerlings by immersion at concentrations of 200-700 mg/L active OTC for 2-6 h. This compound is acidic, and therefore OTC-HCL solutions usually need to be buffered to prevent or minimize mortality in treated fish. Anhydrous sodium phosphate dibasic (SPD) is commonly used as a buffer, probably because it is relatively safe to humans (Stecher et al. 1968) and because its buffering effects are relatively easy to control (Fielder 2002). The Michigan Dept. of Natural Resources (MDNR) has recently published step-by-step guidelines for immersion-marking walleye *Sander vitreus* (formerly *Stizostedion vitreum*) fry and fingerlings with OTC-HCL and detecting resultant marks (Fielder 2002). Building on MDNR's solution-preparation and buffering information, we conducted a study to show (a) pattern and magnitude of pH decrease when OTC-HCL is incrementally added to a "source" water to produce a 700-mg/L active OTC solution and (b) pattern and magnitude of pH increase when SPD is incrementally added to buffer a 700-mg/L active OTC solution to pH 7.0.

Methods: Test articles used in our study were (a) Oxytetracycline HCl Soluble Powder-343 (343 g of active oxytetracycline per 454 g premix; Phoenix Scientific, Inc., St. Joseph, MO 64503; FDA-approved in September, 2004) and (b) sodium phosphate dibasic (anhydrous; > 99% pure, water soluble powder; E. M. Science, Gibbstown, N.J.). We used five source waters, ranging from a "cold, highly buffered, hard water" to a "cool water with no buffering capacity and no hardness" (Table 1). Initial pH values of the five source waters ranged from 7.1 to 7.9. Two 2-gal "replicate" water samples were collected from each source water, and each sample was processed as follows. First, fourteen 0.5-g aliquots of OTC-HCL were sequentially added to the water sample to achieve a non-buffered, nominal 700-mg/L active OTC solution. Second, ten 1-g aliquots of SPD were sequentially added to the resultant solution to buffer it to pH 7 (note: to enhance solubility of SPD, each aliquot of SPD was dissolved in 10 mL of microwave-warmed source water before adding it to the solution). The pH of the water sample was measured after each aliquot of OTC-HCL or SPD was added and dissolved. Replicate pH measurements were averaged for each source water to facilitate comparisons of pH change among source waters.

pH measurement

Results: In all five source waters, pH decreased incrementally as aliquots of OTC-HCL were added to achieve non-buffered, 700-mg/L active OTC solutions (Figure 1). Decreases observed in the two waters with "high" natural buffering capacity (cold spring and cold/warm spring waters) were more gradual and of less overall magnitude (2.3 and 2.6 pH units, respectively) than decreases observed in the two waters with moderate natural buffering capacity (cold/warm spring water diluted 1:1 or 1:2 with distilled water) or in distilled water (4.4, 4.5, and 4.4 pH units, respectively). In addition, minimum pH values observed in cold spring and cold/warm spring waters (5.3 and 5.2, respectively) were much higher than the minimum pH values observed in cold/warm spring waters diluted with distilled water or in distilled water (3.5, 3.1, and 2.7, respectively). Finally, the active OTC concentration at which pH was first observed to be unsuitable for aquatic life (pH ≤ 6.5, according to the U.S. Environmental Protection Agency) was highest in cold spring and cold/warm spring waters (250 mg/L in both), intermediate in cold/warm spring waters diluted with two levels of distilled water (150 and 100 mg/L, respectively), and lowest in distilled water (50 mg/L).

In all five source waters, pH increased incrementally as aliquots of SPD were added to buffer nominal 700-mg/L active OTC solutions to pH 7 (Figure 1). Increases observed in cold spring and cold/warm spring waters were more gradual and of less overall magnitude (1.8 and 1.9 pH units, respectively) than increases observed in cold/warm spring waters diluted with distilled water or in distilled water (3.6, 4.0, and 4.4 pH units, respectively). However, regardless of source water, 4-5 g of SPD (a 0.6-0.7:1 ratio with OTC-HCL) were needed to buffer pH to levels suitable for aquatic life (pH ≥6.5), and 8-9 g of SPD were needed to buffer pH to 7.0 (a 1.1 - 1.3:1 ratio with OTC-HCL). Finally, regardless of source water, maximum pH values achieved were never higher than 7.1, even after all 10 g of SPD had been added to a 700-mg/L active OTC solution.

Discussion and Conclusions: Our results demonstrated that the natural buffering capacity of source water substantially affects pattern and magnitude of pH change when OTC-HCL is added to water to achieve a non-buffered, 700-mg/L active OTC solution, and when SPD is subsequently added to buffer such a solution to pH 7.0. Natural buffering capacity can best be estimated by measuring total alkalinity, as opposed to measuring total hardness (Bain 1999). Total alkalinity and total hardness (both usually expressed as mg/L $CaCO_3$) will be similar if limestone is the source for both (Wurts and Durborow 1992). However, these two water quality parameters can differ considerably in soft waters that have high alkalinity, e.g., soda lakes (Cole 1979) or in hard or soft waters that have low alkalinity, e.g., some ground waters or already acidic waters (Wurts and Durborow 1992). The American Fisheries Society uses the following criteria to characterize source waters based on total alkalinity: (a) minimally acceptable buffering = 20 mg/L; (b) poorly buffered < 25 mg/L; (c) moderately buffered = 25-75 mg/L and (c) highly buffered > 75 mg/L (Bain 1999). Therefore, we suggest measuring the total alkalinity of a source water before preparing and buffering an OTC-HCL solution, and we agree with Fielder's (2002) advice to measure pH before and during immersion-marking sessions.

In addition, our results indicated that (a) most OTC-HCL solutions will need to be artificially buffered to maintain pH at levels safe for fish and that (b) such buffering is relatively easy to achieve with SPD. For example, in the five source waters used in our study, pH levels ≤ 6.5 were first observed in non-buffered solutions at active OTC concentrations of only 50-250 mg/L — far lower than the FDA-approved maximum of 700 mg/L active OTC. However, both our results and those of Fielder (2002) showed that an approximate 1:1 ratio (weight:weight) of SPD to OTC-HCL should be sufficient to buffer virtually any 700-mg/L active OTC solution to pH 7.0 and that addition of "excess" SPD will not increase pH much

above 7.0. It is important to note that Fielder (2002) warns that "overuse" of SPD can be toxic to treated fish.

Finally, it should be cautioned that our results and inferences may have limited application to "real-world" immersion-marking sessions because our study was conducted under controlled laboratory conditions with only two test articles, five source waters, and no fish. However, the information in the MDNR guide (Fielder 2002) can be adapted to a variety of "real-world" immersion-marking situations. Therefore, we encourage our readers to read the MDNR guide and to conduct their own "experiments" to determine how their test articles, source waters and fish interact. Note: The MDNR guide can be obtained in pdf format (3.5 MB) at: http://www.michigandnr.com/publications/pdfs/ifr/ifrlibra/technical/reports/2002-1tr.pdf

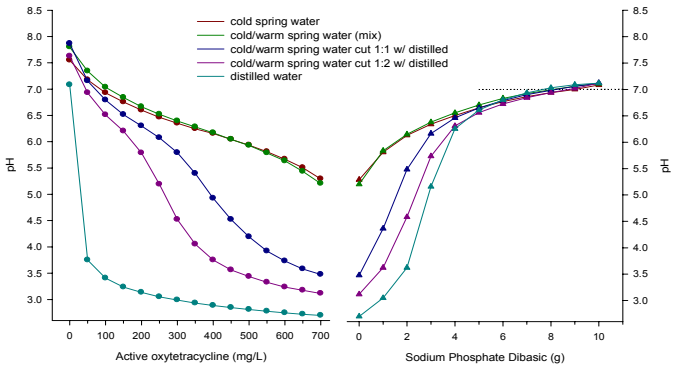
Table 1. Source Water Quality[1]

Source-water[2]	Initial pH	Total alkalinity (mg/L $CaCO_3$)	Total hardness (mg/L $CaCO_3$)	Temp (°C)
cold spring	7.6	185	200	6.9
cold/warm spring (mix)	7.8	165	197	12.0
cold/warm spring (cut 1:1 with distilled)	7.9	81	110	13.4
cold/warm spring (cut 1:2 with distilled)	7.6	56	74	13.9
distilled	7.1	0	0	15.2

Footnotes: 1. Water quality values reported are the means of two "replicate" water samples per source water.

2. **cold spring =** cold, highly buffered, hard; c**old/warm spring (mix) =** cool, highly buffered, hard; c**old/warm spring cut 1:1 (vol:vol) with distilled =** cool, moderately to highly buffered, moderately hard; **cold/warm spring cut 1:2 (vol:vol) with distilled =** cool, moderately buffered, soft to moderately hard; and **distilled =** cool, no buffering capacity, no hardness.

Figure 1. The pH of a solution was recorded after each 0.5-g aliquot of OTC-HCL (50 mg/L active OTC per aliquot) was added to achieve a non-buffered, 700-mg/L active OTC concentration (left portion of graph). The pH of a solution was also recorded after each 1-g aliquot of SPD was added (10 g total) to buffer the solution to pH 7.0 (right portion of graph). The pH values reported are means of two replicate water samples per source water.



References:

Bain, M. B. 1999. Interpreting chemical data. Pages 181-192 *in* M. B. Bain and N. J. Stevenson, editors. *Aquatic Habitat Assessment: Common Methods.* American Fisheries Society, Bethesda, Maryland.

Cole, G. A. 1979. *Textbook of Limnology*. The C. V. Mosby Company, St. Louis, Missouri.

Fielder, D. G. 2002. *Methodology for Immersion Marking Walleye Fry and Fingerlings in Oxytetracycline Hydrochloride and its Detection with Fluorescence Microscopy.* Michigan Department of Natural Resources, Fisheries Technical Report Number 2002-1. Michigan Department of Natural Resources, Lansing, Michigan.

Stecher, P. G., M. Windholz, D. S. Leahy, D. M. Bolton, and L. G. Eaton, editors. 1968. *The Merck Index: an Encyclopedia of Chemicals and Drugs*, eighth edition. Merck and Company, Rathway, New Jersey.

Wurts, W. A., and R. M. Durborow. 1992. *Interactions of pH, Carbon Dioxide, Alkalinity, and Hardness in Fish Ponds.* Publication No. 464, Southern Regional Aquaculture Center, Stoneville, Mississippi.

INAD INFORMATION & STATUS

The U.S. Fish & Wildlife Service currently administers numerous INADs via the AADAP office in Bozeman, Montana. In our last Newsletter, we focused on treatment options for various diseases and other drug uses (e.g., anesthesia). In this edition, we focus attention on an area of particular importance to the Service, our partner INAD-investigators, and FDA; i.e., on adherence to INAD study protocols. We consider the study protocol to be the foundation for the use of any investigational drug under a Service INAD.

No doubt about it, the INAD process is complicated, and at times, can be somewhat onerous. Although we have attempted to make participation in Service INADs as easy as possible, from the initial and routine paperwork perspective, as well as the actual use of the investigational drug as you conduct supportive studies, basic INAD requirements must still be met.

All Service INAD study protocols are developed by AADAP staff, and reviewed by FDA's Center for Veterinary Medicine (CVM). There is a conscious effort to make the protocols accommodating to real-life production settings and as user-friendly as possible. At the same time, the protocols are designed to be sufficiently robust so that there is a high probability that data generated will be applicable and acceptable when submitted to CVM as part of a New Animal Drug Application package. Equally important, the protocols are developed to instill confidence that use of these "yet-to-be-approved drugs" will not jeopardize worker safety, the treated fish, the environment, or those who may be exposed to or consume treated fish.

Therefore, it is important to follow the protocols as closely as possible. It is essential that such specifications as treatment dose/concentration, duration of treatment and entrance criteria be followed. If, for any reason, you feel that you cannot adhere to any aspect of the protocol, please contact us to discuss your situation before you proceed.

Unfortunately, "the work ain't done until you've completed the paperwork." Not only is adherence to protocol specifications essential, but the associated forms must be completed properly. It is from the information on these forms that we insert accountability into the entire INAD process.

It can't be emphasized enough that the INAD process, of which we are all a part, is probably outside of CVM's normal zone of comfort. The "compassionate" INADs held by the Service, although strictly above-board and legal, are (by the inherent nature of aquatic species production) operated differently from most traditional INADs held by pharmaceutical companies. As an example, the number of animals typically treated and authorized for slaughter under a traditional drug company INAD (often a mere handful) would pale when compared to the millions of animals treated (and authorized for slaughter or release into public waters) under Service INADs. We assume that CVM

highly scrutinizes aquaculture INADs; their "Aquaculture Drug Plan" from the mid-90s provided internal guidance and additional requirements, about the issuance, implementation and conduct of aquaculture INADs.

In short, the aquaculture community has been given tremendous latitude to assist in gaining new drug approvals, but to compensate for that, we are being patiently watched very closely. Not only do we have to strictly adhere to the rules, but we need to do so all within a "window of opportunity" that will close if accountable and legitimate progress is not made.

AADAP will continue to do our part to make the road to new approved aquaculture drugs as easy as possible within the constraints of the regulations. We, however, can not do it alone; we need your help and cooperation. So thanks in advance!!

FEATURED INAD DRUG

Terramycin® 100 for Fish: *Status of an expanded approval to include currently approved uses at temperatures less than 9°C, and to control mortality in freshwater-reared salmonids caused by two additional fish diseases.*

Terramycin® 100 for Fish (TF100) is a broad-spectrum anti-microbial especially formulated for incorporation into fish feed. It has been proven highly effective in controlling diseases caused by Gram-positive and Gram-negative bacteria that adversely affect salmonids and catfish.

TF100 is currently approved for use to control mortality in (1) catfish caused by bacterial hemorrhagic septicemia and pseudomonas disease and (2) salmonids caused by ulcer disease, furunculosis, bacterial hemorrhagic septicemia, and pseudomonas disease. It is also approved for (3) use in Pacific salmon feed as a marking agent. All use in salmonids is restricted to fish reared or held at water temperatures greater than 9°C.

Recently, Phibro Animal Health Corporation (PAHC; Ridgefield Park, NJ) began work to reformulate their TF100 and to expand the indications of the current label claim. The company is in the final stages of the product reformulation process, which has included studies to demonstrate that the reformulated oxytetracycline is stable. Once the product has been reformulated and stability studies have been accepted by the U.S. Food and Drug Administration's Center for Veterinary Medicine, the product label will be updated to reflect this change.

During this process, PAHC, Roz Schnick, and researchers from USGS's Upper Midwest Environmental Sciences Center have been trying to address several mandatory antimicrobial safety issues. It is hoped that data from PAHC Terramycin products used in terrestrial food animals will address one of the issues (noted above), and that the aforementioned entities will be able to address the other issue (noted above). If all goes well, these issues should be addressed within the next calendar year (2006) and PAHC will proceed with an Administrative New Animal Drug Application for expanding the approved uses of TF100.

In addition to the existing approved uses, the expanded label will allow TF100 to be used (1) for the previously approved purposes on fish at temperatures less than 9°C, and (2) at the current dosage and duration to control mortality of (a) freshwater reared salmonids caused by coldwater disease (causative agent *Flavobacteria psychrophilum*), and (b) freshwater reared steelhead trout *Oncorhynchus mykiss* caused by columnaris (causative agent *F. columnare*).

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.

- **AQUI-S® vs. MS-222:** If you are in the fish business, chances are you have used tricaine methansulfonate (i.e., MS-222 or tricaine) to sedate fish. You may have also heard about the experimental fish anesthetic AQUI-S®. We've used/tested AQUI-S® extensively over the past few years and would like to pass along a few tips.

  Q *Are MS-222 and AQUI-S® used at the same concentration?*

  A No. Research to gain FDA approval of MS-222 and AQUI-S® were done in different eras, and the proposed maximum allowable safe and efficacious concentration of AQUI-S® will be lower than the allowable concentrations of MS-222.

  Q *So what if the concentrations of MS-222 and AQUI-S® are different?*

  A When using AQUI-S®, more time will likely be required to sedate fish to a desired level of anesthesia. We've also found that fish sedated with AQUI-S® tend to require more time to recover than fish sedated with MS-222.

  Q *Are there differences in how to prepare a working solution of MS-222 and AQUI-S®?*

  A You bet. With MS-222, you can shake, pour, or scoop out the desired amount, drop it into a tub of water, stir, and you're on your way. We recommend preparing a stock solution of AQUI-S® (which is a viscous, oily liquid) in a small volume of water before pouring it into a tub of water (for mixing instructions see AADAP Newsletter, Volume 1-1 at http://fisheries.fws.gov/aadap/archives.htm). Such stock solution will be "milky" white. Now you can stir the contents of the tub and be on your way.

Pouring stock solution into exposure tub

  Q *Will AQUI-S® decrease the pH of water like MS-222 does?*

  A Not to our knowledge. We've done many comparisons under many different environmental conditions and have found that addition of AQUI-S® does not alter the pH of water (for those of you who sedate fish in water with low buffering capacity...take note!).

  Q *AADAP is in the final stages of trying to complete the efficacy technical section for AQUI-S®. What does this mean to you?*

  A Well...we can still use any additional safety and efficacy data that you have or will be willing to generate. We encourage biologists to use AQUI-S® (even if on a small scale) on a variety of fish species under the INAD and submit data to us.

  Q *For which fish species/life-stages are AQUI-S® data needed?*

  A We'll take AQUI-S® data for any and all fish species/life-stages. Chances are that, even if we have a sufficient quantity of data for a specific fish species, conditions under which you sedate fish will be different than conditions under which we (or others) sedated the

same species. We're on the prowl for a higher quality data package, which should include fish species and size sedated, AQUI-S® target concentration, description of the desired level of anesthesia, times to and from the desired level of anesthesia, water temperature, dissolved oxygen concentration, behavior of fish on initial immersion into a solution of AQUI-S® and on recovery in freshwater, and mortality attributed to exposure to AQUI-S®.

Q *Is AQUI-S® intended to replace MS-222?*

A Unequivocally, no! Tricaine has been, and will continue to be, a very useful aquaculture and fisheries management tool. However, the 21-d withdrawal period often legally precludes returning treated catchable-size fish to public waters. Our hope is that AQUI-S® will ultimately be approved with a 0-d withdrawal.

Southern Regional Aquaculture Center Fact Sheets: The Southern Regional Aquaculture Center (SRAC) is one of five USDA Regional Aquaculture Centers whose operations and activities are coordinated by USDA's Office of Aquaculture. The Regional Aquaculture Centers encourage cooperative and collaborative research and extension education programs in aquaculture that have regional or national application. The SRAC has produced a series of Fact Sheets, many of which can be quite useful to those administrating approved and investigational drugs. The SRAC Fact Sheets can be found on their website at: http://srac.tamu.edu/. Of particular interest may be those under the following categories: treatments (http://srac.tamu.edu/index.cfm?catid=20) and diseases (http://srac.tamu.edu/index.cfm?catid=26).

PARTNERS' CORNER

Hubbs-SeaWorld Research Institute: Along with the further development of salmonid aquaculture, eyes have been turning to new candidates for culture. With the dwindling natural stocks of commercial species in our oceans, the culture of these new species has generated tremendous interest. In the development of these animals for farming, the emergence of pathogens usually follows and the producers need to manage them.

Due to the limited number of tools available to treat disease, even the simplest of outbreaks can be devastating. Marine aquaculture may be even more susceptible, as most utilize surface water supplies with the potential of carrying pathogens from feral fish. A true need exists for those involved with marine aquaculture to be proactive in the development of drug usage requirements and associated drug label claims.

The Hubbs-SeaWorld Research Institute operates two facilities dedicated to the development of rearing techniques for several marine species including white seabass, California halibut, California sheephead, yellowtail and several species of rockfish (*Sebastes* spp.). It is our involvement in species development that has driven our participation in the AADAP and INAD programs. Historically, the labeling of drugs has focused mainly on traditional freshwater species, mainly salmonids and catfish. We at Hubbs recognized a need for our involvement to understand the system, assist in drug development where possible and give marine aquaculture a presence in the process.

Rockfish larvae 2-days after release
photo courtesy Hubbs-SeaWorld

With the AADAP coordinators instituting a "Piggy-Backin" program all those years ago, they have opened the doors to facilities like ours to participate in the process, not only benefiting our livestock with the availability of valuable disease treatment tools, but also allowing us to assist in the labeling process. The marine finfish sector of the U.S. aquaculture industry is very small and it is very difficult to find opportunities like that offered by AADAP.

For those of you involved in marine aquaculture, it is time to get involved in programs like AADAP, to ensure that tools will be available for new emerging culture species. If you are interested in getting involved, contact the AADAP coordinator and see how you can contribute. *Paul Curtis, Research Biologist; Hubbs-SeaWorld Research Institute; San Diego, CA 92109*

MEETINGS, ETC.

Upcoming meetings

Eastern Fish Health Workshop; 13-17 June 2005; Shepherdstown, West Virginia: The 30th Annual Eastern Fish Health Workshop will be hosted this year by the USGS National Fish Health Research Laboratory (Leetown, WV). The workshop will be held 13-17 June at the Clarion Hotel and Conference Center in Shepherdstown, West Virginia. Abstracts are due by 1 May 2005. For details see http://fisheries.fws.gov/aadap/2_05Files/30th%20Annual%20Eastern%20Fish%20Health%20Workshop%20announcement.pdf. For more information, contact Dr. Rocco Cipriano: phone 304-724-4432; fax 304-724-4435 or rocco_cipriano@usgs.gov.

Western Fish Disease Workshop; 28-29 June 2005; Boise, Idaho: The annual Western Fish Disease Workshop is being held at the Doubletree Riverside in Boise, Idaho. On Monday 27 June, the American Fisheries Society – Fish Health Section will be conducting a continuing education session. The workshop is being sponsored by the Idaho Department of Fish & Game, the University of Idaho and Clear Springs Foods. Details will be forthcoming on the AFS-FHS webpage (http://www.fisheries.org/fhs).

Aquaculture Canada℠ 2005; 3-6 July 2005; St. John's, Newfoundland: The 22nd annual meeting of the Aquaculture Association of Canada will be held at the Delta St. John's Hotel and Conference Center. The conference comprises several thematic areas, including (but not limited to) an international mussel forum, an international cod symposium, developments in aquatic animal health, and alternate species aquaculture. For other information see: www.aquacultureassociation.ca/ac05.

American Fisheries Society – Fish Health Section; 27-29 July 2005; Minneapolis, Minnesota: The annual meeting of the Fish Health Section will be held at the Ramada Inn Airport at the Mall of America. In conjunction with the meeting will be a continuing education course, "Current Topics in Aquatic Toxicology," which will be held on Saturday, 30 July. For more information see: http://www.fisheries.org/fhs.

11th Annual FWS Drug Approval Coordination Workshop; 2-3 August 2005; Bozeman, Montana: This year's Workshop will be held in Bozeman, MT on 2-3 August 2005. The 11th Annual Workshop will be co-hosted by USGS's Upper Midwest Environmental Sciences Center. In addition to the 2-day Drug Approval Workshop, a JSA sponsored "Biologics in Aquaculture Workshop" and the JSA's "Aquaculture Drug Research Forum" will be held. Each of these will be a 1-day session (1 August and 4 August, respectively). The second announcement for the Workshop has been sent, so please notify Molly Bowman (molly_bowman@fws.gov) if you've not received a copy of the

announcement and/or if you would like to be added to the mailing list. The second announcement includes detailed lodging information and a registration form, and will be available at: http://fisheries.fws.gov/aadap/inadworkshop.html.

AADAP's 2004 Aquaculture Drug Approval Workshop

Aquaculture Europe 2005; 5-9 August 2005; Trondheim, Norway: The theme for Aquaculture Europe 2005 will be "Lessons from the Past to Optimize the Future." The purpose of the conference is to address the sustainability of aquaculture. Sessions include the contributions of aquaculture to sustainable fisheries, health management, sustainable feed resources and reliability of hatchery production. There will also be a trade show (Aqua Nor 2005). The conference secretariat can be contacted by email at ae2005@aquaculture.cc and by FAX: +3259321005. The meeting announcement, call for contributions, registration form and additional information can be found at http://www.easonline.or/agenda/en/AquaEuro2005/default.asp.

Sixth Symposium on Diseases in Asian Aquaculture (DAA VI); 25-28 October 2005; Colombo, Sri Lanka: The theme of this year's symposium is "Aquatic Animal Health – Facing New Challenges." The foci of the symposium include technological advancements, biosecurity, risk analysis, food safety, international trade, finfish health, mollusc health, crustacean health, and stakeholder participation and regional cooperation. Abstracts are due 30 August 2005. Registration and other information can be found at http://www.daasix.org.

Vibrio 2005 - The Biology of Vibrios: Biodiversity, Genomics, Disease/Epidemiology, Ecology, and Applications; 7-8 November 2005; Ghent, Belgium: Vibrios are an important group of aquatic bacteria with diverse impacts, positive and negative, on aquaculture. The deadline for submission of an abstract is 1 July 2005. Registration is open to the public, but only limited numbers will be accepted. For more information see: http://lmg.ugent.be/vibrio2005.

Recently held meetings

Special session - Aquaculture Drug Research, 19 January 2005; New Orleans, LA (Aquaculture America 2005): Jim Bowker co-hosted a special session on current aquaculture drug research. The topics covered ranged from Phish-Pharm, a searchable database of pharmacokinetics data in fish; effects and safety of a variety of therapeutants (e.g., florfenicol, copper sulfate); and AQUI-S® efficacy, safety, and related measures of stress.

17-α methyltestosterone mini session, 18 January 2005; New Orleans, LA (Aquaculture America 2005): AADAP facilitated a small meeting/workshop attended by Rangen Feeds (sponsor of 17MT), federal and state agency researchers, private researchers, representatives of the American Tilapia Association and Rosalie Schnick (National Coordinator for Aquaculture NADAs). Although the mini session was focused on specific technical sections for the NADA not yet complete, we also felt that there was a need to "re-energize" the entire process. In essence, the goal of the session was to establish where have we been, where are we now, what's left to do, who's going to finish that which remains, and to establish timelines for completion of remaining work. Overview presentations were provided from several perspectives, and were followed by a panel discussion. A status report was produced in March 2005 and will be available soon on the AADAP website.

Minor Use Minor Species (MUMS) meeting with FDA's Center for Veterinary Medicine; 6 December 2004; Rockville, Maryland: A set of meeting notes, including a list of the attendees, has been assembled and reviewed by CVM; these notes are available at: http://fisheries.fws.gov/aadap/2_05Files/Meeting%20Notes%20with%20CVM%206dec04%20ver4.pdf.

ROZ'S CORNER

I have been active in trying to obtain funding for approval of AQUI-S® as a zero-withdrawal anesthetic. The North Central Regional Aquaculture Center (NCRAC) is providing $150,000 for residue depletion studies and the International Association of Fish and Wildlife Agencies approved a National Conservation Need that may result in funding for analytical methods development, target animal safety studies, and NADA coordination. If these funding initiatives are successful, all the known data requirements will be covered by immediate funding so that all final study reports could be submitted in 2008 for an approval.

Company sponsors and others have become more active and interactive as we move closer to aquaculture drug approvals: (1) Bimeda, Inc. obtained an Investigational New Animal Drug (INAD) exemption for their erythromycin product, Aquamycin® and has requested a meeting with CVM in May to discuss the remaining data requirements; (2) Eka Chemicals, Inc. submitted microbial safety letters on their hydrogen peroxide product, Perox-Aid®, and is preparing labeling. UMESC and I met in April with CVM to resolve any remaining issues related to the environmental assessment (EA) on hydrogen peroxide; (3) Axcentive SARL is preparing their product chemistry submission for their chloramine-T product, Halamid®. UMESC and I met with CVM in April to discuss the remaining issues related to the proprietary Halamid® EA and public environmental summary on chloramine-T. UMESC received word that the confirmatory method for detecting chloramine-T in fish tissue and the target animal safety for all coolwater and warmwater fish were accepted in March; (4) Phibro Animal Health obtained an INAD for their oxytetracycline product, Terramycin® and is working on revising its label to reflect a change in their formulation; and (5) Rangen, Inc. requested a CVM review in April 2005 of the analytical method to detect 17-α methyltestosterone in feed that was developed with NCRAC funds. *Rosalie (Roz) Schnick, National Coordinator for Aquaculture New Animal Drug Applications, Michigan State University, La Crosse, Wisconsin.*

CVM'S NOTES

CVM's Office of Research (OR) has been working on a variety of studies to support the development of aquaculture drugs. The aquaculture (AQ) research group has submitted the final report of a Good Laboratory Practices (GLP) study describing the effectiveness of formalin to control mortality associated with saprolegniasis on rainbow trout and is developing a protocol for a preliminary (to be followed by a GLP) study to evaluate the effectiveness of formalin to control mortality associated with saprolegniasis on channel catfish. The OR is working with CVM's Office of New Animal Drug Evaluation (ONADE) to

develop methods to detect aquaculture drugs in edible tissue and water to (1) support the development of aquaculture drugs conducted primarily by the public sector, (2) facilitate the import tolerance provisions of ADAA and (3) support the HACCP activities of FDA/CFSAN. Specifically, OR is supporting multiple incurred residue studies in representative major cultured species.

The AQ research group has developed NCCLS/CLSI methods for disc diffusion and broth microdilution antimicrobial susceptibility, and validated each method with an international multi-laboratory study. These will be published in CLSI documents (one is already a report; the other will be published summer 2005). They have also developed a relational database of information on pharmacokinetic parameters in fish that provides rapid access to data about the metabolism, accumulation, and elimination of drugs or chemicals in fish tissues. This database incorporates information from over 300 articles and will be published on-line in the Journal of the Association of American Pharmaceutical Scientists, http://www.aapsj.org.

Currently the research group is coordinating and collaborating on a study to compare a chemical method and a microbiological method for detecting erythromycin in fish tissues. Also under development is an internal parasite infection model in largemouth bass. These fish contain an internal parasite that will be used to test the effectiveness of various drugs for the treatment of the infection. Finally, the research group determined *in vitro* metabolic profiles of multiple fish species in collaboration with a university partner. The profiles are being compared with *in vivo* residue profiles of model drugs in several species to determine if *in vitro* data can help support generalizations regarding drug metabolism and residue predictions in multiple fish species. *For further information contact Dr. Donald A. Prater, Leader, Aquaculture Drugs Team at 301-827-7567 or dprater@cvm.fda.gov.*

TRIBUTE TO TOMMIE CRAWFORD

Tommie in Canada doing what he loved most
Photos courtesy of JR Booth, Missouri Department of Conservation

The drug approval community lost a dear friend and colleague on April 22, 2005. Tommie Crawford's presence at the annual INAD Workshop will be sorely missed. Although Tommie was typically rather reserved, his measured comments were always valued for being "right on the mark." Tommie was normally up for a brew after a long drug approval session, and could be counted on to keep the conversations lively and upbeat - never did he utter a bad word about anybody.

The following is excerpted from material provided by JR Booth of the Missouri Department of Conservation. Thanks to JR for his words, the above photos and allowing us to reproduce these in the Newsletter.

Tommie Gene Crawford, 49, passed away at his home, April 22, 2005. Tommie was born in 1956 in Springfield, Missouri, and graduated there in 1974 from Glendale High School. Growing up in the Ozarks, his love of fishing and the outdoors developed early. He received a B.S. in Wildlife Conservation/Management (Aquatics Option), as well as a B.S. in Soil Science from Southwest Missouri State University in 1978, and an M.S. in Biology (Aquaculture Option) from Arkansas State University in 1980.

Tommie's career took him to three states. From 1983 through 1986 he worked for the Arkansas Game & Fish Commission (AGFC), first as a Hatchery/Extension Biologist and then he directed and supervised all fish culture activities at the Spring River Hatchery. He also carried out disease diagnostic programs for seven AGFC hatcheries. From 1986 through 2000 Tommie worked for the Kansas Department of Wildlife and Parks (KDWP) as the Hatchery Manager at the Milford Fish Hatchery. While in Kansas he also developed and initiated methodologies for successful production of domesticated striped bass broodfish, and was also responsible for KDWP INAD programs. From 2000 until the time of his death, Tommie worked for the Missouri Department of Conservation (MDC). His first duty in Missouri was to serve as the Aquaculture Coordinator, coordinating annual fish requests and fish production assignments. As the Aquaculture Coordinator, he also managed MDC's INAD activities, provided technical assistance to public and private fish hatcheries, developed aquaculture educational programs, coordinated the statewide harvest and stocking of advanced fingerling channel catfish, and supervised MDC's traveling "Show Me Missouri Fish - Mobile Aquarium Program." In January of 2003, Tommie was promoted to MDC's Warmwater Hatcheries Supervisor, directly supervising four warmwater hatcheries. He also provided input on statewide/national policy issues that involved aquaculture and water quality issues.

Tommie genuinely cared about his employees. He had a quirky sense of humor and he loved to tell jokes and share funny stories. He considered his coworkers as an extended family, and he touched many many lives throughout his career. He attributed the bliss of never marrying to the fact that he had never run across a woman that would put up with his obsessive fishing addiction. He loved the water, and was nearly always willing to head out onto the lake after work to spend a few hours fishing. It didn't matter what was biting; he was content catching whatever happened to be biting that day. Tommie's good natured personality gained him friends wherever he went. He was always willing to help out his family and friends in any way that he could. He was a very generous and loyal man who will be missed by everyone that knew him.