

## **17-Alpha Methyltestosterone Clinical Field Trials - INAD 11-236**

### **Year 2008 Annual Summary Report on the Use of 17-Alpha Methyltestosterone in Field Efficacy Trials**

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#### **Summary**

Various techniques have been developed for the control of sexual differentiation in a variety of fish species, including the use of the androgen, 17-alpha Methyltestosterone (MET). Specifically, this compound has been used effectively in the U. S. under compassionate INAD Exemption #11-236 on early life stage tilapia to produce predominately male populations (i.e., sex reversal). In calendar year 2008 (CY08) the efficacy of MET was evaluated in 90 trials involving approximately 14.2 million early life stage tilapia for sex reversal. Trials were conducted at nine commercial tilapia culture facilities. The compassionate study protocol under which treatments were administered allowed the investigator to administer MET at a dosage of 9 mg MET/kg of fish biomass for 28 consecutive days (60 mg MET/kg feed when fed at a rate of 15% body weight). Overall, results of trials conducted in CY08 indicated that treatments appeared efficacious in approximately 8% of the trials or were characterized as inconclusive in 42% of the trials. In the remaining 50% of the trials, the Investigators

were not required to report efficacy data because the effectiveness technical section for the specific claim has been completed and accepted by CVM.

## **Introduction**

Larval stages of many teleost species contain both ovarian and testicular tissue, and sexual differentiation commences shortly after hatching or after the initiation of feeding (Yamamoto, 1969; Donaldson and Hunter, 1982; Yamazaki, 1983). Various techniques have been developed for the control of sexual differentiation in a variety of fish species (Donaldson and Hunter, 1982). These techniques have typically involved the use of either androgen or estrogen treatment to override the endogenous mechanisms of sex determination in developing larval stages and direct sexual differentiation toward the production of either males or females (i.e., sex reversal). Treatment regimens have generally involved immersion of larval stages in water containing a steroid, incorporation of a steroid in the larval diet, or both. As numerous factors such as dosage, timing, duration, and environmental conditions often influence efficacy, results have been somewhat variable.

Although the gonadal tissue of tilapia remains undifferentiated at hatch, tilapia generally attain sexual maturity by three to six months of age, and begin to immediately reproduce. This somewhat precocious reproduction behavior is the primary impediment to the development of successful strategies for the commercial production of tilapia. In

response to this problematic issue, strategies for monosex male culture have been evaluated including: 1) manual separation of the sexes by visual examination; 2) hybridization; and 3) sex reversal/direction via steroid administration.

1. Manual separation of the sexes via visual examination (or hand sexing) has been found to be a tedious and time consuming process, prone to significant human error, and less productive than other methods. Currently, hand sexing of tilapia is practiced by only a limited number of fish farmers in underdeveloped countries.
2. Hybridization involves the crossing of two different species of tilapia that can result in the production of 95 -100% male offspring. The most frequently used crosses suitable for aquaculture production are *Tilapia nilotica* (F) x *T. hornorum* (M); *T. mossambica* (F) x *T. hornorum* (M); and *T. nilotica* (F) x *T. aurea* (M). However, there are limitations to hybridization that include behavioral incompatibilities between two species that lead to significantly decreased fingerling production; the high potential for the contamination of the broodfish populations; and the high costs associated with the sophisticated equipment and techniques necessary to ensure and confirm broodfish purity. Hybridization is currently practiced on only a limited basis worldwide.

3. Gonadal differentiation in tilapia typically occurs between 8 to 25 days post-hatch, dependent upon environmental conditions. It has been demonstrated that the oral administration of the synthetic androgen 17-alpha methyltestosterone (MET) to newly hatched tilapia fry (3 -12 days old) for ~28 consecutive days results in populations comprised of greater than 90% males (Green et al., 1997; Rani and Macintosh, 1997; and Teichert-Coddington et al., 2000). The excess androgen that is introduced into the early life stage fish overrides endogenous hormones and directs sexual differentiation towards the formation of testis. The use of orally administered MET has been shown to be an efficacious, cost-effective, and efficient methodology to produce populations of male tilapia.

Of the three above-described methods/strategies for the monosex culture of male tilapia, oral administration of MET is the most effective and economic option, and is best suited for successful tilapia production in the United States.

### **Purpose of Report**

The purpose of this report is to summarize the results of calendar year 2008 (CY08) MET field efficacy trials conducted under INAD #11-236. Furthermore, it is expected that data from these trials will be used to enhance the existing MET effectiveness database that has been established from previous years studies for the purpose of developing an appropriate label claim for the use of MET in aquaculture.

## **Facilities, Materials, and Treatment Procedures**

### **1. Facilities**

Nine commercial tilapia culture facilities used MET for sex reversal of early life stage tilapia. Mean water temperature during all trials was 82.1 °F, and water temperature ranged between 74.0 - 88.0 °F at the different testing facilities.

Note: One facility was removed from the INAD for failing to submit the required reports. Please see the attached termination letter that was sent to this facility.

### **2. Test article used**

The MET used during the reporting period was 17 beta-hydroxy-17-methylandrosta-4-ene-3-one. All MET medicated feed used in INAD trials was manufactured and supplied by Rangen Inc, P.O. Box 706, Buhl, ID. The source of MET used by Rangen Inc. to prepare medicated feed was supplied by Hawkins, Inc. Pharmaceutical Group, 3000 East Hennepin Ave, Minneapolis, MN.

### **3. Treatment regimen**

As described in the Study Protocol, Investigators were allowed to only use MET at a dosage of 9 mg MET/kg of fish biomass for 28 consecutive days (i.e., the

industry standard); equivalent to 60 mg MET/kg feed when fed at a rate of 15% body weight.

**Protocol Deviation** - Two trials were administered where fish were fed at 9 mg MET/kg of fish biomass for 14 - 28 days. The Investigators noted that feed trials ended early due to not enough MET medicated feed on hand. The Investigators will ensure enough MET medicated feed is available prior to the start of future trials.

### **Fish Species Involved in CY08 Trials**

#### **1. Species of fish treated**

Tilapia were the only fish species treated during CY08. Treated fish ranged in length from 2 - 14 mm. Tilapia strains that were treated included:

1. Nile Tilapia *Oreochromis niloticus*
2. Tilapia Aurea *O. aureus*
3. California Mozambique *O. mossambica*
4. Red Hybrid Tilapia *O. mossambica* x *O. urolepis*

## Data Collected

### 1. **Primary response variable (gross examination of features characteristic of mature males/females)**

The efficacy of MET medicated feed to produce populations of tilapia comprised of > 90% males was the primary indicator of clinical field efficacy trials success. Investigators were allowed to perform a gross visual examination of external features characteristic of either male or female tilapia of fish that had reached or were near reaching sexual maturity. Results from gross visual examinations were submitted with seven trials.

Based on correspondence with FDA, the following efficacy technical section has been completed:

1. Effectiveness of MET at a dosage of 9 mg MET/kg of fish/day for 28 consecutive days. Administration should begin before fish reach 14 days post-hatch. We refer to your file number INAD I-011236-P-0039-EF dated December 10, 2008.

As a result of the completed technical section, efficacy data are no longer required when Investigators administer MET at a dosage of 9 mg MET/kg of fish/day for 28 consecutive days.

## **2. Secondary response variables**

Secondary parameters include general observations on fish behavior and response to routine culture activities. Secondary parameters of interest included evaluating feeding activity, feed consumption, apparent level of stress, negative fish behavior, and mortality.

## **3. Drug accountability data**

Drug receipt reports, drug use reports, and treatment reports (including adverse effects/toxicity observations), and fish disposition reports were prepared by study Investigators. Such reports were routed through the Study Monitor for review, and then sent to the AADAP Office for review, data analysis and report writing, entering data into a database, and archiving in permanent files.

## **Discussion of Study Results:**

### **1. General observations on the efficacy of MET to produce populations**

**comprised of greater than 90% male fish** (Note: Table 1 provides a summary of all trials characterized as effective; Table 2 provides a summary of all trials characterized as inconclusive; Table 3 provides a summary of all trials where efficacy data was not required; Table 4 provides summary data for all trials; and Table 5 provides a summary of all trials conducted during CY08 under INAD #11-236).

### **A. Efficacy at 9 mg/kg bw/day for 14 - 28 days**

Fish were treated 9 mg/kg bw/day for 14 - 28 days in 90 trials (Tables 1 - 3). Treatments were determined to be effective by the Investigators visually evaluating the treated fish a few months after the treatment period ended. Effectiveness of some of the treatments were characterized as inconclusive because (1) Investigators did not determine fish sex while fish were on station, or (2) young fish were sold to a commercial grow out facility before they could be sexed. Results from all trials in which sex was determined using gross visualization techniques showed that MET treatments were effective (i.e., such techniques were used to determine sex of fish in 7 trials). Treatment effectiveness was characterized as inconclusive 38 trials (because sex determination was not performed by the Investigator) and not reported in 45 trials (due to the efficacy packet being complete).

## **2. Observed Toxicity**

No toxicity or adverse effects relating to MET treatment were reported in any of the trials.

### **Current Study Protocol for MET INAD #11-236**

Please see the attached current study protocol for MET INAD #11-236. Please note no changes have occurred to this study protocol.

### **Facility Sign-up List**

Please see “Table 6. Facilities and Names of Investigators” for facilities that signed-up to participate in the MET INAD #11-236 during CY08. Facilities not listed in Appendix III-a of the current MET INAD #11-236 during CY08 study protocol have been highlighted.

The following facilities had MET on-hand during CY08 but never used the drug:

1. Arizona Mariculture Associates
2. Friendly Aquaponics, Inc
3. Minkota Fisheries
4. Simaron Fresh Water Fish, Inc
5. St. Croix Campus

### **Correspondence sent to MET INAD #11-236 Participants**

Please see the attached correspondence that was sent to all MET participants after the AADAP Office received their sign-up form for calendar year 2008.

### **Number of Treated Fish under Treatment Use Authorization**

Total number of fish treated during CY08 was 14,188,936. The total number of treated fish to count against the current treatment use authorization dated December 20, 2007 is 14,523,936.

## Summary of Study Results

MET was administered at 9 mg/kg bw/day for 14 - 28 days on four different strains of tilapia and involved approximately 14.2 million fish. Treated fish ranged in length from 2 - 14 mm. Water temperature during treatment ranged from 74.0 - 88.0 °F, with a mean treatment temperature of 82.1 °F. Overall results in all cases in which gross visual exams were performed to determine sex of fish showed that treatment appeared effective. However, such techniques to determine sex were only used in 8% of trials. Treatments were characterized as inconclusive in 42% of the trials and in the remaining 50% of the trials, the Investigators were not required to report efficacy data because the effectiveness technical section for the specific claim has been completed and accepted by CVM. No evidence of toxicity or adverse effects related to MET treatment were reported in any of the trials. Data from these studies will be considered as ancillary data because of a general lack of quality control criteria essential for pivotal or supportive studies, such as use of untreated control fish, dose verification, replication, and randomization. None-the-less, the data described above should provide useful corroborative data to support a label claim for MET for the sex reversal in tilapia. It is anticipated that additional ancillary efficacy data will continue to be collected under INAD #11-236 until such a time that the INAD is terminated. In future trials conducted under INAD #11-236, efforts will continue to be directed towards the generation of high quality data.

## References

- Donaldson, E. M. and Hunter, G.A. 1982. Sex control in fish with particular reference to salmonids. *Can. J. fish Aquat. Sci.* Vol. 39:99-110.
- Green, B.W., Veverica, K.L., and M.S. Fitzpatrick. 1997. Fry and fingerling production, pp. 215-243. *In* H.S. Engo and C.E. Boyd, editors; Dynamics of pond aquaculture. CRC Press, Boca Raton, Florida, USA.
- Rani, A. and D.J. Macintosh. 1997. An evaluation of the effects of hormone concentration, treatment period, feeding regime, and rearing salinity on the production of all-male Nile tilapia (*Oreochromis niloticus*) fry using 17alpha methyltestosterone, pp. 791-804. *In* K. Fitzsimmons, editor; Tilapia aquaculture: Proceedings from the fourth international symposium on tilapia in aquaculture. Northeast Regional Agricultural Engineering Service, Cooperative Extension Service. Ithaca, New York, USA.
- Teichert-Coddington, D., B. Manning, and J. Eya. 2000. Concentration of 17alpha methyltestosterone in hormone-treated feed: Effects of analytical technique, fabrication, and storage temperature. *J. World Aquaculture Soc.* Vol. 31, No. 1:42-49.
- Yamamoto, T. 1969. Sex differentiation, pp. 117-175. *In* W.S. Hoar and D.J. Randall, editors; Fish Physiology, Vol. III. Academic Press, New York and London.
- Yamazaki, F. 1983. Sex control and manipulation in fish. *Aquaculture*, 33:329-354.

**Table 1. Summary of CY08 MET Treatment Results - Effective Trials**

Hatchery	Number of Trials	Tilapia Species	Fish Size (mm)	Number of Treated Fish	Treatment Duration (Days)	Dose (mg/kg fish bw)	Temp. (°F)
Americulture	1	Nile Tilapia	9.00	150,000	28	9	84.0
Canyon Farm - SeaPac of Idaho	2	California Mozambique	5.00	460,000	28	9	82.0
Kent SeaTech Corp	1	California Mozambique	14.00	80,000	28	9	78.0
Pacific Aquafarms	3	California Mozambique	5.00	1,254,261	28	9	78.8 - 82.0

**Table 2. Summary of CY08 MET Treatment Results - Inconclusive Trials**

Hatchery	Number of Trials	Tilapia Strain	Fish Size (mm)	Number of Treated Fish	Treatment Duration (Days)	Dose (mg/kg fish bw)	Temp. (°F)
Aquasafrá	30	Nile Tilapia Tilapia Aurea	6.00	7,708,000	28	9	80.0 - 82.0
McLean County Aquaponics	6	California Mozambique	2.00	17,000	28	9	74.0 - 78.0
Smith Creek Hatchery	1	Nile Tilapia	5.00	250,724	17 - 28	9	80.0
Tilapia Farm	1	Nile Tilapia	5.00	300,000	28	9	?

**Table 3. Summary of CY08 MET Treatment Results - Trials where efficacy data was not needed**

Hatchery	Number of Trials	Tilapia Strain	Fish Size (mm)	Number of Treated Fish	Treatment Duration (Days)	Dose (mg/kg fish bw)	Temp. (°F)
AmeriCulture	11	Nile Tilapia	9.00	1,791,444	28	9	84.0
Aquasafra	1	Nile Tilapia	6.00	300,000	28	9	80.0
Canyon Farm	20	Red Hybrid Tilapia	3.00	1,082,000	28	9	88.0
Chirico Farms	1	Nile Tilapia	7.00	3,000	28	9	78.0
Kent SeaTech Corp.	10	California Mozambique	14.00	788,007	14 - 28	9	78.0
McLean County Aquaponics	2	California Mozambique	2.00	4,500	28	9	76.0 - 78.0

**Table 4. Summary Data Regarding Summary of CY08 MET Treatment Trials**

**Total Fish Treated:** **14,188,936**

Number of fish treated in efficacious trials	1,944,261
Number of fish treated in inconclusive trials	8,275,724
Number of fish where efficacy was not needed	3,968,951

**Total number of trials:** **90**

Efficacious trials	7
Inconclusive trials	38
Efficacy was not needed	45

**Treatment Regimen Used:**

9 mg/kg bw/day for 14 - 28 days	90 trials
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**Treatment Water Temperature (°F):**

Temperature Range	74.0 - 88.0
Mean Temperature	82.1

**Size of Treated Fish:**

Size Range	2 - 14 mm
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**Tilapia Strains Treated:**

**Tilapia**

Nile Tilapia *Oreochromis niloticus*

Tilapia Aurea *O. aureus*

California Mozambique *O. mossambica*

Red Hybrid Tilapia *O. mossambica* x *O. urolepis*