

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

## **Year 2007 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 2007 (CY07) the efficacy of MET was evaluated in 144 trials involving approximately 20.5 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 from trials conducted in CY07 indicated that treatment appeared either effective (in approximately 66% of the trials) or was characterized as

inconclusive (in the remaining 34% of the trials). Based on results from CY07, it appears that MET is an effective compound for sexual reversal of early life stage tilapia.

## **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 2007 (CY07) 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 81.6 °F, and water temperature ranged between 78.0 - 88.9 °F at the different testing facilities.

### **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** - One trial was administered where fish were fed at 9 mg MET/kg of fish biomass for 13 days. The Investigator noted that the feed trial

was ended early due to high mortality experienced by treatment day 13. It was unknown what caused the high mortality to occur.

## **Fish Species Involved in CY06 Trials**

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

Tilapia were the only fish species treated during CY07. Treated fish ranged in length from 2 - 19.5 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 (microscopic examination of gonadal tissue reports or 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.

The sex of individual fish was determined by evaluation of gonadal tissue according to procedures of the gonadal squash technique as described by Guerrero and Shelton, 1974. Investigators were required to send 60 fish to the

U. S. Fish and Wildlife Service's Aquatic Animal Drug Approval Partnership Program's research office in Bozeman, MT where gonads were examined and classified as testis, ovary, or ovotestis (gonads containing both ovarian and testicular tissue). However, in CY07, samples were sent from a limited number of Investigators, and subsequent microscopic examination of gonadal tissue reports were available for only 1 of 144 (1%) trials. Investigators were also 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 94 of 144 (65%) of the trials. Overall, a report of sex determination was submitted for 95 of 144 (66%) of the trials.

## **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.

### **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 summary data for all trials; and Table 4 provides a summary of all trials conducted during CY07 under INAD #11-236).

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

Fish were treated the industry standard dose for 28 days in 142 trials (Tables 1 & 2). 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 94 trials). Treatment effectiveness was characterized as inconclusive in the remaining 48 trials because sex determination was not performed by the Investigator.

#### **B. Efficacy at 9 mg/kg bw/day for 13 days**

Fish were treated at the industry standard for 13 days in one trial (Table 2). The Investigator noted that the feed trial was ended early due to high mortality experienced by treatment day 13. It was unknown what caused the high mortality to occur. Treatment effectiveness was characterized as inconclusive because sex determination was not performed by the Investigator.

**C. Efficacy at 9 mg/kg bw/day for 28 days under Pivotal Research Protocol  
MT-05-EFF**

Fish were treated at 9 mg/kg bw/day for 28 days in 1 trial (Table 1). Sex of fish was determined by the Investigator who performed histology on the treated fish a few months after the treatment period. Please refer to pivotal submission numbers MT-05-EFF.3-04 for more details on this study. Treatment was considered effective in this trial.

**2. Observed Toxicity**

No toxicity or adverse effects relating to MET treatment were reported in 143 trials; however, it was unknown what caused high mortality to occur in one study on treatment day 13. The investigator noted mortality may have been due to disease, water quality, or a new bag of MET feed that was opened a few days prior to the onset of the mortality.

**Number of Treated Fish under Slaughter Authorization**

Total number of treated fish during CY07 was 20,532,060. The total number of treated fish to count against the slaughter authorization dated June 4, 2004 (valid through December 19, 2007) is 62,655,119. The total number of treated fish to count against the current slaughter authorization dated December 20, 2007 is 335,000. No changes have occurred to the current MET INAD #11-236 study protocol.

## **Facility Sign-up List**

Please see “Table 5. Facilities and Names of Investigators” for facilities that signed-up to participate in the MET INAD #11-236 during CY07. Facilities not listed in Appendix III-a of the current MET INAD #11-236 study protocol have been highlighted.

The following facility received MET during CY07 but never used the drug:

1. Chirico Farms

## **Summary of Study Results**

MET was administered at 9 mg/kg bw/day for 13 - 28 days on four different strains of tilapia and involved approximately 20.5 million fish. Treated fish ranged in length from 2 - 19.5 mm. Water temperature during treatment ranged from 78.0 - 88.9 °F, with a mean treatment temperature of 81.6 °F. Overall results in all cases in which microscopic or 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 66% of trials. Consequently, treatment results in the remaining 34% of the trials were characterized as inconclusive. No evidence of toxicity or adverse effects related to MET treatment were reported in 143 of the trials. The trials conducted under the pivotal research protocol MT-05-EFF included use of control fish, detailed histology reports documenting the male to female sex ratio during the post-treatment periods, and

will likely be accepted by CVM as pivotal or supportive. Data from the other 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

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**Table 1. Summary of CY07 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	12	Nile Tilapia	9.00	3,020,250	28	9	84.0
Canyon Farm - SeaPac of Idaho	26	Red Hybrid Tilapia	3.00	1,445,000	28	9	88.0
Canyon Farm - SeaPac of Idaho (Pivotal Study)	1	Red Hybrid Tilapia	11.00	1,600	28	9	88.9
Kent SeaTech Corp	35	California Mozambique	14.00	673,100	28	9	78.0
Pacific Aquafarms	21	California Mozambique	5.00	1,831,110	28	9	78.0 - 82.4

**Table 2. Summary of CY07 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)
Aquasafra	34	Nile Tilapia Tilapia Aurea	6.00	7,889,000	28	9	80.0 - 82.0
Arizona Mariculture Associates	11	Nile Tilapia	9.00	4,950,000	28	9	82.0
Canyon Springs Fish Farm	1	Red Hybrid Tilapia	2.00	40,000	28	9	80.0
Lake Geneva Fisheries	1	Nile Tilapia	10.00	600,000	28	9	78.0
Minkota Fisheries	2	Nile Tilapia	9 - 19.5	82,000	13 - 28	9	80.0

**Table 3. Summary Data Regarding Summary of CY07 MET Treatment Trials**

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<b>Total Fish Treated:</b>	<b><u>20,532,060</u></b>
Number of fish treated in efficacious trials	6,971,060
Number of fish treated in inconclusive trials	13,561,000
<b>Total number of trials:</b>	<b>144</b>
Efficacious trials	95 (66%)
Microscopic evaluation	1 of 95 trials
Gross visual examination	94 of 95 trials
Inconclusive trials	
No sex determination techniques used	49 (34%)
<b>Pivotal Study:</b>	
Study Number: 11-236-07-56	
<b>Treatment Regimen Used:</b>	
9 mg/kg bw/day for 28 days	143 trials
9 mg/kg bw/day for 13 days	1 trial
<b>Treatment Water Temperature (°F):</b>	
Temperature Range	78.0 - 88.9
Mean Temperature	81.6
<b>Size of Treated Fish:</b>	
Size Range	2 - 19.5 mm
<b>Tilapia Strains Treated:</b>	
<u>Tilapia</u>	
Nile Tilapia <i>Oreochromis niloticus</i>	
Tilapia Aurea <i>O. aureus</i>	
California Mozambique <i>O. mossambica</i>	
Red Hybrid Tilapia <i>O. mossambica</i> x <i>O. urolepis</i>	