

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

## **Year 2009 Annual Summary Report on the Use of 17-Alpha Methyltestosterone in Field Efficacy Trials**

Prepared by:

Bonnie Johnson, Biologist  
U.S. Fish and Wildlife Service  
Aquatic Animal Drug Approval Partnership Program  
Bozeman, Montana

### **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 2009 (CY09) the efficacy of MET was evaluated in 74 trials involving approximately 13.9 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 CY09 indicated that treatments were characterized as inconclusive in 3% of the trials. In the remaining 97% 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 CY09 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 82.6 °F, and water temperature ranged between 78.0 - 88.0 °F at the different testing facilities.

Note: Two facilities were removed from the INAD for failing to submit the required reports. Please see the attached termination letters that were sent to these facilities.

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

The MET used during the reporting period was 17 beta-hydroxy-17-methylandroster-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 2 - 25 days. In the trial where the fish were fed for 25 days, the Investigator noted the feed trial ended early due to not enough MET medicated feed on hand. The Investigator will ensure enough MET medicated feed is available prior to the start of future trials. In the trial where fish were fed for two days, the trial was terminated after treatment day 2. The investigator noted the Tilapia's water supply was abundant with plankton and the fish were not eating the medicated feed. The study was terminated and all other MET treatments have been put on hold until the water supply can be re-plumbed.

## **Fish Species Involved in CY09 Trials**

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

Tilapia were the only fish species treated during CY09. Treated fish ranged in length from 2 - 10 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. No results from gross visual examinations were submitted in CY09.

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 list of all trials characterized as inconclusive; Table 2 provides a list of all trials where efficacy data was not required; Table 3 provides summary data for all trials; and Table 4 provides a summary of all trials conducted during CY09 under INAD #11-236).

### **A. Efficacy at 9 mg/kg bw/day for 2 - 28 days**

Fish were treated 9 mg/kg bw/day for 2 - 28 days in 74 trials (Tables 1 - 2). Effectiveness of some of the treatments were characterized as inconclusive because (1) Investigator did not determine fish sex while fish were on station, or (2) the trial was terminated on treatment day 2 due to Tilapia feeding on natural food instead of the MET medicated feed. Treatment effectiveness was characterized as inconclusive two trials and not reported in 72 trials (due to the efficacy packet being complete).

### **2. Observed Toxicity**

No toxicity or adverse effects relating to MET treatment were reported in CY09.

### **3. Observed Withdrawal Period**

All withdrawal times were either met or exceeded in 72 trials and was unknown in two trials due to the data not being submitted to the AADAP Office. However, due to fish age at time of treatment the 120 day withdrawal period should have been met.

### **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 CY09. Facilities not listed in Appendix III-a of the current MET INAD #11-236 during CY09 study protocol have been highlighted. Please note all of these facilities are in compliance with their reporting requirements to the NPDES authority; and have been approved by CVM’s environmental team to participate under the MET INAD.

The following facilities had MET on-hand during CY09 but never used the drug:

1. Pacific Aquafarms
2. 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 CY09.

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

Total number of fish treated during CY09 was 13,908,949. The total number of treated fish to count against treatment use authorization dated December 20, 2007 is 28,432,885.

## **Summary of Study Results**

MET was administered at 9 mg/kg bw/day for 2 - 28 days on four different strains of Tilapia and involved approximately 13.9 million fish. Treated fish ranged in length from 2 - 10 mm. Water temperature during treatment ranged from 78.0 - 88.0 °F, with a mean treatment temperature of 82.6 °F. Treatments were characterized as inconclusive in 3% of the trials and in the remaining 97% 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. 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

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- 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.
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- 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. List of CY09 MET Treatment Results - Inconclusive Trials**

Hatchery	Tilapia Species	Fish Size (mm)	Number of Treated Fish	Dose (mg/kg fish bw)	Treatment Duration (Days)	Observed Withdrawal Time	Temp. (°F)
Arcola Fisheries	TIN	4	33,000	9	2	>120 days	86.00
Smith Creek Hatchery	TIN	5	300,000	9	25	4 months	86.0

**Table 2. List of CY09 MET Treatment Results - Trials where efficacy data was not needed**

Hatchery	Tilapia Species	Fish Size (mm)	Number of Treated Fish	Dose (mg/kg fish bw)	Treatment Duration (Days)	Observed Withdrawal Time	Temp. (°F)
AmeriCulture	TIN	9	181,300	9	28	210 days	84.00
AmeriCulture	TIN	9	229,690	9	28	210 days	84.00
AmeriCulture	TIN	9	157,500	9	28	210 days	84.00
AmeriCulture	TIN	9	186,000	9	28	210 days	84.00
AmeriCulture	TIN	9	210,700	9	28	210 days	84.00
AmeriCulture	TIN	9	150,500	9	28	210 days	84.00
AmeriCulture	TIN	9	168,000	9	28	210 days	84.00
AmeriCulture	TIN	9	249,900	9	28	210 days	84.00
AmeriCulture	TIN	9	250,000	9	28	210 days	84.00
AmeriCulture	TIN	9	220,500	9	28	210 days	84.00
AmeriCulture	TIN	9	167,000	9	28	210 days	84.00
AmeriCulture	TIN	9	142,100	9	28	210 days	84.00
Aquasafra	TAU	6	309,000	9	28	120 days	81.00
Aquasafra	TAU	6	144,000	9	28	120 days	81.00
Aquasafra	TAU	6	335,000	9	28	120 days	81.00
Aquasafra	TAU	6	150,000	9	28	120 days	81.00
Aquasafra	TAU	6	193,000	9	28	120 days	81.00
Aquasafra	TAU	6	300,000	9	28	120 days	81.00
Aquasafra	TAU	6	260,000	9	28	120 days	81.00
Aquasafra	TAU	6	353,000	9	28	120 days	81.00
Aquasafra	TAU	6	240,000	9	28	120 days	81.00

**Table 2. List of CY09 MET Treatment Results - Trials where efficacy data was not needed - continued**

Hatchery	Tilapia Species	Fish Size (mm)	Number of Treated Fish	Dose (mg/kg fish bw)	Treatment Duration (Days)	Observed Withdrawal Time	Temp. (°F)
Aquasafra	TAU	6	200,000	9	28	120 days	81.0
Aquasafra	TAU	6	287,000	9	28	120 days	81.00
Aquasafra	TIN	6	200,000	9	28	120 days	81.00
Aquasafra	TIN	6	288,000	9	28	120 days	81.00
Aquasafra	TIN	6	200,000	9	28	120 days	81.00
Aquasafra	TIN	6	210,000	9	28	120 days	81.00
Aquasafra	TIN	6	345,000	9	28	120 days	81.00
Aquasafra	TIN	6	180,000	9	28	120 days	81.00
Aquasafra	TIN	6	245,000	9	28	120 days	81.00
Aquasafra	TIN	6	186,000	9	28	120 days	81.00
Aquasafra	TIN	6	202,000	9	28	120 days	81.00
Aquasafra	TIN	6	176,000	9	28	120 days	81.00
Aquasafra	TIN	6	181,000	9	28	120 days	81.00
Aquasafra	TIN	6	329,000	9	28	120 days	81.00
Aquasafra	TIN	6	123,000	9	28	120 days	81.00
Aquasafra	TAU	6	344,000	9	28	120 days	80.00
Aquasafra	TAU	6	340,000	9	28	120 days	80.00
Aquasafra	TAU	6	310,000	9	28	120 days	80.00
Aquasafra	TAU	6	296,000	9	28	120 days	80.00
Aquasafra	TIN	6	330,000	9	28	120 days	80.00
Aquasafra	TIN	6	226,000	9	28	120 days	80.00
Aquasafra	TIN	6	270,000	9	28	120 days	80.00
Arizona Mariculture Associates	TIN	9	35,000	9	28	210 days	82.0
Arizona Mariculture Associates	TIN	9	120,000	9	28	210 days	82.00
Arizona Mariculture Associates	TIN	9	105,000	9	28	210 days	82.00
Arizona Mariculture Associates	TIN	9	83,000	9	28	210 days	82.00
Arizona Mariculture Associates	TIN	9	58,000	9	28	210 days	82.00
Arizona Mariculture Associates	TIN	9	189,000	9	28	210 days	82.0
Arizona Mariculture Associates	TIN	9	275,000	9	28	210 days	82.00

**Table 2. List of CY09 MET Treatment Results - Trials where efficacy data was not needed - continued**

Hatchery	Tilapia Species	Fish Size (mm)	Number of Treated Fish	Dose (mg/kg fish bw)	Treatment Duration (Days)	Observed Withdrawal Time	Temp. (°F)
Arizona Mariculture Associates	TIN	9	255,000	9	28	210 days	82.00
Arizona Mariculture Associates	TIN	9	87,000	9	28	210 days	82.00
Canyon Farm	RHT	3	54,000	9	28	250 days	88.00
Canyon Farm	RHT	3	39,000	9	28	250 days	88.00
Canyon Farm	RHT	3	38,500	9	28	250 days	88.00
Canyon Farm	RHT	3	75,000	9	28	250 days	88.00
Canyon Farm	RHT	3	100,000	9	28	250 days	88.00
Lake Geneva Fisheries	TIN	10	500,000	9	28	365 days	78.00
McLean County Aquaponics	MZT	2	3,500	9	28	120 days	78.00
McLean County Aquaponics	TIA	2	3,500	9	28	120 days	78.00
McLean County Aquaponics	TIA	2	3,500	9	28	120 days	78.00
McLean County Aquaponics	MZT	2	3,500 - estimated	9	28	120 days - based on fish size	
Pacific Corp Arizona Organic Fisheries	TIN	2	45,000	9	28	250 days	83.00
Pacific Corp Arizona Organic Fisheries	TIN	2	5,000 - estimated	9	28	120 days - based on fish size	
Smith Creek Hatchery	TIN	5	300,000	9	28	120 days	86.0
Smith Creek Hatchery	TIN	5	142,296	9	28	120 days	86.00
Smith Creek Hatchery	TIN	5	120,963	9	28	120 days	86.00
Smith Creek Hatchery	TIN	5	150,000	9	28	120 days	86.00
Smith Creek Hatchery	TIN	5	120,000	9	28	120 days	86.00
Smith Creek Hatchery	TIN	5	200,000	9	28	120 days	86.00
Smith Creek Hatchery	TIN	5	200,000	9	28	120 days	86.00
Smith Creek Hatchery	TIN	5	200,000	9	28	120 days	86.0

**Table 3. Summary Data Regarding Summary of CY09 MET Treatment Trials**

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**Total Fish Treated:** **13,908,949**

Number of fish treated in inconclusive trials 333,000  
Number of fish where efficacy was not needed 13,575,949

**Total number of trials:** **74**

Inconclusive trials 2  
Efficacy was not needed 72

**Treatment Regimen Used:**

9 mg/kg bw/day for 2 - 28 days 74 trials

**Treatment Water Temperature (°F):**

Temperature Range 78.0 - 88.0  
Mean Temperature 82.6

**Size of Treated Fish:**

Size Range 2 - 10 mm

**Tilapia Strains Treated:**

**Tilapia**

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