

**DRUG APPROVAL RESEARCH ON 17 $\alpha$ -METHYLTESTOSTERONE**

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**Industry Advisory Liaison:** Rosalie A. Schnick, National Aquaculture NADA Coordinator, La Crosse, Wisconsin

**Extension Liaison:** Joseph E. Morris, Iowa State University

**Funding Request:** \$50,000

**Duration:** 2 years (November 1, 2004- October 31, 2006)

**Objectives:**

1. Interact with the Center for Veterinary Medicine (CVM) to determine the study design and protocol.
2. Submit the study protocol to CVM and gain acceptance from CVM for the study protocol.
3. Conduct a target animal safety study using 17 $\alpha$ -methyltestosterone (MT) on tilapia according to CVM guidelines for a target animal safety study in feed under good laboratory practices (GLP).
4. Write the final study report and submit to CVM through the MT Investigational New Animal Drug (INAD) Coordinator at Auburn University.
5. Provide progress reports to the North Central Regional Aquaculture Center (NCRAC).
6. Gain acceptance from CVM for the target animal safety study on MT in tilapia.

**Proposed Budget:**

<b>Institution</b>	<b>Principal Investigator</b>	<b>Objectives</b>	<b>Year 1</b>	<b>Year 2</b>	<b>Total</b>
Southern Illinois University-Carbondale	Anita M. Kelly	1-6	\$23,730	\$26,270	\$50,000
<b>Totals</b>			\$23,730	\$26,270	\$50,000

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## JUSTIFICATION

Sexual differentiation in teleosts is diverse and labile (Francis 1992) allowing for endocrine sex inversion in many species (Pandin and Sheela 1995). Hormonal induction of sex inversion is a valuable tool to not only understand sex differentiation, but also to produce monosex populations for the aquaculture industry (Guerrero 1975; Hunter et al. 1983; Shelton 1986; Pandin and Sheela 1995). Treatment protocols for 47 species using one of 31 natural or synthetic steroids are reviewed by Pandin and Sheela (1995).

In most teleosts, males grow faster than females or are more colorful in the case of ornamental fishes. Accordingly, protocols for masculinization are more numerous than for feminization. Among the androgens, MT is the most widely used hormone for sex inversion and has been tested on more than 25 species belonging to Salmonidae, Cichlidae, Cyprinidae, Anabantidae, Poeciliidae, Cyprinodontidae (Pandin and Sheela 1995) and Percidae (Malison et al. 1986). It is most often provided to fish in the diet (Hunter et al. 1983) either by homogenous mixing of the steroid with ingredients (Yamamoto 1953) or more often by the alcohol evaporation method (Guerrero 1975).

Congress has charged the U.S. Food and Drug Administration (FDA) with the control of the use of drugs. Although extensive testing is required for both food fishes and for nonfood fishes, additional data on metabolites, residues, and residue persistence must be submitted for the establishment of a minimum tolerance for chemicals applied to food fish (Meyer and Schnick 1989). The Food, Drug, and Cosmetic Act and corresponding regulations (21 CFR 511) do not permit the use of an unapproved new animal drug unless an exemption, i.e., an INAD exemption, has been granted. Currently, Auburn University holds the INAD for MT for sex reversing tilapia. The INAD is associated with the generation of data to support approval of a New Animal Drug Application (NADA). A data base, apparently sufficient for a NADA on MT for tilapia is currently missing the Target Animal Safety Study (R. Schnick, National Aquaculture NADA Coordinator, personal communication). Target Animal Safety Studies address concerns regarding the safety of the drug to the animal to which it is being applied. Simply stated, does MT have any adverse effects on the fish (tilapia in this case)?

A Target Animal Safety Study must be conducted under Good Laboratory Practice (GLP) regulations (21 CFR 58) which are minimum requirements for conducting a study to assure the quality and integrity of data generated. Briefly, the GLP regulations require that each and every procedure is fully documented as a Standard Operating Procedure (SOP), all facilities and equipment have written procedures and maintenance documented, the use and distribution of the test article is controlled and documented, the procedures are monitored by quality assurance personnel, and that personnel are properly trained for their assigned duties and responsibilities. Documentation of all procedures followed, reasons for any deviations from SOPs, the protocol, all correspondence pertaining to the study, quality assurance audit reports and findings, and the final report of the study with all data generated, must be kept in a designated, separate archive area in a fireproof cabinet for a minimum of two years, and usually for the duration of the new animal drug registration. In addition to the facility quality assurance personnel, the sponsor also monitors the study for compliance with GLP regulations and adherence to the written, approved (by FDA) protocol. Lastly, a final report is written by the testing facility personnel, reviewed by the sponsor, and submitted to the FDA as a part of the registration package.

## RELATED CURRENT AND PREVIOUS WORK

A Target Animal Safety Study for human chorionic gonadotropin was completed at Southern Illinois University-Carbondale (SIUC) in which Anita M. Kelly was the investigator. This study was sponsored by Intervet, Inc., conducted under GLP compliance, and was subsequently approved by the FDA for use in food fish.

A Target Animal Safety Study for common carp pituitary was recently completed by Anita M. Kelly. This study was sponsored by Spirit Lake Fisheries, conducted under GLP compliance, and the results will be submitted to the FDA in the near future.

Both of the above Target Animal Safety studies required approximately 50 SOPs, a housing facility fully accredited by the American Association of Laboratory Animal Care, specialized training of key personnel, and

American College of Veterinary Medicine board certified veterinarian for necropsies, and either a American College of Veterinary board-certified veterinarian pathologist or an American Fisheries Society Certified Fish Pathologist for histological examinations.

### **ANTICIPATED BENEFITS**

The results of this study will complete the package being promulgated by Auburn University to gain FDA approval for use of MT for sex inversion in tilapia. The production of all male populations of tilapia will greatly enhance their aquaculture potential because males grow significantly faster and larger than females. Accordingly, this study has the potential to significantly benefit the aquaculture industry in the North Central Region where tilapia are emerging as an aquaculture species.

### **OBJECTIVES**

1. Interact with the Center for Veterinary Medicine (CVM) to determine the study design and protocol.
2. Submit the study protocol to CVM and gain acceptance from CVM for the study protocol.
3. Conduct a target animal safety study using 17 $\alpha$ -methyltestosterone (MT) on tilapia according to CVM guidelines for a target animal safety study in feed under good laboratory practices (GLP).
4. Write the final study report and submit to CVM through the MT Investigational New Animal Drug (INAD) Coordinator at Auburn University.
5. Provide progress reports to the North Central Regional Aquaculture Center (NCRAC).
6. Gain acceptance from CVM for the target animal safety study on MT in tilapia.

### **PROCEDURES**

#### **Interact with CVM to Determine the Study Design and Protocol (Objective 1)**

The principal investigator will contact CVM and discuss the study design that would ultimately be used for the target animal safety study. The Principal Investigator has previous experience working with the FDA in designing study protocols for target animal safety studies. The protocol will be written based on the CVM publications CVM Guidance Document for Target Animal Safety Studies, CVM Guidance Document for Target Animal Safety Studies in General, and CVM Guidance Document for Protocols, and based on previously approved protocols for other drugs that were previously submitted and approved by FDA for the principal investigator.

#### **Submit the Study Protocol to CVM and Gain Acceptance From CVM for the Study Protocol (Objective 2)**

Following discussions with CVM personnel, a protocol will be developed and submitted to CVM for final approval prior to the initiation of the study.

#### **Target Animal Safety Study (Objective 3)**

The procedures that follow are written in the format required as a protocol for FDA approval for a Target Animal Safety Study. Accordingly, there is some redundancy with other sections of this proposal namely RELATED CURRENT AND PREVIOUS WORK, FACILITIES, and REFERENCES. The appendices are part of the protocol and are thus included in this section.

1      Title: SAFETY OF 17  $\alpha$ -METHYLTESTOSTERONE FOR INDUCTION OF SEX INVERSION IN

TILAPIA.

- 2 Purpose and Objective: To assess the safety of 17 a-methyltestosterone (MT) fed to tilapia (family Cichlidae) fry for induction of sex inversion to produce all male populations. The results of the safety study will be used to support a New Animal Drug Application to the U.S. Food and Drug Administration to approve the use of MT for this indication for the family Cichlidae.
- 3 GLP Compliance: The study will be conducted in compliance with the Good Laboratory practices (GLP) for nonclinical laboratory studies at 21 CFR Part 58.
- 4 Sponsor  
  
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- 6 GLP Monitor

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## 7 Test Facilities

7.1 Fisheries Research Laboratory  
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Carbondale, IL 62901-6501

7.2 Vivarium  
Southern Illinois University-Carbondale  
Mailcode 6506  
Carbondale, IL 62901

7.2.1 All fish will be maintained in a single room of the Vivarium in 37.9 L glass aquaria with individual biofiltration and aeration supplied via ceramic air stone for maintenance of water quality. The room will be solely dedicated to this study.

7.2.2 The Southern Illinois University-Carbondale facilities above are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

## 8 Test Animals

Tilapia (*Oreochromis niloticus*) fry at the onset of exogenous feeding from the family Cichlidae. Eggs will be removed from the mouth of the female and placed into hatching containers. Once the fry have absorbed their yolk sac (approximately 3 days post hatch) the fry will be fed a commercial trout diet for 7 days prior to initiation of the diets that will be used in this study.

### 8.1 Justification

*Oreochromis* species are characterized by a precocious sexual maturity and a high reproductive efficacy, resulting in overpopulation in ponds. A monosex male population is a solution to control reproductive activity and also to increase production because the male grows faster than the female in these species (Ruwet et al. 1976; Hanson et al. 1983). Sex reversal with synthetic androgens is one of the most frequently applied techniques to produce monosex male populations in tilapia. This involves the administration of synthetic androgens or estrogens to differentiating fry either by injection, immersion, or via the feed. Use of steroid-treated feeds for the production of all-male populations is widespread in tilapia aquaculture (MacIntosh and Little 1995). This technique results generally in 100% masculinization both at experimental and commercial stages (Guerrero 1975; Berger and Rothbard 1987; Mc Geachin et al. 1987; Guerrero and Guerrero 1988; Vera-Cruz and Mair 1994; Melard et al. 1995). However, the FDA has not approved the use of MT for sex inversion in fish, which will eventually be consumed by humans. Therefore the objective of this study is to conduct a target animal safety study to determine if MT is harmful to tilapia when provided in the feed.

### 8.2 Procurement

Tilapia fry will be obtained from brood stock maintained at Southern Illinois University-Carbondale.

## 9 Test Article

17 $\alpha$ -methyltestosterone (MT) will be incorporated into #1 salmon starter feed and #1 crumble, Batch Numbers #tba. Feed size must be increased as fish grow. Because this study will be conducted for 28 days, various feed sizes will need to be used.

9.1 Source: Feed will be purchased from Rangen, Inc., Buhl, Idaho. Methyltestosterone will be added to the feed by mixing it with ethanol and stirring into the dry diet. The hormone-ethanol solution will be thoroughly mixed with a numerically equal volume of the feed (e.g. 100 mL hormone solution with 100 g of feed). Uniform distribution of the hormone will be accomplished by thoroughly mixing the solution with the feed. The wet diet will be dried and three samples from various areas within the batch will be analyzed for MT concentration prior to feeding to the tilapia fry, 30 days into the study and upon completion of the feeding trials. Appropriate amounts of MT will be added to the dry diets to achieve MT concentrations of 0, 60, 180, and 300-mg/kg feed. Feed will be stored frozen until fed. Certification of MT content in feed will be provided by either the Biochemistry Department at University of Wisconsin at Madison or by the Chemistry Department at Southern Illinois University-Carbondale by using an FDA approved method for determination of MT in feed (currently under development, Terence Barry, University of Wisconsin-Madison, personal communication). Feeds that are within  $\pm 10\%$  of the nominal will be utilized in this study.

## 9.2 Dosage

Feeds will contain 0, 60, 180 and 300 mg/kg MT. Fish will be fed 15% of their wet body weight daily. MT will be administered at a dosage of 0, 9, 27, or 45 mg/kg of fish biomass.

### 9.2.1 Duration of treatment

MT medicated feed will be fed to tilapia fry for 28 consecutive days.

### 9.2.2 Concomitant therapy

Because this is a target animal safety study in which data on the effects of MT are being collected, there should be little or no concomitant therapy. Preferably no treatment should occur in the seven days prior to the initiation of the feeding trials or during the feeding trials. Any fish that dies during the course of the study will have an immediate necropsy performed and tissue samples sent for histopathological analysis.

### 9.2.3 Justification

Tilapia aquaculture in the United States has grown dramatically since 1991. In 2003, domestic tilapia production was 9200 mt, which is less than half of the 100,000 mt of tilapia that was imported in the same year (American Tilapia Association). The market for tilapias in the United States, more so than in developing countries, demands the production of larger fish. However, excessive reproduction is a problem in tilapia culture where overcrowding of young causes competition for the available food and represses the growth of the entire population.

Many approaches for controlling reproduction have been tried and one of the more promising techniques is hormone induction of monosex populations (Guerrero 1975). In addition to eliminating the problem of unwanted reproduction, all-male populations have a greater growth potential (Kirk 1972). One method of producing all male populations is to treat juvenile fish with androgens to induce phenotypic sex inversion resulting in 100% male offspring.

Various steroids are differentially potent and can be influenced by the mode of administration (Yamamoto 1969). It is considered more efficient to administer sex hormones orally than by injection or immersion. The addition of the hormones to the feed is thus a convenient mode of presentation. The androgen dosage that has been consistently effective for sex inversion in tilapia is methyltestosterone at 60 mg/kg of feed (Guerrero 1975; Sanico 1975; Tayamen 1977). Lower levels have been less effective, or ineffective. Although initial studies fed the hormone for 60-120 days, the typical hormone treatment period is 28 days.

The dosages to be used in this safety study are 0, 1, 3, and 5x the proposed standard dosage.

## 9.4 Inventory: Appendix I.

10 Placebo: #1 salmon starter and #1 crumble which have been mixed with ethanol that does not contain MT.

10.1 Source: Rangen, Inc.

10.2 Dosage

Fish will be fed at 15% of their body weight daily a feed that does not contain MT.

10.2.1 Justification: The feeding regime for placebo is consistent with the feeding regime for test article.

10.3 Inventory: Appendix II

11 Experimental design

Twelve 37.9-L aquaria each containing 20 fish will be utilized in this study. Because the MT may leach into the water and contaminate the water in aquaria containing control fish or fish of different treatment levels, each aquarium will be maintained on its own filtration system. A randomized block design will be used to account for any differences that would be accountable to location of the tanks within the room.

11.1 Blinding

Fish will be randomly assigned to a block of aquaria and randomly assigned to treatment group within the block design. The diets will be assigned a color code by the study monitor. The color code will not be revealed to the investigator doing the observations. This will eliminate the potential of bias based on treatment group. The necropsy of the fish will also be blind in that the veterinarian will be told only the color and not the treatment dose.

12 Procedure

12.1 Fish will be anesthetized, and five fish will be counted, weighed and measured before being randomly assigned to the twelve experimental tanks. The twelve tanks will be stocked with five fish before the next batch of five fish is stocked. This will be repeated until each tank is stocked with 20 fish.

12.2 Each group of four tanks will be assigned to the placebo or test article groups following a randomized block design. Fish will be color coded to correspond to the placebo or test article feed group. Fish will be fed the assigned diets for 30 days. This is the standard duration for administration of the drug.

12.3 An analysis of starting water in the aquaria will be conducted to determine water temperature, hardness, alkalinity, dissolved oxygen, nitrate, nitrite, ammonia, chloride, carbon dioxide, and pH will be measured prior to the initiation of the study. Water will also be tested for MT concentration by either the Biochemistry Department at University of Wisconsin at Madison or by the Chemistry Department at Southern Illinois University-Carbondale by using an FDA approved method for determination of MT in feed (currently under development, Terence Barry, University of Wisconsin-Madison, personal communication). Thereafter, water quality and temperature will be maintained at optimum levels according to standard aquaculture practices. Dissolved oxygen and temperature will be measured daily with an YSI Model 55/12 FT (Yellow Springs Instruments, Inc., Yellow Springs, Ohio, USA). The pH of experimental tank water will be measured daily with a Beckman 210 pH meter (Beckman Instruments, Inc., Fullerton, California, USA). Alkalinity and hardness will be measured titrimetrically (APHA 1995) once a week. Photoperiod will be maintained at 14 h light and 10 h dark using an Intermatic timer that is directly wired into the electrical system of the room. Photoperiod will begin at 700 h. Water temperature will be maintained at  $28 \pm 1^\circ\text{C}$  for the duration of the study.

12.4 All fish will be fully acclimated to the facilities during yolk-sac absorption before treatments are administered.



- 12.5 On day 0, each tank of fish will be anesthetized using 0.05% MS-222, weighed as a group and individually measured. Feed calculations will be determined from the initial weights. Feed will be offered 3-4 times daily. The amount of feed offered will initially be based on the biomass in the experimental tanks at allocation and then fish will be weighed every two weeks to adjust the feeding amount.
- 12.6 After weighing, each tank of fish will be fed their assigned feeds and fish will be observed once daily for 30 days (Days 1-30) for any unusual clinical signs. Technicians offering the feed and making the observations will be unaware of the treatment assigned to each tank. Fish that die during the period will be subjected to complete gross and histopathological examination (see below).
- 12.7 On Day 31, all fish in the experimental tanks (20 per tank) will be euthanized by an overdose of MS-222. Ten fish per tank will be subjected to full necropsy. Three fish will be submitted for histopathological analysis. If lesions or changes are found, in the ten fish subjected to necropsy, then 10 fish will be subjected to histopathological analysis. Any fish not selected for necropsy or histopathology, but having clinical signs of gross lesions will also be submitted for histopathological analysis. Each fish will be opened and examined grossly by a veterinarian. Due to the relatively small size of the fish, individual organs and organ systems will not be weighed. The veterinarian conducting the gross pathological exam will be unaware of the treatment assignment when gross necropsies are performed. After gross necropsies are performed, the fish will be preserved in 10% formalin. Whole fish will be processed and sent to the pathologist who will receive a copy of the treatment code before histological examination of tissues. Tissues to be examined during histopathology include liver, anterior (head) kidney, posterior (trunk) kidney, spleen, heart, gill, pituitary, and gonads. Tissues and residual carcasses will be incinerated.

### 13 Data Collection and Recording

The following data will be collected on each fish and recorded on the data record forms in Appendices III-VII:

- 13.1 Aquaria No., temperature, and amount fed
- 13.2 Twice-daily observation Days 1-30 for any unusual clinical signs
- 13.3 Weights and lengths of fish per tank on day 0 and day 30
- 13.4 Date of euthanization, body weight, length, and general condition of fish at time of euthanization
- 13.5 gross appearance of major organ/organ systems:
- 13.5.1 Viscera
  - 13.5.2 Heart
  - 13.5.3 Gastrointestinal tract
  - 13.5.4 Liver
  - 13.5.5 Spleen
  - 13.5.6 Kidney (appearance only)
  - 13.5.7 Urogenital tract
- 13.6 Liver somatic index
- 13.7 Complete histopathology report

14 Data Analysis

14.1 Primary response variables:

Unanticipated clinical signs during Days 1-30  
Body weight change during Days 1-30  
Body length change during Days 1-30  
Gross appearance of organs/organ systems  
Liver-somatic index  
Histopathological findings  
Feed consumption

14.1.1 Methods of analysis

Continuous variables (feed consumption and growth) will be analyzed by Mixed Model ANOVA, using tank (for feed consumption) or fish nested within tank (for growth) as the experimental unit using the Statistical Analysis System version 8.1 software (SAS Institute, Cary, North Carolina, USA). To control for the potential of a variance component for any random variable equaling zero in the mixed model, the degrees of freedom will be automatically adjusted by the Satterthwaite option so that it is not necessary to drop the random term and refit the statistical model. Least squares means will be run to compare growth and feed consumption at each dose versus control. Contrast statements will be used to evaluate linear trends in growth and feed consumption among all doses simultaneously. For histological data, if it is deemed necessary, a logistical regression will be used to analyze potential categorical data. The variables for the histological data will be normal or abnormal and are, therefore, dicotomous. If used, quantitative data will be analyzed by the Logistic Model (maximum likelihood estimation) using SAS. A significance level of  $P \leq 0.10$  will be used for all comparisons.

Differences between treated and control fish for the following variables will be analyzed in analyses of variance with treatment as main effect:

Body weight change during Days 1-30  
Body length change during Days 1-30  
Liver-somatic index  
Feed consumption

14.2 Other data: Tabulated in final report

15 Anticipated Starting Date: Nov 1, 2004

16 Anticipated Completion Date: Oct 31, 2006

17 Approval Signatures

17.1 Sponsor

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Dave Erdahl, Ph.D. Date  
USFWS

17.2 Study Director

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Anita M. Kelly, Ph.D. Date  
Fisheries Research Laboratory  
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### 17.2.1 Investigators

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### 17.3 GLP Monitor

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William L. Muhlach, Ph.D. Department of Zoology Southern Illinois University-Carbondale	Date
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### 18 Citations

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Appendix II

Safety of 17 $\alpha$ -methyltestosterone For Induction Of Sex-Inversion In Tilapia

Feed Consumption Record

INSTRUCTIONS: Complete one form for each tank of fish in the study - attach extra pages if necessary. For feed code, state the color code, type of feed (i.e. #1 starter diet, #1 crumble). Observations of appetite need to be made after each feeding. Feeding should be coded as follows: 1 = all feed on bottom; 2 = one half of feed on bottom; 3 = no feed on bottom. Return completed form to: Anita M. Kelly, Ph.D., Fisheries and Illinois Aquaculture Center, Southern Illinois University-Carbondale, Mailcode 6511, Carbondale, IL 62901-6511.

Tank No. \_\_\_\_\_ Feed Code/type \_\_\_\_\_ Batch No. \_\_\_\_\_

Expiration Date \_\_\_\_\_

Date	Weight of feed (g) prior to feeding	Weight of feed (g) after feeding	Weight of feed fed	Observation	Comments	Initials

Remainder (check one): Destroyed  Returned  Date \_\_\_\_\_

Investigator signature \_\_\_\_\_ Date \_\_\_\_\_

Study Director \_\_\_\_\_ Date \_\_\_\_\_

Appendix III

**Safety of 17 $\alpha$ -methyltestosterone For Induction Of Sex-Inversion In Tilapia**

PROCUREMENT AND TREATMENT RECORD

INSTRUCTIONS: Complete one form for each tank of fish in the study - attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Anita M. Kelly, Ph.D., Fisheries and Illinois Aquaculture Center, Southern Illinois University-Carbondale, Mailcode 6511, Carbondale, IL 62901-6511.

Tank No. \_\_\_\_\_ Fish Qty \_\_\_\_\_

Combined initial weight of 20 fish \_\_\_\_\_

ACQUISITION

Date yolksac fry \_\_\_\_\_

Fish No.	Length (mm)	Gen. Condition
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		

Holding Temp \_\_\_\_\_ °C

Additional Comments \_\_\_\_\_

Received By (signature) \_\_\_\_\_ Date \_\_\_\_\_

Study Director \_\_\_\_\_ Date \_\_\_\_\_

Appendix IV

ACCLIMATION PERIOD OBSERVATION RECORD

INSTRUCTIONS: Complete one form upon completion of the feeding study for each tank of fish in the study (twice daily)- attach extra pages if necessary. Fill in all blanks - use "N/A" if not applicable. In the observation column, fish distribution and the flight or fright response should be recorded along with any other observations. Fish distribution will be defined as: (1) Dispersed (fish generally dispersed throughout the tank); (2) Crowded (fish generally crowded at the water flow inlet); or (3) Podded (fish generally congregated in a shaded area of the tank). The flight/fright response is to be assessed by slowly waving an arm over each tank. The flight/fright response will be categorized as follows: (1) Lethargic (fish seem unresponsive with little to no flight/fright response); (2) Normal (fish are dispersed throughout the water column and react to movement by podding or rapid movement away from the movement, then appear to calm relatively quickly); or (3) Frantic (fish make erratic bursts to evade the movement source, seem to bounce off the walls). If additional forms are necessary, please attach. Return completed form(s) to: Anita M. Kelly, Ph.D., Fisheries and Illinois Aquaculture Center, Southern Illinois University-Carbondale, Mailcode 6511, Carbondale, IL 62901-6511.

Tank No. \_\_\_\_\_ Treatment Code: \_\_\_\_\_

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

Investigator: \_\_\_\_\_ Date: \_\_\_\_\_ Study Director: \_\_\_\_\_ Date: \_\_\_\_\_



Appendix V

**Safety of 17 $\alpha$ -methyltestosterone For Induction Of Sex-Inversion In Tilapia**

TREATMENT OBSERVATION RECORD

INSTRUCTIONS: Complete one form for each tank of fish in the study – attach extra pages if necessary. Fill in all blanks – use “N/A” if not applicable. In the observation column, fish distribution and the flight or fright response should be recorded along with any other observations. Fish distribution will be defined as: (1) Dispersed (fish generally dispersed throughout the tank); (2) Crowded (fish generally crowded at the water flow inlet); or (3) Podded (fish generally congregated or podding in a shaded area of the tank). The flight/fright response is to be assessed by slowly waving an arm over each tank. The flight/fright response will be categorized as follows: (1) Lethargic (fish seem unresponsive with little to no flight/fright response); (2) Normal (fish are dispersed throughout the water column and react to movement by podding or rapid movement away from the movement, then appear to calm relatively quickly); or (3) Frantic (fish make erratic bursts to evade the movement source, seem to bounce off the walls). Return completed form to: Anita M. Kelly, Ph.D., Fisheries and Illinois Aquaculture Center, Southern Illinois University-Carbondale, Mailcode 6511, Carbondale, IL 62901-6511.

Tank No. \_\_\_\_\_ Treatment Code: \_\_\_\_\_

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

DATE	COMMENTS/OBSERVATIONS
	Fish Distribution:
	Flight or Fright Response:
	Additional Comments:

Investigator: \_\_\_\_\_ Date: \_\_\_\_\_ Study Director: \_\_\_\_\_ Date: \_\_\_\_\_

Appendix VI

**Safety of 17 $\alpha$ -methyltestosterone For Induction Of Sex-Inversion In Tilapia**

POSTMORTEM RECORD

INSTRUCTIONS: Complete one form for each fish in each tank of the study - attach extra pages if necessary. If lesions are found they should be scored as follows: (0) no lesions; (1) mild lesions; (2) moderate lesions; (3) severe lesions. Location of lesion should be noted in the comment column. Lesion should be removed and placed into 10% formalin and sent for histological analysis. Fill in all blanks - use "N/A" if not applicable. Return completed form to: Anita M. Kelly, Ph.D., Fisheries and Illinois Aquaculture Center, Southern Illinois University-Carbondale, Mailcode 6511, Carbondale, IL 62901-6511.

Date \_\_\_\_\_ Aquaria No. \_\_\_\_\_ Treatment Code: \_\_\_\_\_

Fish No.	Weight (g)	Length (mm)	Liver Somatic Index	Comments
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

Additional Comments \_\_\_\_\_

Data Recorded By \_\_\_\_\_

Date \_\_\_\_\_

Veterinarian Signature \_\_\_\_\_

Date \_\_\_\_\_

Study Director \_\_\_\_\_

Date \_\_\_\_\_

## Appendix VII

**Safety of 17 $\alpha$ -methyltestosterone For Induction Of Sex-Inversion In Tilapia**

## HISTOLOGY RECORD

Instructions: The following organs need to be histologically examination from the whole body slices submitted. Mark the box under tissue appearance with an "x" indicating whether lesions are present or absent. (0) no lesions; (1) mild lesions; (2) moderate lesions; (3) severe lesions. Location of lesion should be noted in the comment column.If abnormal state findings in the comment column. Any additional tissue sent for analysis should be listed under other. Indicate the tissue name in the space provided. If additional sheets are necessary, please attach.

Organ	Lesions on Tissue	Comments
Gonads	<input type="checkbox"/> Present <input type="checkbox"/> Absent	
Pituitary	<input type="checkbox"/> Present <input type="checkbox"/> Absent	
Liver	<input type="checkbox"/> Present <input type="checkbox"/> Absent	
Spleen	<input type="checkbox"/> Present <input type="checkbox"/> Absent	
Gills	<input type="checkbox"/> Present <input type="checkbox"/> Absent	
Kidney (anterior and posterior)	<input type="checkbox"/> Present <input type="checkbox"/> Absent	
Thyroid	<input type="checkbox"/> Present <input type="checkbox"/> Absent	
Other (list):	<input type="checkbox"/> Present <input type="checkbox"/> Absent	

Pathologist Signature \_\_\_\_\_

Date \_\_\_\_\_

Study Director \_\_\_\_\_

Date \_\_\_\_\_

**Write the Final Study Report and Submit to CVM Through the MT INAD Coordinator at Auburn University (Objective 4)**

This objective will be accomplished by following the CVM Guidance Document on Final Study Reports and follow final reports submitted by the principal investigator that have been previously approved by FDA.

**Provide Progress Reports to NCRAC (Objective 5)**

Annual progress reports will be submitted to the Director of the NCRAC. Additional reports will be provided upon request.

**Gain Acceptance from CVM for the Target Animal Safety Study on MT in Tilapia (Objective 6)**

The final report for the Target Animal Safety Study will be sent to Auburn University for submission to the FDA. If the report is found to be deficient, additional information will be provided via a revised final report.

**FACILITIES**

All fish will be maintained in Room 51A of the SIUC Vivarium in 37.9-L glass aquaria with individual biofiltration and aeration supplied via ceramic air stone to maintain water quality and to prevent exposure of fish receiving various treatments of MT to water containing other concentrations of MT. The room will be solely dedicated to this study and have restricted access. A freezer in the room will be used for storing feed and any dead fish. The SIUC Vivarium is under the direction of an AVCM board-certified veterinarian, and is fully accredited by the American Association of Laboratory Animal Care.

Necropsies will be conducted in Room 41 of the SIUC Vivarium. This room will be designated solely to this study and will have restricted access. The room is designed as a dry lab and contains stainless steel countertops. A Fisher Model 1200D electronic balance will be used for weighing the fish ( $\pm 0.1$ g). A fireproof file cabinet is in place and will be used for archiving project documents.

## REFERENCES

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- Guerrero, R.D., 1975. Use of androgens for the production of all male *Tilapia aurea* (Steindachner). *Transactions of the American Fisheries Society* 104:342–348.
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- Ruwet, J.C., J. Voss, L. Hanon, and J.C. Micha. 1976. Biologie et élevage du tilapia. Pages 332-364 *in* FAO, Eds., *Symposium on Aquaculture in Africa*, 30 September-2 October 1975, Accra, Ghana. CIFA Technical Paper 4.
- Vera-Cruz, E.M.V., and G.C. Mair. 1994. Conditions for effective androgen sex-reversal in *Oreochromis niloticus* (L.). *Aquaculture* 122:237– 248.

**PROJECT LEADER**

<u>State</u>	<u>Name/Institution</u>	<u>Area of Specialization</u>
<b>Illinois</b>	Anita M. Kelly Southern Illinois University-Carbondale	Aquaculture/Reproductive Physiology

**PARTICIPATING INSTITUTION AND PRINCIPAL INVESTIGATOR**

**Southern Illinois University-Carbondale (SIUC)**

Anita M. Kelly

**BUDGET**

ORGANIZATION AND ADDRESS Southern Illinois University-Carbondale Carbondale, IL 62901			USDA AWARD NO. Year 1: Objectives 1-6			
PROJECT DIRECTOR(S) Anita M. Kelly			Duration Proposed Months: <u>12</u>  <b>Funds Requested by Proposer</b>	Duration Proposed Months: ____  <b>Funds Approved by CSREES (If different)</b>	Non-Federal Proposed Cost-Sharing/Matching Funds (If required)	Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)
<b>A. Salaries and Wages</b> 1. No. of Senior Personnel			<b>CSREES FUNDED WORK MONTHS</b>			
			Calendar	Academic	Summer	
a. ____ (Co)-PD(s) .....						
b. <u>1</u> Senior Associates .....			5.1			\$8,277
2. No. of Other Personnel (Non-Faculty)						
a. ____ Research Associates-Postdoctorates ...						
b. ____ Other Professionals .....						
c. ____ Paraprofessionals .....						
d. ____ Graduate Students .....						
e. ____ Prebaccalaureate Students .....						
f. ____ Secretarial-Clerical .....						
g. ____ Technical, Shop and Other .....						
<b>Total Salaries and Wages</b> ..... →						\$8,277
B. Fringe Benefits (If charged as Direct Costs)						\$6,802
<b>C. Total Salaries, Wages, and Fringe Benefits (A plus B)</b> ..... →						\$15,079
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies						\$1,000
F. Travel						
G. Publication Costs/Page Charges						
H. Computer (ADPE) Costs						
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)						
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)						\$7,651
<b>K. Total Direct Costs (C through I)</b> ..... →						\$23,730
L. <b>F&amp;A/Indirect Costs.</b> (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)						
<b>M. Total Direct and F&amp;A/Indirect Costs (J plus K)</b> ..... →						\$23,730
N. Other ..... →						
<b>O. Total Amount of This Request</b> ..... →						\$23,730
<b>P. Carryover -- (If Applicable)</b> .....			<b>Federal Funds: \$</b>	<b>Non-Federal funds: \$</b>	<b>Total \$</b>	
<b>Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)</b>						
Cash (both Applicant and Third Party) ..... →						
Non-Cash Contributions (both Applicant and Third Party) ..... →						
<b>NAME AND TITLE</b> (Type or print)	<b>SIGNATURE</b> (required for revised budget only)				<b>DATE</b>	
<b>Project Director</b>						
<b>Authorized Organizational Representative</b>						
<b>Signature (for optional use)</b>						

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing the reviewing the collection of information.



UNITED STATES DEPARTMENT OF AGRICULTURE  
 COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE  
**BUDGET**

OMB Approved 0524-0039  
 Expires 03/31/2004

ORGANIZATION AND ADDRESS Southern Illinois University-Carbondale Carbondale, IL 62901			USDA AWARD NO. Year 2: Objectives 1-6			
PROJECT DIRECTOR(S) Anita M. Kelly			Duration Proposed Months: <u>12</u>	Duration Proposed Months: _____	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
<b>A. Salaries and Wages</b> 1. No. of Senior Personnel			CSREES FUNDED WORK MONTHS			
			Calendar	Academic	Summer	
a. ___ (Co)-PD(s) .....						
b. <u>1</u> Senior Associates .....			5.1			\$8,277
2. No. of Other Personnel (Non-Faculty)						
a. ___ Research Associates-Postdoctorates . . .						
b. ___ Other Professionals .....						
c. ___ Paraprofessionals .....						
d. ___ Graduate Students .....						
e. ___ Prebaccalaureate Students .....						
f. ___ Secretarial-Clerical .....						
g. ___ Technical, Shop and Other .....						
<b>Total Salaries and Wages</b> .....						\$8,277
B. Fringe Benefits (If charged as Direct Costs)						\$6,802
<b>C. Total Salaries, Wages, and Fringe Benefits (A plus B)</b> .....						\$15,079
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies						\$750
F. Travel						\$2,342
G. Publication Costs/Page Charges						
H. Computer (ADPE) Costs						
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)						
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)						\$8,099
<b>K. Total Direct Costs (C through I)</b> .....						\$26,270
L. F&A/Indirect Costs. (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)						
<b>M. Total Direct and F&amp;A/Indirect Costs (J plus K)</b> .....						\$26,270
N. Other .....						
<b>O. Total Amount of This Request</b> .....						\$26,270

P. Carryover -- (If Applicable) .....	Federal Funds: \$	Non-Federal funds: \$	Total \$
---------------------------------------	-------------------	-----------------------	----------

Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)		
Cash (both Applicant and Third Party) .....	→	
Non-Cash Contributions (both Applicant and Third Party) .....	→	

NAME AND TITLE (Type or print)	SIGNATURE (required for revised budget only)	DATE
Project Director		
Authorized Organizational Representative		
Signature (for optional use)		

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing the reviewing the collection of information.

UNITED STATES DEPARTMENT OF AGRICULTURE  
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039  
Expires 03/31/2004

**BUDGET**

ORGANIZATION AND ADDRESS Southern Illinois University-Carbondale Carbondale, IL 62901			USDA AWARD NO.    Years 1 & 2: Objectives 1-6			
			Duration Proposed Months: <u>24</u>	Duration Proposed Months: _____	Non-Federal Proposed Cost-Sharing/ Matching Funds (If required)	Non-federal Cost-Sharing/ Matching Funds Approved by CSREES (If Different)
PROJECT DIRECTOR(S) Anita M. Kelly			Funds Requested by Proposer	Funds Approved by CSREES (If different)		
<b>A. Salaries and Wages</b>			<b>CSREES FUNDED WORK MONTHS</b>			
1. No. of Senior Personnel			Calendar	Academic	Summer	
a. ___ (Co)-PD(s) .....						
b. ___ Senior Associates .....			10.2			\$16,554
2. No. of Other Personnel (Non-Faculty)						
a. ___ Research Associates-Postdoctorates . . .						
b. ___ Other Professionals .....						
c. ___ Paraprofessionals .....						
d. ___ Graduate Students .....						
e. ___ Prebaccalaureate Students .....						
f. ___ Secretarial-Clerical .....						
g. ___ Technical, Shop and Other .....						
<b>Total Salaries and Wages</b> .....						\$16,554
B. Fringe Benefits (If charged as Direct Costs)						\$13,604
C. <b>Total Salaries, Wages, and Fringe Benefits (A plus B)</b> .....						\$30,158
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)						
E. Materials and Supplies						\$1,750
F. Travel						\$2,342
G. Publication Costs/Page Charges						
H. Computer (ADPE) Costs						
I. Student Assistance/Support (Scholarships/fellowships, stipends/tuition, cost of education, etc. Attach list of items and dollar amounts for each item.)						
J. All Other Direct Costs (In budget narrative, list items and dollar amounts and provide supporting data for each item.)						\$15,750
K. <b>Total Direct Costs (C through I)</b> .....						\$50,000
L. <b>F&amp;A/Indirect Costs.</b> (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs in on/off campus bases.)						
M. <b>Total Direct and F&amp;A/Indirect Costs (J plus K)</b> .....						\$50,000
N. <b>Other</b> .....						
O. <b>Total Amount of This Request</b> .....						\$50,000

<b>P. Carryover -- (If Applicable)</b> .....	<b>Federal Funds: \$</b>	<b>Non-Federal funds: \$</b>	<b>Total \$</b>
--	--------------------------	------------------------------	-----------------

<b>Q. Cost Sharing/Matching (Breakdown of total amounts shown in line O)</b>		
Cash (both Applicant and Third Party) .....	→	
Non-Cash Contributions (both Applicant and Third Party) .....	→	

<b>NAME AND TITLE</b> (Type or print)	<b>SIGNATURE</b> (required for revised budget only)	<b>DATE</b>
<b>Project Director</b>		
<b>Authorized Organizational Representative</b>		
<b>Signature (for optional use)</b>		

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average 1.00 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing the reviewing the collection of information.

## BUDGET JUSTIFICATION FOR SOUTHERN ILLINOIS UNIVERSITY-CARBONDALE

(Kelly)

- A. Salaries and Wages.** Years 1 and 2: one three quarter-time technician to assist in conducting the study. Due to the nature of the study, it is not practical to use graduate students.
- B. Fringe Benefits.** Years 1 and 2: the SIUC rate for medical/life benefits is \$1,133/person/month; the retirement rate is 12.37%.
- E. Materials and Supplies.** Year 1: these funds will be used for acquisition of feeds (\$250), MT (\$150), and maintenance of holding systems for the study (\$600). Year 2: feeds (\$250) and maintenance of holding systems (\$500).
- F. Travel.** Year 2: transportation, lodging, and meal expenses for the PI to attend two 1-day drug approval workshops at destinations to be determined.
- I. Other Direct Costs.** Year 1: feed analysis (\$7,500), postage (\$100), telephone (\$38), and fax (\$13). Year 2: postage (\$100), telephone (\$37), fax (\$12), histological pathology (\$7,500), and report preparation (\$450).

## **SCHEDULE FOR COMPLETION OF OBJECTIVES**

Objectives 1-6: Initiated in Year 1 and completed in Year 2.

**PRINCIPAL INVESTIGATOR**

**Anita M. Kelly**, Southern Illinois University-Carbondale

## VITA

Anita M. Kelly  
Fisheries and Illinois Aquaculture Center  
Southern Illinois University-Carbondale  
Mailcode 6511  
Carbondale, IL 62901-6511

Phone: (618) 453-6099  
Fax: (618) 453- 6095  
E-mail: akelly@siu.edu

### EDUCATION

B.S. University of Iowa, 1987, Biology  
M.S. Southern Illinois University-Carbondale, 1990, Zoology/Aquatic toxicology  
Ph.D. Southern Illinois University-Carbondale, 1995, Zoology/Aquaculture, Physiology

### POSITIONS

Assistant Professor (2003-present), Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University-Carbondale  
Assistant/Associate Professor (1998-2003), Department of Wildlife and Fisheries, Mississippi State University  
Vice President of Operations and Plant Manager (1996-1998), The Great Black Creek Fish Company, Black Creek, Wisconsin

### SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society (Fish Culture, Fish Physiology, and Education Sections)  
World Aquaculture Society and the U.S. Chapter-World Aquaculture Society  
Phi Kappa Phi Honor Society

### SELECTED PUBLICATIONS

- Kelly, A.M. In press. Broodfish management. *In* Tucker, C.S., and J.A. Hargreaves, editors. Biology and culture of channel catfish. Elsevier Science, Inc. New York, New York.
- Kelly, A.M. In press. Channel catfish culture. *In* Kelly, A.M., and J. Silverstein, editors. Aquaculture in the 21<sup>st</sup> Century. Fish Culture Section, American Fisheries Society, Bethesda, Maryland.
- Kelly, A.M., W. H. Holmes, and T.P. Schultz. In press.. An environmentally benign method to reduce off-flavor terpenoids in aquaculture. *North American Journal of Aquaculture*.
- Kelly, A.M., and C.C. Kohler. 2003. Effects of *Yucca shidigera* extract on growth, nitrogen retention, ammonia excretion, and toxicity in channel catfish, *Ictalurus punctatus* and hybrid tilapia *Oreochromis niloticus* x *O. niloticus*. *Journal of the World Aquaculture Society* 34:156-161.
- Kelly, A.M., and C.C. Kohler. 1999. Cold tolerance and fatty acid composition of striped bass, white bass and their hybrids. *North American Journal of Aquaculture* 61:278-285.