

**ENVIRONMENTAL ASSESSMENT OF USE OF 17 α -METHYLTESTOSTERONE FOR SEX
INVERSION OF NEWLY HATCHED TILAPIA**

A Literature Review to Support the Investigational New Animal Drug Exemption and New
Animal Drug Application for 17 α -Methyltestosterone

Principal Investigators: Ronald P. Phelps and Bartholomew W. Green

Industry Advisory Council Liaison: Rosalie A. Schnick, National Aquaculture NADA
Coordinator

Extension Liaison: Joseph E. Morris, Iowa State University

Funding Request: \$5,000

Duration: 1 Year (December 1, 1996 - November 30, 1997)

Objectives:

1. Conduct a thorough review of computerized scientific literature data bases.
2. Obtain relevant scientific papers and review.
3. Prepare the environmental assessment for submission to FDA's Center for Veterinary
Medicine (CVM).

Proposed Budget:

Institution	Principal Investigator(s)	Objectives	Year 1	Total
Auburn University	Ronald P. Phelps Bartholomew W. Green	1-3	\$5,000	\$5,000
TOTALS			\$5,000	\$5,000

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I - Application for an INAD Exemption for 17 α -Methyltestosterone (12 pages)	
II - Protocol for Clinical Field Trial (15 pages with attachments including four appendices)	
III - Letter or INAD Issuance (five pages with one page enclosure)	

JUSTIFICATION

The commercial tilapia industry in the United States produced nearly 5.9 million kilograms (13 million pounds), worth an estimated \$26 million at the retail level, in 1994 and is expected to produce 7.3 million kilograms (16 million pounds) in 1995, a 23% increase over the previous year. Many producers in the U.S. commercial tilapia industry use all-male populations of tilapia in their production units because of the faster growth of male fish. Hormonal sex inversion of newly hatched tilapia fry, achieved through the oral administration of an androgen, usually 17 α -methyltestosterone (MT), incorporated into a formulated ration, is the principal method used to produce populations of all-male tilapia. However, the use of MT for sex inversion in tilapia destined for human consumption is not approved by the U.S. Food and Drug Administration (FDA).

In response to requests from U.S. commercial tilapia producers, the National Aquaculture NADA Coordinator convened a meeting at FDA's Center for Veterinary Medicine (CVM), Rockville, Maryland in June 1995 that was attended by CVM personnel, the National Aquaculture NADA Coordinator, commercial tilapia farmers, a National Fisheries Institute representative and scientists from the Department of Fisheries and Allied Aquacultures, Auburn University. As a result of that meeting, Auburn University prepared and submitted a request for an Investigational New Animal Drug (INAD) exemption to CVM for the use of MT for tilapia sex inversion (INAD request and clinical field trial protocol appear as Appendices I and II, respectively).

Auburn University is the sponsor and monitor of an INAD exemption for the use of MT for sex inversion of newly hatched tilapia fry. In its letter of INAD issuance (Appendix III), CVM stated that the sponsor, Auburn University, must prepare an action plan to demonstrate how, when and with what source of funding this INAD is going to progress to a New Animal Drug Application (NADA). Areas that must be addressed in a NADA are efficacy and effective dose, environmental safety, human food safety, target animal safety and potential for abuse. The planned approach of Auburn University is to prepare individual action plans for each of the areas required for the NADA and submit these to the appropriate review team within CVM. CVM granted Auburn University a categorical exclusion from preparation of an environmental assessment for the INAD, however, this area must be addressed for the NADA.

The objective of the project we submit to the North Central Regional Aquaculture Center (NCRAC) is to conduct a literature review on the environmental impact of using MT for sex inversion of newly hatched tilapia. Based on results of this literature review, Auburn University will prepare the environmental assessment (EA) and submit the EA to the appropriate office within CVM for review in an effort to move towards the NADA for MT. The direct beneficiaries of MT attaining New Animal Drug status in the United States will be U.S. commercial tilapia farmers. In addition, U.S. consumers will benefit in the area of food safety as all tilapia producers, both domestic and foreign, will have an approved protocol for the use of MT for tilapia sex inversion.

RELATED CURRENT AND PREVIOUS WORK

In its request to CVM for the INAD exemption for MT, Auburn University presented enough information to permit CVM to grant the categorical exclusion for the preparation of an environmental assessment for the INAD. However, CVM does require that the area of environmental impact be addressed in the NADA. In order to prepare the environmental assessment it is necessary to know what work has been accomplished to date on the

environmental impact of the use of MT and related androgens. Thus, the need for the literature review. We are not aware of any available literature review on this subject.

As part of the overall tilapia MT INAD effort, a clinical field trial to collect efficacy data under commercial production conditions is being implemented by commercial producers. This effort is being financed by U.S. commercial tilapia producers participating as clinical investigators in the clinical field trial, through monetary contributions from other U.S. commercial tilapia producers whose scale of operation prohibits them from participating, from related companies, and from contributions from foreign producers. In addition, the National Fisheries Institute provides limited administrative support to the clinical field trial effort at no cost. Presently ten U.S. commercial tilapia farmers participate as clinical investigators in the MT INAD; each clinical investigator pays an annual administrative fee of \$3,500. As of May 9, 1996, contributions to the MT INAD totaling \$5,000 had been received from a total of two domestic and one foreign operations.

ANTICIPATED BENEFITS

Implementation of this project will allow Auburn University to conduct a thorough review of the scientific literature, obtain copies of appropriate scientific papers and prepare an environmental assessment for submission to CVM in support of the NADA for MT. Commercial tilapia farmers throughout the U.S. will be the direct beneficiaries of this project because of its significant contribution to the furtherance of the NADA for MT. Attainment of New Animal Drug status for MT ensures that U.S. tilapia farmers have access legally to the most efficient method presently available for the production of predominantly male fish for stocking into grow-out units.

OBJECTIVES

1. Conduct a thorough review of computerized scientific literature data bases.
2. Obtain relevant scientific papers and review.
3. Prepare the environmental assessment for submission to CVM.

PROCEDURES

Conduct a Thorough Review of Computerized Scientific Literature Data Bases (Objective 1)

Searches will be conducted of computerized data bases of scientific literature accessed through Auburn University Ralph Brown Draughon (RBD) library. The following data bases will be searched for information on the environmental impact of MT and related androgen use: BIOSIS PREVIEWS, EMBASE, MEDLINE, TOXLINE, CAB Abstracts, Aquatic Science Abstracts, CRIS/USDA, CA SEARCH, Pascal and Zoological Record Online. Other data bases will be searched as necessary.

Obtain Relevant Scientific Papers and Review and Prepare the Environmental Assessment for Submission to CVM (Objectives 2 and 3)

Obtain relevant scientific papers from RBD library holdings or through RBD inter-library loan, review and prepare environmental impact action plan.

FACILITIES

The RBD library at Auburn University is a 35,023 m² (377,000 ft²) structure whose collections include more than 1.9 million volumes, more than 2 million items in microformat, 1.4 million government publications and 134,000 maps. Ralph Brown Draughon library is a member of the Association of Research Libraries. The library also is a U.S. government documents depository library and participates in the depository programs of the U.S. Defense Mapping Agency, the U.S. Geological Survey, and the U.S. National Oceanic and Atmospheric Agency. Access to more than 600 data bases from vendors including Dialog, BRS, National Library of Medicine, Orbit, STN International, RGL, Wilson and WESTLAW are available through RBD library.

PROJECT LEADERS

<u>State</u>	<u>Name and Institution</u>	<u>Area of Specialization</u>
Alabama	Ronald P. Phelps Auburn University	Fish Reproduction/Hatchery Management
Alabama	Bartholomew W. Green Auburn University	Aquaculture/Water Quality

BUDGET

ORGANIZATION AND ADDRESS Auburn University Auburn University, Alabama 36849			USDA AWARD NO. Year 1 - Objectives 1-3	
			Duration Proposed Months: <u>12</u>	Duration Awarded Months: _____
PRINCIPAL INVESTIGATOR(S)/PROJECT DIRECTOR(S) Ronald P. Phelps and Bartholomew W. Green			FUNDS REQUESTED by PROPOSER	
A. Salaries and Wages			FUNDS APPROVED BY CSREES (If Different)	
1. No. of Senior Personnel			\$	
a. <u>1</u> (Co)-PI(s)/PD(s)			\$3,293	
b. ___ Senior Associates				
2. No. of Other Personnel (Non-Faculty)				
a. ___ Research Associates-Postdoctorates				
b. ___ Other Professional				
c. ___ Graduate Students				
d. ___ Prebaccalaureate Students				
e. ___ Secretarial-Clerical				
f. ___ Technical, Shop and Other				
Total Salaries and Wages →			\$3,293	
B. Fringe Benefits (If charged as Direct Costs)			\$823	
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →			\$4,116	
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				
E. Materials and Supplies			\$11	
F. Travel				
1. Domestic (Including Canada)				
2. Foreign (List destination and amount for each trip.)				
G. Publication Costs/Page Charges				
H. Computer (ADPE) Costs			\$275	
I. All Other Direct Costs (Attach supporting data. List items and dollar amounts. Details of Subcontracts, including work statements and budget, should be explained in full in proposal.) (Inter-Library Loans, \$75; Telephone/FAX, \$25; FEDEX, \$26; Photocopying, \$472)			\$598	
J. Total Direct Costs (C through I) →			\$5,000	
K. 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.)				
L. Total Direct and Indirect Costs (J plus K) →			\$5,000	
M. Other →				
N. Total Amount of This Request →			\$5,000	
O. Cost Sharing (If Required Provide Details)			\$	
NOTE: Signatures required only for Revised Budget			This is Revision No. →	
NAME AND TITLE (Type or print)		SIGNATURE		DATE
Principal Investigator/Project Director				
Authorized Organizational Representative				

BUDGET JUSTIFICATION FOR AUBURN UNIVERSITY

(Phelps and Green)

Objectives 1-3

- A. Salaries and Wages.** Co-PI Green is supported by soft money. In the past, Green has contributed time to develop and support the MT INAD effort. However, the proposed literature review will demand a significant effort for which Green does not have salary support. Therefore, it is estimated that one month of salary support and fringe benefits are required for this undertaking. Research and teaching responsibilities do not leave Co-PI Phelps with sufficient time to complete the literature review.
- E. Materials and Supplies.** Computer supplies (1 ream of paper for laser printer (\$3), 10% of a toner cartridge (\$7) and 2 diskettes (\$1) for a total of \$11.
- H. Computer (ADPE) Costs.** The cost of the computerized search of the listed databases is estimated at \$275. It is anticipated that the search will yield 150 plus "hits" which cost approximately \$1.50 each to retrieve plus the on-line time used for search and retrieval.
- G. Other Direct Costs.** Other direct costs include funds to obtain an estimated 10 journal articles through inter-library loan at an average cost, according to the RBD inter-library loan office, of \$7.50 per journal article (total = \$75). Long distance telephone/FAX is included at \$25 for consultations with CVM. FEDEX charges of \$26 are included for initial and revised submissions to CVM. CVM requires submission of copies of all papers cited in the literature review. In addition, copies of all materials submitted to CVM must be maintained in Auburn University files. Therefore, a photocopy charge of \$472 (5,900 copies x \$0.08/copy) is included.

SCHEDULE FOR COMPLETION OF OBJECTIVES

Objective 1: Initiated and completed in Year 1.

Objective 2: Initiated and completed in Year 1.

Objective 3: Initiated and completed in Year 1.

LIST OF PRINCIPAL INVESTIGATORS

Bartholomew W. Green, Auburn University

Ronald P. Phelps, Auburn University

VITA

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EDUCATION

Ph. D. Auburn University, 1993
M. S. Auburn University, 1982
B. A. Case Western Reserve University, 1976

POSITIONS

Post-doctoral Fellow (1993 to present); Senior Research Associate (1983 to 1992), Department of Fisheries and Allied Aquaculture, Auburn University

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
Phi Beta Delta (Honor Society for International Scholars)
Gamma Sigma Delta (Honorary Agricultural Society)
World Aquaculture Society (WAS and U.S. Chapter)

SELECTED PUBLICATIONS

- Green, B.W., and C.E. Boyd. 1995. Chemical budgets for organically fertilized fish ponds in the dry tropics. *Journal of the World Aquaculture Society* 26:284-296.
- Green, B.W., and C.E. Boyd. 1995. Water budgets for fish ponds in the dry tropics. *Aquacultural Engineering* 14:347-356.
- Green, B.W., and D.R. Teichert-Coddington. 1994. Growth of control and androgen-treated Nile tilapia during treatment, nursery and grow-out phases in tropical fish ponds. *Aquaculture and Fisheries Management* 25:613-621.
- Teichert-Coddington, D.R., and B.W. Green. 1993. Tilapia yield improvement through maintenance of minimal dissolved oxygen concentrations in experimental grow-out ponds in Honduras. *Aquaculture* 118:63-71.
- Green, B.W., and D.R. Teichert-Coddington. 1993. Production of *Oreochromis niloticus* fry for hormonal sex reversal in relation to temperature. *Journal of Applied Ichthyology* 9:230-236.
- Popma, T.J., and B.W. Green. 1990. Aquaculture production manual: Sex reversal of tilapia in earthen ponds. Research and Development Series 35, International Center for Aquaculture, Auburn University, Alabama.

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EDUCATION

Ph.D. Auburn University, 1975
B.S. Auburn University, 1969

POSITIONS

Associate Professor (1982 to present), Department of Fisheries and Allied Aquaculture, Auburn University
Technical Staff (1981-1982), International Center for Aquaculture, Auburn University
Chief of Party (1977-1980), Technical Assistance Team, Colombia

SCIENTIFIC AND PROFESSIONAL ORGANIZATIONS

American Fisheries Society
World Aquaculture Society (WAS and U.S. Chapter)

SELECTED PUBLICATIONS

- Phelps, R.P., G. Conterras Salazar, V. Abe, and B.J. Argue. 1995. Sex reversal and nursery growth of Nile tilapia *Oreochromis niloticus* (L.), free-swimming in earthen ponds. *Aquaculture Research* 26:293-295.
- Argue, B.J., and R.P. Phelps. 1995. Hapa-based systems for producing *Oreochromis niloticus* fry suitable for hormone sex-reversal. *Journal of Applied Aquaculture* 5(1):21-27.
- Potts, A.C., and R.P. Phelps. 1995. Use of diethylstilbestrol and ethynyldiol to feminize Nile tilapia *Oreochromis niloticus* (L.) in an outdoor environment. *Journal of Applied Ichthyology* 11:111-117.
- Galvez, J. I., P.M. Mazik, R.P. Phelps, and D.R. Mulvaney. 1995. Masculinization of channel catfish *Ictalurus punctatus* by oral administration of trenbolone acetate. *Journal of the World Aquaculture Society* 26(40):378-383.
- Phelps, R.P., E. Arana, and B. Argue. 1993. Relationship between the external morphology and gonads of androgen-treated *Oreochromis niloticus*. *Journal of Applied Aquaculture* 2:103-108.
- Hiot, A.E., and R.P. Phelps. 1993. Effects of initial age and size on sex reversal of *Oreochromis niloticus* fry using methyltestosterone. *Aquaculture* 12:301-308.