IV. PROGRESS REPORTS

A. AQUACULTURE FOOD SAFETY: RESIDUES

Progress Report
For the Period
September 11, 1992 to August 31, 1996

FUNDING LEVEL:

Year 1 .... $100,000
Year 2 .... $155,000
Year 3 .... $101,000
Total $356,000

PARTICIPANTS:

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University of Florida - Cheng I. Wei

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ADMINISTRATIVE ADVISOR:

Dr. Neal Thompson, Professor
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PROJECT OBJECTIVES:

Objective 1: Information on residues is available for many food products and some is available for specific aquaculture products. Various state and federal agencies and private companies have collected data on chemical residues in channel catfish. The quality and quantity of these data is unknown. For instance, distinctions between farm-raised channel catfish and "wild" channel catfish are often overlooked. The exposure of "wild" channel catfish to hazardous compounds is often greater due to point source environmental contamination and data collected from "wild" catfish may not be representative of aquaculture products. In the development of an appropriate data base, related publications and educational programs, distinctions will be made between farm-raised channel catfish and "wild" channel catfish.

Survey and review of data bases for pesticides, PCB and metal residues in farm-raised catfish, crawfish and rainbow trout.

Objective 2: Protocols and guidelines are to be developed and disseminated for a residue monitoring program.

Objective 3: All research scientists participating in the study are to be contacted and requested to send reports and/or any information that they have that could be used in the development of Extension-type fact sheets or brochures. The information received will be catalogued as reference sources so that outlines and fact sheets can be written with this new data. Also, a library search will be conducted for additional information that may be applicable in writing the publications mentioned above.

Objective 4: The University of Georgia will develop a chemical application record system for producers.

Objective 5: The steady growth in per capita consumption of fish and seafood products has caused increased attention to product safety. There is always a potential for problems due to contamination of foods by pesticides, heavy metals, and pharmaceutical compounds either from direct or indirect sources. These potential problems can occur on the farm, during processing, or at wholesale/retail levels. There is a need to minimize potential problems during and following production by determining the influence of processing on residues. The aim of the study is to determine the fate of residues from the farm to the processing plant and finally to a product which would be prepared by the consumer.

Objective 6: The aim of this project is to attempt to improve the available information on residues in farm-raised channel catfish, crawfish and rainbow trout so that consumers can more realistically assess issues related to consumer safety from scientific data.

ANTICIPATED BENEFITS:

Objective 1: The aquaculture industry will have scientifically-generated residue data base to support its contention that aquaculture products are safe for consumption.

Objective 2: Residues will be monitored by producers to increase consumer confidence in the safety of aquaculture products.

Objective 3: Educational programs are invaluable to preventing residue problems in the industry.

Objective 4: Proper record-keeping will also help to insure that pesticides and animal drugs have been used in a safe manner.

Objective 5: The benefits of food processing and preparation for reducing residue levels in edible tissue will be determined.

Objective 6: Pesticide and heavy metal residue data will be made available for selected aquaculture products. Preliminary indications are that the fish sampled in this study are free from residues or have much lower residues than wild fishes.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1: Data included in this report on fish and seafood products were retrieved from FOODCONTAM, a national database consisting of state generated information on pesticide and other toxic chemical residues in human foods. Data are generated by state agriculture, food, and health protection agencies responsible for assuring the quality and safety of foods grown or imparted into their states. The data are presented in four separate computer-generated packets for FY 1986-1989, FY 1990-1992, FY 1993, FY 1994 and FY 1995.

Objective 2: Protocols and guidelines were developed and disseminated for a residue monitoring program. These protocols were used in collecting and analyzing residue data.

Objective 3: Development of educational materials were redirected to the SRAC Publications Work Group.

Objective 4: A chemical application record system was developed in the third year of this project.

Objective 5: After appropriate dosing of pesticides and pharmaceuticals, channel catfish were processed by researchers at the University of Georgia into four consumer-ready products in order to determine the effects of different processing conditions on the residues. Catfish were stunned, beheaded, eviscerated, skinned, filleted and washed. Both fillets from each fish were frozen for subsequent residue analyses either before or after cooking. One of the frozen complimentary fillets was thawed then either: (1) breaded and fried in vegetable oil at 190°C; (2) breaded and baked at 190°C in a conventional oven; (3) injected with a mixture of polyphosphates and citric acid, then frozen for four weeks, thawed, breaded and fried in vegetable oil at 190°C; or (4) brined and smoked in a temperature- and humidity-controlled smoke house to achieve an internal temperature of 71°C for 30 min. Raw and cooked fillets were homogenized following processing into a table-ready item and stored at -80°C until analyses.

Pesticide and pharmaceutical residues were determined in fillets from duplicate fish for each treatment using standard procedures (FDA, PAM-1 Methods, AOAC Methods, etc.). Results of a study to determine oxytetracycline, sulfadimethoxine and ormetoprim residues in catfish using HPLC were reported in the 1995 Progress Report. This work was also published in the Journal of Food Science 60: 1220-1224, and presented at the 1995 annual meeting of the Institute of Food Technologists. The study of oxytetracycline residues in raw and cooked catfish fillets is summarized below.

Channel catfish (average weight = 0.87 kg; SD = 0.05) were held in concrete tanks at 24°C, and fed feeds containing 1.88, 3.75, or 7.50 g oxytetracycline hydrochloride per kg feed. Fish were provided medicated feeds at 2% body weight daily for 10 days. If all the feed was consumed, fish would receive 37.5, 75.0, or 150.0 mg oxytetracycline per kg of fish daily. At 18 hours after the last feeding, fish were stunned with a hammer, beheaded using a band saw, skinned and eviscerated. Both fillets from each fish were collected: one matched fillet was analyzed raw and the other was cooked and analyzed. Fillets from each fish were wrapped in aluminum foil, placed in zip-lock plastic bags, kept under ice, and transported to the University of Georgia, Athens, where they were stored at -23°C prior to further processing. Fillets were prepared by each of the four cooking methods. All cooked samples were labeled, wrapped in aluminum foil, placed in zip-lock plastic bags, and frozen at -23°C until analyzed. Moisture contents of catfish fillets were determined by an AOAC (1990) oven method 950.46. Oxytetracyline in the cooked and uncooked fillets was determined by high performance liquid chromatography.

The moisture content of raw fillets ranged from 70.7 to 78.1%. Cooking the fish muscle caused a significant ($P < 0.05$) reduction in moisture content to 68.6%. Fried fillets had a significantly higher moisture content than baked and smoked sampled. The smoked fillets had the lowest moisture content. The HPLC detection limits for oxytetracycline were 3.5 ng for a standard solution (50 ng/mL, 70-L injection volume) and 0.05 ppm for catfish extracts. Oxytetracycline exhibited a linear response over the range of 0.05 to 1.0 ppm in spiked catfish muscle ($R^2 > 0.999$). The recovery rate of oxytetracycline from spiked catfish was 92.5% over the concentrations of 0.05 - 1.0 ppm.

Oxytetracycline residues were detected in raw catfish 18 hr after oral administration of 37.5, 75.0 or 150.0 mg oxytetracycline/kg of fish for 10 days. Most of the residues exceeded the 0.1 ppm tolerance level, which might be expected since fish were purposely harvested only 18 hr after ending the medicated feed regimen in order to maintain oxytetracycline residues in raw fillets. No corrections for recovery loss were made with these data. Catfish receiving higher doses of oxytetracycline tended to have higher tissue residue levels. Those receiving 150 mg oxytetracycline/kg for 10 days had a higher residue level (0.27 ± 0.26 ppm) $P < 0.05$) than those receiving 37.5 mg/kg (0.12 ± 0.10 ppm). Those receiving 75.0 mg oxytetracycline/kg had a residue level of 0.24 ± 0.29 ppm.

Pairwise comparisons between the raw and cooked fillets for each cooking method and at each feeding level showed that cooking of catfish caused a reduction in oxytetracycline residues. In general, the cooked fillets of the 150 mg/kg group had a higher residue content than that of the 37.5 or 75 mg/kg group. Baking and smoking were, in general, more effective than frying in reducing oxytetracycline in fillets. The longer cooking time used for baking and smoking apparently contributed to the greater loss of oxytetracycline in cooked fillets. Although a temperature of 190°C was used to fry the fillets, the internal temperature was only 71°C. Therefore, frying of catfish fillets at this temperature for only 7-10 min was not as effective as baking or smoking in reducing oxytetracycline residues. Thus, the results confirmed that ordinary cooking procedures by frying, baking, and smoking at 190°C could not completely eliminate high levels of oxytetracycline residues in catfish fillets. The most effective approach to minimize oxytetracycline residues in cooked fillets would be adherence to the FDA's guidelines for oxytetracycline use. Under such feeding protocol, oxytetracycline residues in catfish fillets, if it occurs, will not exceed the legal tolerance level of 0.1 ppm and such low levels may then be destroyed by regular cooking procedures.

Objective 6: Progress for the first three years included: development of standard operating procedures for sample collection, sample preparation, pesticide analysis, metal analysis, and quality assurance; producers' and processors' samples were submitted to the University of Georgia for sample preparation; analysis of samples was completed at the University of Georgia and Mississippi State University. Residue data will be provided to cooperators in the eight states. Farm-raised channel catfish, rainbow trout and red swamp crawfish were collected from commercial ponds and processing sites at intervals for the two-year period. Locations for sample collection are as follows:

Location	Catfish Processors	Catfish Pond Sites	Rainbow Trout Pond Sites	Crawfish Production Sites
Mississippi	3	4	--	--
Alabama	2	3	--	--
Georgia	--	4	2	--
Louisiana	2	3	--	3
Tennessee	--	3	3	--
Florida	2	4	--	--
Texas	2	4	--	2
N. Carolina	--	--	20*	--
TOTAL	11	25	25	5

*Samples were only submitted following a single collection from this location.

Pond sites for channel catfish, rainbow trout and red swamp crawfish were selected to obtain diverse and representative sampling sites from each state. Catfish and rainbow trout were harvested from ponds within each state; fillets, including bellyflap, were collected and frozen. Crawfish were harvested; raw tail flesh was obtained and frozen. In addition, catfish, rainbow trout and crawfish feed were collected for analyses when fish with elevated residues are found. Catfish fillets obtained from commercial processing facilities were collected and frozen. Frozen samples were shipped to the University of Georgia where a composite sample was coded, homogenized, frozen and distributed to analytical facilities. Samples are being maintained below 0°C for five years for future retesting or additional residue analyses. Analyses for the following chemicals have been conducted:

<u>Organochlorines</u>

PCB's (1242, 1248, 1254, 1260)
Chlordane's
BHC's (Lindane, etc.)
Heptachlor
Heptachlor Epoxide
Dieldrin
Endosulfan I & II
Endosulfan sulfate
Endrin
o,p'- and p,p'-DDD, DDE, DDT
Methoxychlor
Toxaphene
Hexachlorobenzene
Mirex

<u>Organophosphates</u>

Chlorpyrifos
Diazinon
Malathion
methyl-Parathion
ethyl-Parathion

<u>Pyrethroids</u>

Cypermethrin
Fenvalerate

Metals

Copper
Cadmium
Lead
Mercury
Arsenic
Selenium
Chromium
Barium
Silver

Since pharmaceutical compounds are approved for use during production, samples of catfish are being maintained below 0°C until such time as the methods have been satisfactorily developed and additional funds become available. Multiples of all samples collected during this study will be maintained below 0°C for five years from collection date for subsequent residue determinations which may be of interest to the industry.

Quality assurance was conducted by the University of Georgia in a facility which is independent of sample analyses. Standard Operating Procedures (SOP) have been developed to ensure the validity of the data generated during this study.

IMPACTS:

Objective 5: Regulatory agencies are currently evaluating animal drugs for use in aquaculture systems. A major impact of this study will be to determine the fate of antibiotics from production through processing. It is not known whether prophylactic treatment with antibiotics will increase the residues in harvested catfish which have or have not been held for prescribed withdrawal times before harvest.

One of the important educational aspects which will result from this study will be a better understanding of the fate of antibiotics used in production. The information generated during this study will be communicated to production and processing segments of the industry to help avoid problems which may occur involving resistant organisms and residues in the processed fish.

Objective 6: The results generated during this objective are likely to have a major positive impact on the aquaculture industry. The data are expected to follow trends from other limited sampling experiments which demonstrate much lower residues in farm-raised products than in wild caught fish. The results from this study will be used to find potential problems relating to elevated residues and solve these problems with the help of the producer or processor. Furthermore, this study will serve as a pilot study for the industry to develop a quality assurance program to routinely monitor for residues in aquaculture products.

Educational opportunities are also expected as a result of this project. First, producers and processors will be made more aware of the importance in reducing residues in aquaculture products. Second, there will be many analytical methods developed from this study which will be useful for people to conduct future testing. Third, undergraduate and graduate students as well as faculty participants will become more aware of aquacultural products and practices and be better able to serve the industry.

PUBLICATIONS, MANUSCRIPTS OR PAPERS PRESENTED:

PUBLICATIONS

Du, Wen-Xian. (1994) Determination of oxytetracycline, sulfadimethoxine, and ormetoprim residues in catfish using high performance liquid chromatography. Master thesis. University of Florida, Gainesville.

Du, W.X., M.R. Marshall, W.B. Wheeler, M. Mathews, D. Gatlin, S.D. Rawles, D.-H Xu, W.A. Rodgers, and C.I. Wei. (1995) Oxytetracycline, sulfadimethoxine, and ormetoprim residues in catfish by HPLC. J. Food Sci. 60:1220-1224.

PUBLICATIONS SUBMITTED

Du, W.X., M.R. Marshall, D.-H. XU, C.R. Santerre, and C.I. Wei. (1996) Effect of cooking on oxytetracycline residues in catfish fillets. J. Food Sci. (submitted).

Xu, D., J.M. Grizzle, W.A. Rogers, and C.R. Santerre. In press. Effect of cooking on residues of ormetoprim and sulfadimethoxine in the muscle of channel catfish. Food Research International 30: in press.

PUBLICATIONS IN PREPARATION

Rawles, S.D., A. Kocabas, C.I. Wei (others from Dr. Wei's lab) and Delbert M. Gatlin III (In preparation) Dietary supplementation of Terramycin and Romet-30 does not enhance growth of channel catfish but does influence tissue residues. Journal of the World Aquaculture Society.

Khanna, N., C.R. Santerre, Dehai Xu and Y.W. Huang. 1996. Influence of Processing of Residues of Dieldrin and p,p'-DDE in Channel Catfish. Journal of Food Protection (in preparation).

Wei, C, et al. 1996. Determination of Oxytetracycline, Sulfadimethoxine and Ormetoprim Residues in Catfish Using HPLC. (in preparation)

Wei C. et al. 1995. Processing Affects on Oxytetracycline, Sulfadimethoxine and Ormetoprim residues in Catfish. (in preparation).

PAPERS PRESENTED

Du, W.X., M.R. Marshall, W.B. Wheeler and C.I. Wei. (1995) Determination of oxytetracycline, sulfadimethoxine, and ormetoprim residues in catfish using high performance liquid chromatography. Presented at the Institute of Food Technologists annual meeting at Anaheim, CA (Abstract 54E-13).

Du, W.X., M.R. Marshall, D.-H Xu, C. R. Santerre, and C.I. Wei. (1996) Effect of cooking on oxytetracycline residues in catfish. Presented at the American Chemical Society 212th annual meeting at Orlando, Florida in August (AGFD Abstract 48).

SUPPORT:

Additional support ($95,048 over two years) has been received by the University of Georgia from the National Biological Survey under a project entitled, "Use of Immune Factors in Fish as Indicators of Environmental Contamination" with R. Reinert as the PI and C. Santerre as CoPI and V. Blazer as the agency coordinator. Results from this research are demonstrating the relationship between residue levels of pesticides in fish and exposure through the diet.

YEAR	SRAC FUNDING	OTHER SUPPORT					TOTAL SRAC+ OTHER SUPPORT
		UNIVERSITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL OTHER SUPPORT	
1	100,000	29,978				29,978	129,978
2	155,000	60,785		95,048		155,833	310,833
3	101,000	48,651				48,651	149,651
Total	356,000	139,414		95,048		234,462	590,462