TREATMENT OF ICHTHYOPHTHIRIASIS IN CHANNEL CATFISH WITH A TRIIODINATED RESIN AND FREE IODINE

by

Michael J. LeValley

B.S., Kansas State University, 1975

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology
Parasitology Group

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1979

Approved by:

John R Kelley, Jr.

Major Professor

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. John R. Kelley, Jr. along with committee members Dr. Merle F. Hansen and Dr. Louis R. Fina who provided information, guidance, and critical evaluation throughout this research project.

The Kansas Agricultural Experiment Station provided funds for this study. Thanks are also due DAVCO, Inc., Ann Arbor, Michigan, who provided the resin. The Kansas Fish and Game Commission provided the fish.

The author also wishes to express his sincere appreciation to his fiancee, Shelley K. Burke, and to his parents, Clayton and Glenna LeValley, for their unwaivering support throughout the course of his graduate studies.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

INTRODUCTION

Almost all species of freshwater fish are susceptible to ichthyophthiriasis or white-spot disease, commonly referred to as "ich." The causative organism is a ciliated, parasitic protozoan, *Ichthyophthirius multifilis* Fouquet (Ciliophora, Hymenostomatida, Ophryoglenidae), cosmopolitan in distribution. The disease is one of the most serious of fish (Bauer 1958, Meyer 1969, Hines and Spira 1973a, 1973b) and probably the most serious disease affecting channel catfish (Meyer 1974).

Annual economic losses due to "ich" among the various fish raising enterprises worldwide are substantial (Hines and Spira 1973a, 1973b). The disease attacks fish of all sizes and is highly contagious. Stress is not a predisposing factor to an epizootic, and if unchecked, heavy mortalities result. "Ich" is seasonal with most cases reported in the spring and fall (Meyer 1978).

Many chemicals have been used in an attempt to control "ich" infections in fish. Cross (1972) gives a good review of the drugs tried and lists advantages and disadvantages of the various treatments. To date, only the vulnerable tomite stage of the parasite is susceptible to the various chemical regimes. Unfortunately, the disease is rarely diagnosed until white spots appear on the fish.

The most widely used chemotherapeutic agents in the United States for treatment of ichthyophthiriasis have been formalin, malachite green, copper sulfate, and a mixture of malachite green and formalin. Enactment of the Federal Environmental Pesticide Control Act (FEPCA), effective in 1972, has required all chemical uses to be covered by

approved registrations granted by the Environmental Protection Agency and the Food and Drug Administration.

All aforementioned compounds, except cooper sulfate, are currently unregistered for use in fish culture. These chemicals may eventually earn registration, but each has several disadvantages.

Formalin has long been used as a general treatment for ectoparasitic, protozoan infections of fish (Herman 1970). However, heavily parasitized fish are often killed at the recommended level of 250 ppm and lower concentrations give limited control (Meyer 1969). Hoffman (1970) warns that applications of this chemical may result in oxygen depletions in ponds. Malachite green (oxalate form) is effective at concentrations around 0.1 ppm, but parameters such as pH and hardness vary the toxicity and efficacy of the drug. High concentrations of the compound are carcinogenic (Leteux and Meyer 1972). Copper sulfate has been recommended by Meyer (1966) at a concentration of 0.5 ppm; however, the toxicity to fish varies directly with water hardness and the drug is ineffective in waters high in calcium carbonate. Mixtures of malachite green and formalin (0.1 ppm malachite green and 25 ppm formalin) have been very effective (Leteux and Meyer 1972, Schachte 1974), but the combination is still unregistered.

Generally, most drugs are too expensive to use on a large scale, give limited control of the disease at best, have a low therapeutic index, that is, the degree of latitude between a concentration controlling the disease and a concentration causing fish mortality, or have varying efficacy and toxicity depending on water chemistry parameters.

Chemotherapy is a neglected area of fisheries research according to Post (1965). Hoffman (1976) states that better therapy for ichthyophthiriasis is needed. The restrictions and curtailments imposed by FEPCA

has necessitated an immediate and concentrated effort to find drugs that are effective and that meet the requirements of the EPA and FDA.

The germicidal capabilities of diatomic iodine are well documented (Wyss and Strandkov 1945, Chang 1958, Hsu et al. 1965). Chang and Morris (1953) reported the effectiveness of iodine in killing the cysts of the protozoan, *Entameba histolytica*. Iodophors (organic iodine complexes) have been shown to be very effective in destroying a variety of viral and bacterial pathogens of fish and fish eggs (Amend 1974). The major drawback to the use of diatomic iodine, iodoforms, or iodophors in treating ectoparasitic infections of fish would seem to be their toxicity. The general use of chlorine, another halogen, as a disinfectant for municipal water supplies has led to many studies documenting its severe toxicity to fish at low concentrations (Zillich 1972, Brungs 1973).

The development of a virtually water-insoluble, demand type disinfectant by Lambert and Fina (1974, 1975) which depends on iodine for its efficacy, seemed a likely candidate for screening as a fish parasiticide. The compound is a triiodinated, strong base quaternary ammonium exchange resin, and possesses remarkable germicidal capabilities (Taylor et al. 1970, Fina and Lambert 1975). Physical and chemical characteristics of various forms of the complex and its preparation are described by Taylor et al. (1970) and Fina and Lambert (1975). One form of the triiodinated resin, provided by L. L. Davis of DAVCO, Inc., Ann Arbor, Michigan, was tested as a possible chemotherapeutic compound against ichthyophthiriasis.

The only citation concerning the toxicity of iodine to fish is reported by Gozlan (1968) who found that 8 ppm iodine was lethal for a marine fish (*Mugil* sp.). Since the resin-triiodide complex minimally elutes diatomic iodine into solution, the toxicity of iodine as well as the complex to channel catfish was determined.

Life History of the Parasite

The techniques, methods, and experiments dealt with in this paper require a thorough background and understanding of the disease itself and the unusual life cycle of the parasite.

Adult stages of the parasite, termed trophozoites or trophonts, are macroscopic and spherical and range in diameter form 0.5 to 1.0 mm. Cilia cover the entire organism and give it a rolling movement. This feature and a large horseshoe-shaped macronucleus easily identify the parasite. The parasite infests the gills and body surface with no preference for either site, except in the golden shiner where individuals localize entirely in the gill tissues (Meyer 1966). The host walls off the parasite with a covering of epithelial and connective tissue resulting in the characteristic white pustules seen on the skin and fins of the fish in advanced stages of the disease.

After maturation in the dermal tissues, the adult emerges and is free swimming for several minutes up to 6 hours (Bauer 1958, Meyer 1974). The trophozoite enlarges during this time and eventually settles on a substrate. A proteinaceous cyst wall is secreted which completely envelopes the cell and attaches it to the substrate (Bauer 1958, Meyer 1969).

Within the cyst the trophont reabsorbs its cytostome (Bauer 1958), undergoes multiple fission, and forms up to 2000 daughter cells referred to as tomites or swarmers. The number of tomites produced depends on the size of the adult at the time of encystment (Meyer 1974). These immature stages are oval and ciliated and range in diameter from 20 µm (Bauer 1958) to 45 µm (Meyer 1969). Fission can occur in unencysted adults but is limited to one or two divisions (Bauer 1958).

After fission is completed, the tomites rupture the cyst wall, escape, and actively search out a host. Limited food reserves (glycogen,

fats, and proteins) accumulated during the parasitic stage are used until a host is found (Ychehckar and Uspenskaja 1964). The infective stage must find a host within 24 (Meyer 1974) to 96 hours (Johnson 1974) or die. Development is temperature-dependent and at 25 C the lifespan of the tomite is 12 hours (Meyer 1974).

Once a host is located, the tomite burrows into the skin of the fish using a ciliary motion (Bauer 1958, Meyer 1974). Burrowing is facilitated by the secretion of hyaluronidase which depolymerizes hyaluronic acid, the basic constituent of the connective tissue filling the intercellular spaces in the integument (Uspenskaja 1963). When the parasite is finally localized between the epithelium and the connective tissue, the fish walls off the organism with epithelial tissue and mucus, the tomite matures into the trophozoite and the cycle is repeated.

The entire life cycle takes 2 days at 23.9 to 26.6 C (Johnson 1974) and more than 5 weeks at 10 C (Meyer 1969, Johnson 1974). Butcher (1947) reported a life cycle of 14-16 days at 15.6 to 16.7 C. Bauer (1958) states that fission does not occur above 27 C; however, Johnson (1969) found that reproduction continues up to 32.2 C. Bauer et al. (1969) has observed reproduction in wintering ponds at water temperatures of 2 to 4 C.

Though "ich" may cause death in large fish, the disease is most detrimental to small individuals. Damage is most severe when the organism occurs in the gills. Hines and Spira (1973a) reported instances of gills totally devoid of an epithelial surface. Consequently, gill surface area is significantly reduced and respiration impaired. Invasion of the epithelial tissue in the skin of the host results in deformation of productive cells and deterioration of the fine blood vessels (Bauer et al. 1969). In addition, enzymes secreted by the parasite and products of cell decomposition are toxic (Bauer et al. 1969). Bauer et al. (1969) found changes

in blood chemistry including a sharp drop in hemoglobin. Ichthyophthiriasis also opens the host to secondary infections by opportunistic fungi and bacteria.

The objectives of this research project consist of (1) investigating the effects of iodine on channel catfish, (2) investigating the effects of iodine on the life stages of "ich," and (3) evaluating a triiodinated resin as a potential therapeutic for controlling ichthyophthiriasis.

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental Fish

Channel catfish, *Ictalurus punctatus* (Rafinesque), were used as the principal host for "ich" and in toxicity tests of iodine and the triiodinated resin. Fingerlings were obtained from the Kansas Fish and Game Commission and cultured in 150 liter stainless steel flow-through troughs. Fish were put on a maintenance feeding schedule to insure a fingerling size of 60 to 100 mm total length and 2.0 to 7.3 g total weight. Large numbers of this size fish could be held in a relatively small area and using this size lessens the chance that the fish had contacted "ich" previously and developed a partial immunity (Beckert and Allison 1964).

Channel catfish were the preferred test animal for the following reasons: (1) they are scaleless fish (a morphological feature that greatly facilitates the removal of the parasites without the associated large amounts of mucus), (2) they are very susceptible to "ich", and (3) they are an important commercial species in the United States.

Parasite Culture

A supply of parasites was maintained in a manner similar to the method described by Beckert (1967) with several modifications. Parasitized catfish were held in 150 liter troughs at 18 C. Healthy fish were added periodically to sustain the culture and all dead hosts were removed from the tank within 24 hours. Parasites were also cultured in 60 liter aquaria at 26 C but the troughs proved to be the method of choice. Temperature could only be maintained at 26 C in aquaria and some very virulent infections killed all fish in 24 hours. Several cultures were lost and had to be restarted. A substrate of rocks and soil placed in the troughs more easily maintained "ich," but no such requirement is

cited in the literature. Aeration was continuously supplied to the troughs. Ychehckar and Uspenskaja (1964) found that tomites have an oxygen requirement of above 0.8 mg/liter.

Cultures were started from the following sources: a commercial grower in the spring of 1978, the Kansas Fish and Game Commission Farlington hatchery in January of 1979, and various species of tropical fish. Several attempts at infection with tropical fish strains of "ich" failed, suggesting that this strain is different than the catfish strain (Meyer 1966).

Trophozoites were collected using the technique of Beckert (1967). Infected fish were pithed and placed in 102 mm diameter fingerbowls. Parasites were allowed to leave the fish and fish were removed after one hour to prevent oxygen depletions. This method typically yielded around 2000 adult stages per fish. If parasites were not used immediately, they were placed in a refrigerator and kept at 5.5 C. This depressed the fission rate and allowed use of the parasites at a later date. Parasites maintained at this temperature underwent fission rapidly when brought to 25 C and they exhibited no loss of viability or function. Trophozoites were then collected with a pipette or eye dropper and transferred to experimental vessels or allowed to form cysts and tomites in fingerbowls.

Toxicological Assays with Fish

Stock solutions were made as needed by dissolving 1 g of iodine crystals (analytical reagent grade) in 1 liter of distilled water at 60 C. This yielded typical concentrations of 0.72 to 1.00 mg/l. Stock preparations were stored in the dark to limit photodecomposition. As a matter of routine, solutions were checked before each use by analyzing for iodine according to the method of Palin (1967) modified by the Hach Chemical Company (Method Manual 12th edition, 1973).

Preliminary bioassays of iodine against channel catfish were run in 4 liter aquaria to delimit a range of concentrations giving between 10 and 90% mortality. Final bioassays were carried out in 60 liter glass aquaria for intervals of 0.5, 1, 2, 10, and 24 hours. All test fish were starved for 3 days prior to and during any bioassay to lessen metabolites in solution. Fish were also acclimated for at least 2 hours before introduction of the chemical. At the end of each test, viable fish were placed in 30 liter recovery aquaria and observed for 48 hours.

Dissolved oxygen, temperature, pH, and total alkalinity were measured initially and at the end of each assay. Dissolved oxygen and temperature were determined using a Yellow Springs Instrument Company model 54A meter. A Corning model 12 pH meter was used to measure pH. Total alkalinity was determined using standard titrametric methods. Lethal concentration (LC) 16, 50, and 84 values for the toxicity of free iodine were determined using the technique of Litchfield and Wilcoxon (1949). Typically, iodine concentraions of 0.5 and 1 hour assays remained constant. In all other tests, iodine concentration was monitored at 1 hour intervals and extra chemical introduced to adjust for losses of iodine due to photodecomposition and organic matter.

Toxicity of the triiodide resin-complex to channel catfish was determined using two methods. Fish in 60 liter aquaria were exposed for 96 hours to water recirculated through glass columns containing differing amounts of the resin. These columns were 3.5 cm in diameter and 30 cm in length, and contained from 70 to 100 g (wet volume 49 to 70 ml) of resin. Water for both experimental and control aquaria was pumped at rates varying from 70 to 100 ml per hour.

The other method consisted of layering the resin complex on the bottom of 4 and 60 liter aquaria. Four liter aquaria contained 215 to 240 g (wet

volume 150 to 168 ml) of resin and 60 liter aquaria contained 600 to 800 g (wet volume 420 to 560 ml) of resin. This gave a uniform stratum of resin 4 to 5 cm thick in both types of aquaria. Fish were then placed in these aquaria and observed for 96 hours.

In both methods, water chemistry parameters such as dissolved oxygen, temperature, pH, total alkalinity, initial I_2 concentration and final I_2 concentration were measured.

Toxicological Assays with "Ich" Life Stages

Tomites, trophozoites, and cysts of "ich" were challenged with varying concentrations of iodine in 25 x 170 mm petri plates. All assays were run for 1 hour at 25 C with 20 ml of known concentrations of chemical added to each plate. Only 1 hour tests were performed since iodine was found earlier to disappear after 60 minutes. Percent mortality at each level was calculated by averaging 2 replicates. LC 16, 50, and 84 values were arrived at by employing the method of Litchfield and Wilcoxon (1949). Tomite numbers were found by counting the number in 3, 0.1 ml samples and averaging. Total tomite numbers were calculated by multiplying the number per 0.1 ml times the total ml used. After 1 hour, subsamples of 10 ml were taken and percent mortality calculated. Non-motility of the tomite was used as the criteria for death.

Trophozoites and cysts were counted directly by using a dissecting microscope. Trophozoites were allowed to form cysts in the experimental plate before introduction of the chemical to guard against mechanical damage incurred to the cyst wall during transfer with a pipette. Cysts usually had greater than 25 daughter cells before exposure to iodine. Trophozoites and cysts were judged nonviable in the absence of cytoplasmic streaming.

The effects of the triiodinated resin on the life stages of "ich" were determined in 25 x 170 mm petri plates which contained 12 g (wet volume 8.4 ml) of material. Each plate contained 20 ml of dechlorinated tap water and each assay was run at 25 C. Known numbers of tomites, trophozoites, and cysts were added to 3 experimental and 1 control plate in each test. Initial tomite numbers and percent mortality were calculated as before.

Final verification of the iodinated resin's effects on the 3 stages of "ich" was carried out in 4 liter aquaria. Three healthy channel catfish fingerlings were challenged with 50 viable tomites in an aquarium containing 220 g (wet volume 154 ml) of resin and observed for symptoms of the disease for 1 week. Trophozoites and cysts subjected to the resin in petri plates were collected and introduced into 2 aquaria containing 5 healthy fish each. If external symptoms of the disease did not appear, the fish were examined microscopically for parasites.

Efficacy Tests

Three experimental lots of 10 moderately infected channel catfish each (~300 parasites per fish) were placed in 2, 60 liter aquaria fitted with columns of the same dimensions as those used in the toxicity tests. These columns contained 100 g (wet volume 70 ml) of the triiodinated resin and water was recirculated from the aquaria through the resin at a flow rate of 100 ml per hour. An untreated control group with a similar infection was observed at the same time.

Evaluation of the resin as a prophylactic treatment and as a cure was carried out in 4 liter aquaria containing 240 g (wet volume 168 ml) of resin and in 60 liter aquaria containing 600 g (wet volume 420 ml) of resin.

The first experiment consisted of introducing 9 healthy and 1 parasitized fish (excluding gills around 300 parasites per fish) into aquaria containing the resin-triiodide complex to determine if the disease could be transmitted. A control aquarium containing no resin was maintained in a like manner.

The second experiment tested the effectiveness of the resin as a cure. Three lots of 10 fish, each moderately infected, were placed in 60 liter aquaria and observed for 1 week. Three lots of 10 heavily infected fish ($\sim$1000 trophozoites per fish) were introduced into 60 liter aquaria containing the triiodinated resin. In each case, 2 aquaria, with no resin, were used as controls. Scrapings of the skin were taken to determine parasitism if no pustules were visible externally.

Diatomic iodine was tested as a therapeutic for "ich" in 3 separate experiments. Thirty channel catfish (mean length 97 mm) were separated into 3 groups of 10 fish each. These groups were judged subjectively as having light, moderate, and heavy "ich" infections. A treatment level of 1.00 mg/liter iodine was administered 3 times daily for 7 days in 30 liter aquaria. Temperature remained constant at 25 C and the fish were observed for 3 days after treatment had stopped. Fish which died during the first 4 hours after application of the chemical were considered to have died of toxicosis. In each experiment, a control group of 5 fish with a similar infection was maintained. If no pustules were visible externally, fish were examined microscopically for trophozoite stages of the parasite.

Survival data were evaluated using analysis of variance and least significant difference statistics (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

Some General Observations

Bauer (1958) observed fission in unencysted adults of "ich" and stated that fission is limited to 1 or 2 divisions. Though rare, the phenomenon was also observed during this study. Fission was limited to 1 division and took place while the parasite was still imbedded in the fish. Whether these forms fall off and undergo normal reproduction is unclear.

Free-floating cysts also occurred. Cell division proceeded normally in these forms and it is assumed that the daughter cells produced were as infective as tomites formed by attached cysts.

Several attempts at infection using "ich" from tropical fish failed, suggesting the strain phenomenon cited by Meyer (1966). Transfer of the disease was attempted at 18 C. Different strains may have evolved to show a host or temperature preference.

Infections were started by allowing tomites to be formed in the original fingerbowl, and introducing them into holding troughs containing "clean" fish. Tomites were successfully kept in fingerbowls for periods not exceeding 24 hours. Mass mortality of tomites was found to occur when holding periods exceeded 24 hours. Meyer (1974) observed mortalities among tomites kept under similar conditions.

In culture troughs the life cycle was completed in 5 days at 18 C. After infection of fish by "ich" tomites, death eventually followed. Results of this study indicate that the parasite is eurythermal. Rapid temperature changes were withstood by most of the parasites with no loss of viability. It is unclear which stage or stages of the organism are able to withstand the severe thermal shocks. In one instance, several tomites accidentally frozen overnight in a fingerbowl, were viable the following day.

Toxicological Assays

LC 50 values for 0.5, 1, 2, 10, and 24 hour exposures of channel catfish to iodine were 11.00, 3.00, 2.10, 0.98, and 0.44 mg/liter respectively (Table 1). No mortality of catfish occurred at 4.25 (0.5 hr), 1.50 (1 hr), 1.20 (2 hr), 0.50 (10 hr), and 0.10 (24 hr) mg/liter of iodine. Concentrations above 14.30, 7.22, 4.30, 1.52, and 0.72 mg/liter resulted in 100% mortality for exposures of 0.5, 1, 2, 10, and 24 hours respectively. LC 16, 50, and 84 values (Table 1) show a high tolerance of channel catfish to iodine for 30 minute bioassays. In all other tests, the doses became progressively lower as one would expect. One and 2 hour LC 50's are reasonably close as are the values for the 10 and 24 hour tests. Mortality was absent in the control groups demonstrating that iodine was the toxic substance, and it exerts a toxic effect directly on the fish since water chemistry parameters were similar in both experimental and control groups (Table 2).

Mortalities occurred within 2 hours of dosing at all iodine concentrations in 0.5, 1, and 2 hour tests. Death of fish in the 10 and 24 hour bioassays occurred at all iodine levels 4-12 hours after dosing. Inactivity, loss of equilibrium, color fading, "piping," and massive gill damage were symptomatic of iodine poisoning. Color fading in channel catfish is symptomatic of severe oxygen stress. In all toxicity tests channel catfish never recovered after loss of equilibrium and most fish died within 30 minutes of equilibrium loss. Gill damage consisted of proliferative lesions and severe hemorrhaging in the gill filaments.

Varying the amount of resin in columns and changing the circulation rate had no toxic effects on channel catfish (Table 3). No significant changes existed for dissolved oxygen, temperature, total alkalinity, or pH in 6 experimental and 1 control aquarium (Table 3).

Table 1. LC 16, 50 (with 95% confidence intervals), and 84 values in mg/l for free iodine toxicity to channel catfish.

Duration of Test	No. Tested	LC 16	LC 50	LC 84
30 minutes	48	6.80	11.00 (7.80 - 15.51)	13.40
1 hour	60	1.35	3.00 (2.22 - 4.05)	6.60
2 hour	40	1.20	2.10 (1.63 - 2.71)	3.70
10 hour	48	0.68	0.98 (0.89 - 1.07)	1.37
24 hour	50	0.30	0.44 (0.40 - 0.49)	0.62

Table 2. Range of initial and final water chemistry parameters observed in exposures of free iodine to channel catfish.

Parameter	30 minute[a]	1 hour[a]	2 hour[b]	10 hour[a]	24 hour[b]
Dissolved oxygen m/l					
Initial	8.1- 8.3	8.2- 8.8	7.5- 8.3	7.9- 8.5	8.2- 9.0
Final	7.8- 8.3	8.0- 8.5	7.5- 8.2	8.0- 8.5	8.1- 9.0
Temperature C					
Initial	20.2-22.3	21.0-21.7	20.5-21.3	21.6-22.4	20.3-21.0
Final	20.4-21.6	21.1-21.7	20.5-21.3	21.6-22.4	20.3-21.2
Total Alkalinity mg/l					
Initial	62.7-68.0	67.0-72.0	64.0-68.0	65.0-67.0	65.0-72.0
Final	63.1-67.3	65.0-69.0	63.0-70.0	64.6-68.9	63.4-70.6
pH					
Initial	7.3- 7.6	7.4- 7.5	7.2- 7.4	7.3- 7.4	7.2- 7.3
Final	7.3- 7.4	7.3- 7.4	7.3- 7.4	7.3- 7.4	7.2- 7.4

[a]Water from 6 experimental aquaria and 1 control aquarium.

[b]Water from 4 experimental aquaria and 1 control aquarium.

Table 3. Summary of water quality parameters and mortality rates of channel catfish exposed to water recirculated through triiodinated resin columns for 96 hours.

Parameter	1	2	3	4	5	6	7 (control)
Amount of resin g	70	70	70	100	100	100	0
Wet volume of resin ml	49	49	49	70	70	70	0
Flow rate ml/hour	80	90	100	70	80	90	100
Free iodine mg/l							
Initial	0.10	0.18	0.12	0.20	0.27	0.90	0
Final	0.00	0.17	0.00	0.00	0.25	0.00	0
Dissolved oxygen mg/l							
Initial	7.9	8.2	8.5	8.1	8.8	8.2	8.1
Final	8.0	8.3	8.2	8.1	8.3	8.2	8.0
Temperature C							
Initial	22.3	21.3	20.7	20.5	20.1	20.7	21.5
Final	22.0	21.7	21.0	20.5	20.6	21.4	22.3
Total Alkalinity mg/l							
Initial	67.4	63.0	68.0	64.6	63.7	69.5	68.3
Final	65.3	65.0	72.0	66.5	69.2	66.2	63.7
pH							
Initial	7.3	7.4	7.3	7.2	7.3	7.4	7.2
Final	7.4	7.4	7.3	7.4	7.3	7.2	7.4
% Mortality	0	0	0	0	0	0	0

Initial elution values for free iodine were highly variable. Table 3 shows that a column containing less resin and subjected to less flow (Test 1) eluted substantially less iodine than a column containing more resin and subjected to a greater flow (Test 6). In 2 instances (Tests 2 and 5), initial and final concentrations of iodine were similar. In all tests, the fish were healthy and active, and readily accepted feed at the conclusion of the exposure interval. Microscopic examination of the gills indicated no hemorrhaging or "clubbing" of the filaments.

Layering of the triiodinated resin in 2 sizes of aquaria (4 and 60 liter) in differing amounts also had no toxic effects on channel catfish (Table 4). No free iodine was present initially or at the end of the 96 hour exposure period in the test water. Water chemistry was essentially unchanged (Table 4).

Free iodine was substantially more toxic to "ich" tomites than trohpozoites or cysts. LC 50 values for 1 hour exposures were 0.58, 21.00, and 38.00 mg/liter iodine for tomites, trophozoites, and cysts respectively (Table 5). No mortality of tomites, trophozoites, and cysts occurred at concentrations of 0.10, 5.00, and 9.20 mg/liter respectively. Concentrations above 1.20, 32.00, and 65.00 mg/liter iodine resulted in 100% mortality for tomites, trophozoites, and cysts respectively. Mortality in untreated controls during each test was minimal. Data for bioassays of iodine against tomites and trophozoites was significantly heterogeneous; however, the results clearly indicate the extreme sensitivity of the infective stage of "ich" to the compound.

Mortality of tomites was preceded by motility loss and lysis of the cell wall. Loss of cytoplasmic streaming and lysis of the cell wall preceded death of the trophozoites. Mortality of cysts was characterized by (1) dissolution of the cyst wall, (2) loss of cytoplasmic streaming in daughter cells, and

Table 4. Summary of water quality parameters and observed mortality rates of channel catfish exposed to triiodinated resin layered on aquaria bottoms for 96 hours.

Parameter	4 liter aquaria			60 liter aquaria		
	1	2	3 (control)	1	2	3 (control)
Amount of resin g	215	240	0	600	800	0
Wet volume of resin ml	150	168	0	420	560	0
Free iodine mg/l						
Initial	0.00	0.00	0	0.00	0.00	0
Final	0.00	0.00	0	0.00	0.00	0
Dissolved oxygen mg/l						
Initial	8.3	7.6	8.7	8.4	8.3	8.3
Final	8.4	7.7	8.3	8.1	8.6	8.3
Total alkalinity mg/l						
Initial	62.8	64.5	68.9	61.0	67.7	69.5
Final	67.5	65.7	72.6	63.0	65.3	67.1
pH						
Initial	7.3	7.2	7.2	7.7	7.3	8.1
Final	7.4	7.4	7.5	8.0	7.3	8.1
% Mortality	0	0	0	0	0	0

Table 5. LC 16, 50 (with 95% confidence intervals), and 84 values for 1 hour tests of free iodine against "ich" life stages at 25 C.

		Concentration values (mg/l)		
Life Stage	No. Tested	LC 16	LC 50	LC 84
Tomites[a]	360	0.36	0.58 (0.37 - 0.90)	0.92
Trophozoites[a]	205	15.00	21.00 (14.00 - 31.50)	27.00
Cysts	36	26.00	38.00 (31.50 - 45.20)	56.00

[a]Confidence interval adjusted to compensate for significantly heterogeneous data (Litchfield and Wilcoxon 1949).

(3) rupture of daughter cell walls. Many cysts had viable daughter cells in the core of the cyst while daughter cells at the periphery of the cyst were killed.

Tomites were unaffected by the resin complex in plates (Table 6). Tomites on control plates exhibited a greater mortality rate than the plates containing the resin. Trophozoites exposed to experimental plates had 100% mortality after 15 minutes while trophozoites in controls had a 90% survival rate (Table 6). Cysts in contact with the resin were rendered nonviable or killed after an exposure of 60 minutes while cysts in untreated controls had a 93% survival rate. Death in trophozoites and cysts was prefaced by the suspension of cytoplasmic streaming followed by contraction of the cell wall.

Channel catfish challenged with 50 viable tomites in an aquarium containing 220 g (wet volume 154 ml) of the triiodinated resin developed an infection in 2 days. Fish challenged with trophozoites and cysts that had contacted the resin-complex in plates had no external symptoms of the disease after 1 week of exposure. Subsequent microscopic examination of scrapings taken from the integument of the fish were negative for "ich" trophozoites.

Efficacy Tests

Treatment of ichthyophthiriasis using columns was ineffective. The disease ran its course in both experimental and control groups and all fish eventually died. Use of the triiodinated resin as a prophylactic therapy was effective. The disease was not transmitted from a moderately infected host to healthy fish in aquaria containing 240 g (wet volume 168 ml) of material. Untreated controls developed a moderate infection 5 days after introduction of 1 infected host. The resin complex was ineffective in curing already infected fish. Heavily parasitized channel catfish died

Table 6. Killing capability of the triiodinated resin against life stages of *Ichthyophthirius multifilis*.

Life Stage	No. Tested		% Mortality		Time to Death
	Drugged	Control	Drugged	Control	
Tomites	210	90	4.8	6.7	----
Trophozoites	64	20	100.0	10.0	15 minutes
Cysts	35	14	100.0	7.1	60 minutes

within 24 hours and moderately infected fish became progressively worse. Even after a treatment period of 10 days, no visible signs of disease abatement were evident. Necropsy revealed greater than 3000 parasites per fish and trophozoites and cysts were clearly in contact with the compound.

The LC 84 value for "ich" tomites during exposures to free iodine for 1 hour is 0.92 mg/liter. The LC 16 value for exposures of channel catfish to free iodine over a 1 hour interval is 1.35 mg/liter. A treatment level of 1 ppm iodine was chosen from this toxicity differential and found to be effective in curing channel catfish with a light infection. Out of 30 fish tested, 22 survived and microscopic examination revealed no parasites (Table 7). Thirty control fish had a 3.3% survival rate (Table 7). Moderately infected fish had a 50% survival rate in the treated groups while untreated controls had a 0% survival rate. Both treated and untreated groups of heavily infected channel catfish had similar mortality rates (Table 7). Only 1 treated fish in this group survived.

Survival of channel catfish exposed to a treatment level of 1 ppm iodine depends on the parasite load carried by the fish. Analysis of variance (Table 8) resulted in a F value of 31.23 which is highly significant at the 99% level. Least significant difference comparisons between survival means indicates that all 3 treatment groups are statistically different at the 99% level (LSD = 3.01).

The toxicity of iodine to channel catfish appears to be similar to the toxicity of chlorine to channel catfish. Marking and Bills (1977) found the 96 hour LC 50 for chlorine against channel catfish to be 0.156 mg/liter. This compares with a 24 hour LC 50 of 0.44 mg/liter for iodine against channel catfish (Table 1). It seems reasonable to assume that lower concentrations of iodine will exert toxic effects similar to those for chlorine over a 96 hour test period. Ideally, 96 hour bioassays for iodine against

Table 7. Survival of channel catfish with varying degrees of "ich" infections after 7 days of treatment with 1 ppm of iodine. (Three applications/day, observations recorded for 7 days, 10 fish/experiment.)

Treatment Group	Experiment 1	Experiment 2	Experiment 3	Total surviving of 30 fish
Light*	7	6	9	22
Control	0	1	0	1
Moderate*	5	4	6	15
Control	0	0	0	0
Heavy*	0	1	0	1
Control	0	0	0	0

*Light $\geq$ 100 parasites/fish; moderate $\geq$ 300 parasites/fish; heavy $\geq$ 1000 parasites/fish.

Table 8. Analysis of variance of survival of channel catfish grouped by level of infection.

Source	Degrees of Freedom	Sums of Squares	Mean Square	F
Between Groups	2	76.22	38.11	31.23*
Within Groups	6	7.34	1.22	
Total	8	83.56		

*($P < 0.01$)

channel catfish would have been performed; however, iodine is readily decomposed by light and organic matter and the precision needed to detect changes in iodine concentration and adjust accordingly over 96 hours was lacking.

Diatomic iodine undergoes the following reaction in water:

$$I_2 + H_2O \rightleftharpoons HIO + H^+ + I^-$$

At a pH of 7, HIO and I_2 are present in approximately equal parts. At a pH of 8, there is 88% HIO and 12% I_2 (Black et al. 1965, Wyss and Strandkov 1945). The range of pH in the toxicity tests for channel catfish indicates that both I_2 and HIO were present in the test water. However, it is unclear which of the 2 chemicals exerts the greater toxic effect. Chlorine is believed to kill fish by causing severe gill damage and eventual asphyxiation. Hypochlorous acid (HOCl) is the compound responsible. Hypoiodous acid (HIO) then, may be responsible for the hemorrhaging and lesions observed in the gill filaments of the bioassay fish. Destruction of the gill tissue is great enough to significantly impair respiration and death by anoxia results.

The complete absence of toxic effects on fish due to triiodinated resin is evidently a result of (1) minimal elution of I_2 by the material and/or (2) rapid light and metabolite decomposition of I_2 to the relatively harmless I^- form.

Toxicity tests in plates suggest that the trophozoites and cysts must contact the resin to be rendered nonviable. Trophozoites evidently take up a dose greater than 27 mg/liter iodine from the resin and cysts take up a dose greater than 56 mg/liter (Table 5). It is unclear why the material worked in vitro but not in an aquaria situation. Contact time was adequate but possible explanations may include (1) insufficient amount of resin resulting in too low a dose, (2) free-floating cysts which did not contact

the compound, or (3) mortalities in plates due to damage to the cell wall incurred during transfer with a pipette. The effects of the resin on tomites which had contacted the complex were not determined when it became apparent during the efficacy tests that the free-swimming tomites were not likely to encounter the resin-triiodide.

The vulnerable infective stage cannot be attacked by either of the 2 treatment methods employed. Columns do not elute sufficient iodine residual to kill the tomites and recirculation of aquaria water can never assure that all individuals will contact the compound before they find a host. Total lack of contact between the tomite and resin layered on aquaria bottoms insures the survival of this stage of "ich."

The triiodinated resin may be used in aquaria as a prophylactic treatment. The material is restricted to such use because of its expense and the difficult engineering problems inherent in adapting the complex to large fish culture operations. Introducing the substance into aquaria also has potential inherent difficulties. It would probably have to remain in aquaria indefinitely and it may have detrimental effects on the bacteria responsible for antifouling of the water in such aquaria.

The use of free iodine as a cure for ichthyophthiriasis in aquaria or holding tank situations is a viable possibility. The treatment would be economical costing 1 cent for each 300 liters of water treated. Losses of the chemical due to light and organic matter decomposition probably restrict its use to aquaria or holding tanks. Iodine has no value in treating the trophozoite and cyst stages of "ich" since the doses required to kill these forms greatly exceeds the lethal dose for channel catfish.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

A summary of the more important findings of this research project are contained in items 1 through 3.

1. Iodine is severely toxic to channel catfish and approaches the toxic range of another halogen, chlorine.

2. The triiodinated resin is ineffective as a cure for ichthyophthiriasis but may have value as a prophylactic since it is nontoxic to channel catfish.

3. Free iodine is useful as a treatment for ichthyophthiriasis in aquaria and holding tanks and may have potential use in ponds.

4. Black et al. (1965) has proposed the use of iodine in disinfecting public water supplies. Increasing evidence of the carcinogenic effects of residual chlorine species (monochloramine, dichloramine) may increase the use of iodine for treatment purposes. The data presented here may help determine safe dilution levels for aquatic life.

5. Further testing of free iodine as a general treatment for fish ectoparasites is needed. It appears that it could be used on food fish since it rapidly disappears from the environment. Testing of the triiodinated resin as a prophylactic fungicide or bactericide with applications in fish culture is also warranted. The material is not toxic to at least one species of fish and could probably meet EPA and FDA requirements.

LITERATURE CITED

LITERATURE CITED

Amend, D. 1974. Comparative toxicity of two iodophors to rainbow trout eggs. Trans. Am. Fish. Soc. 103:73-78.

Bauer, O. N. 1958. Parasitic disease of cultured fishes and methods of their prevention and treatment. In: Parasitology of Fishes. V. A. Dogiel, G. K. Petrushevski, and Y. I. Polyanski (eds.). Oliver and Boyd, London 1961. pp. 265-298.

Bauer, O. N., V. A. Musselius, and Yu. A. Strelkov. 1969. Diseases of Pond Fishes. Israel Program Sci. Trans. 1973. U. S. Dept. Comm. TT-72-50070. pp. 72-78.

Beckert, H. 1967. Culture of some common fish parasites for experimental studies. Zool.-Entom. Dept. Series Fisheries No. 5. Ag. Exp. Sta. Auburn Univ.

Beckert, H., and R. Allison. 1967. Some host responses of white catfish to *Ichthyophthirius multifilis* Fouquet. Proc. of 18th Ann. Conf. of the Southeast. Assoc. Game and Fish Comm. October, 1964. 18:438-441.

Black, A. P., R. N. Kinman, W. C. Thomas, G. Freund, and E. D. Bird. 1965. Use of iodine for disinfection. J. Am. Wat. Works Assoc. 57:1401-1421.

Brungs, W. A. 1973. Effects of residual chlorine on aquatic life. J. Wat. Poll. Cont. Fed. 45:2180-2193.

Butcher, A. D. 1947. Ichthyophthiriasis in an Australian trout hatchery. Prog. Fish Cult. 9:21-26.

Chang, S. L., and J. C. Morris. 1953. Elemental iodine as disinfectant for drinking water. Ind. and Eng. Chem. 45:1009-1012.

Chang, S. L. 1958. The use of active iodine as a water disinfectant. J. Am. Pharm. Assoc. 47:417-423.

Cross, D. C. 1972. A review of methods to control ichthyophthiriasis. Prog. Fish Cult. 34:165-170.

Fina, L. R., and J. L. Lambert. 1975. A broad-spectrum water disinfectant that releases germicide on demand. Second World Congress, Int. Wat. Res. Assoc. 2:53-59.

Gozlan, R. S. 1968. Isolation of iodine-producing bacteria from aquaria. Antonie van Leeuwenhoek. 34:226.

Herman, R. L. 1970. Chemotherapy of fish diseases: a review. J. Wildl. Dis. 6:31-34.

Hines, R. S., and D. T. Spira. 1973a. *Ichthyophthirius multifilis* (Fouquet) in the mirror carp, *Cyprinus carpio* L. I. Course of infection. J. Fish. Biol. 5:385-392.

Hines, R. S., and D. T. Spira. 1973b. Ichthyophthiriasis in the mirror carp: leucocyte response. J. Fish Biol. 5:527-534.

Hoffmann, G. L. 1970. Control and treatment of parasite diseases of freshwater fishes. U. S. Bureau of Sport Fish and Wildl., Fish Disease Leaflet 28. 7 pp.

Hoffmann, G. L. 1976. Protozoan diseases of freshwater fishes, advances and needs. Proc. Int. Wildl. Dis. Conf. 3.

Hsu, Y., S. Nomura, and C. W. Kruse. 1965. Some bactericidal and virucidal properties of iodine not affecting infectious RNA and DNA. Am. J. Epidem. 82:317-328.

Johnson, S. K. 1974. Ich disease of fishes. Tex. Ag. Ext. Ser. Leaflet 1210. 4 pp.

Lambert, J. L., and L. R. Fina. 1974. Method of disinfecting water and demand bactericide for use therein. U. S. Patent No. 3,817,860.

Lambert, J. L., and L. R. Fina. 1975. Demand bactericide for disinfecting water and process of preparation. U. S. Patent No. 3,923,665.

Leteux, F., and F. P. Meyer. 1972. Mixtures of malachite green and formalin for controlling *Ichthyophthirius* and other protozoan parasites of fish. Prog. Fish Cult. 34:21-26.

Litchfield, J. T., Jr., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharm. Exp. Ther. 96:99-113.

Marking, L. L., and T. D. Bills. 1977. Chlorine: its toxicity to fish and detoxification of antimycin. U. S. Fish and Wildl. Ser. Invest. Fish Control. 74. 5 pp.

Meyer, F. P. 1966. A review of the parasites and diseases of fishes in warm-water ponds in North America. Proc. World Symp. on Warm-water Pond Fish Cult. FAO Fish. Report. 44:290-318.

Meyer, F. P. 1969. Parasites of freshwater fishes. II. Protozoa. 3. *Ichthyophthirius multifilis*. U. S. Bureau of Sport Fish. and Wildl., Fish Disease Leaflet 2. 4 pp.

Meyer, F. P. 1974. Parasites of freshwater fishes. II. Protozoa. 3. *Ichthypohthirius multifilis*. U. S. Fish and Wildl. Ser., Fish Disease Leaflet (revised) 2. 5 pp.

Meyer, F. P. 1978. Incidence of disease in warm-water fish farms in the south-central United States. Mar. Fish. Rev. 40:38-41.

Palin, A. T. 1967. Diethyl-p-Phenylene Diamine (DPD) method for residual iodine. J. Inst. Wat. Eng. 21:545-547.

Post, G. 1965. A review of advances in the study of diseases of fish: 1954-1964. Prog. Fish Cult. 27:3-12.

Schachte, J. W., Jr. 1974. A short term treatment of malachite green and formalin for the control of *Ichthyophthirius multifilis* on channel catfish in holding tanks. Prog. Fish Cult. 36:103-104.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State Univ. Press, Ames. 593 pp.

Taylor, S. L., L. R. Fina, and J. L. Lambert. 1970. New water disinfectant: an insoluble quaternary ammonium resin-triiodide combination that releases germicide on demand. J. Appl. Micro. 20:720-722.

Uspenskaja, A. V. 1963. Hyaluronidase in the various stages of the life history of *Ichthyophthirius multifilis*. Doklady Akademii Nauk SSSR. 5:1476-1478.

Wyss, O., and F. B. Strandkov. 1945. The germicidal action of iodine. Arch. Biochem. 6:261-267.

Ychehckar, A. B., and A. V. Uspenskaja. 1964. Reserve materials, RNA, DNA, and respiratory enzymes at different stages of the life cycle of *Ichthyophthirius multifilis*. Acta. Protozool. 2:175-194.

Zillich, J. A. 1972. Toxicity of combined chlorine residuals to freshwater fish. J. Wat. Poll. Cont. Fed. 44:212-220.

TREATMENT OF ICHTHYOPHTHIRIASIS IN CHANNEL CATFISH WITH A TRIIODINATED RESIN AND FREE IODINE

by

Michael J. LeValley

B.S., Kansas State University, 1975

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Division of Biology
Parasitology Group

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1979

ABSTRACT

This study evaluated a triiodinated, strong base, quaternary ammonium exchange resin and free iodine as parasiticides for *Ichthyophthirius multifilis*. Minimal elution of diatomic iodine by the compound necessitated bioassays of iodine against channel catfish. Tests of free iodine and the resin against "ich" life stages were carried out in vitro and in practical situations with aquaria. Lethal concentration (LC) levels were determined using a standard dose-effect method.

Iodine was found to be extremely toxic to channel catfish with the 24 hour LC 50 being 0.44 mg/l. Fish were able to withstand much higher iodine levels for a short exposure period (30 minutes). The toxicity differential of free iodine between channel catfish and tomites resulted in use of the compound as an aquarium treatment. Fish with a light parasite load were effectively treated with 1 ppm iodine 3 times daily for 7 days.

The triiodinated resin was effectively used as a prophylactic treatment for ichthyophthiriasis, but was found to be ineffective as a cure. Further testing of the resin as a fungicide or bactericide is warranted since it was found to be not toxic to channel catfish. Additional testing may prove iodine to be an effective treatment against other fish ectoparasites in aquaria and holding tanks.