

## Prevention of an Initial Infestation of *Ichthyophthirius multifiliis* in Channel Catfish and Blue Tilapia by Potassium Permanganate Treatment

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**Abstract.**—Potassium permanganate (KMnO<sub>4</sub>) has been used to control infestations of *Ichthyophthirius multifiliis*, but its effectiveness has not been reported from controlled efficacy studies. The purpose of this study was to determine the acute toxicity of KMnO<sub>4</sub> to the *I. multifiliis* theront and the concentration needed to prevent an initial infestation of *I. multifiliis* in juvenile channel catfish *Ictalurus punctatus* and blue tilapia *Tilapia aurea*. *Ichthyophthirius multifiliis* theronts were exposed to concentrations of KMnO<sub>4</sub> in 100 µL of well water in 96-well plates and observed for 4 h to determine the acute toxicity. A concentration of 0.9 mg KMnO<sub>4</sub>/L caused greater than 95% mortality of the theronts in 4 h in well water; the 4-h LC50 (concentration lethal to 50% of test animals) value was estimated to be 0.77 mg/L. Juvenile channel catfish were exposed to 10,000 theronts/L of well water and immediately treated with a single dose of KMnO<sub>4</sub>. Infestation occurred in controls 6 d after exposure. The lowest effective dose of KMnO<sub>4</sub> was 1.0 mg/L. Juvenile blue tilapia were exposed and treated in the same manner as the channel catfish. Infestation occurred in controls by day 8 after exposure. The lowest effective dose of KMnO<sub>4</sub> was 0.5 mg/L. An additional experiment (without KMnO<sub>4</sub>) indicated that channel catfish were 33-fold more susceptible to *I. multifiliis* infestation than blue tilapia. These results indicate that KMnO<sub>4</sub> is toxic to *I. multifiliis* theronts at low concentrations in clean water. However, effective treatment of pond water will be strongly influenced by detoxication of KMnO<sub>4</sub> (based on the concentration of easily oxidizable substances in the water) and water temperatures controlling the lifecycle of the parasite.

Ichthyophthiriasis is caused by an external protozoan parasite, *Ichthyophthirius multifiliis*, that invades the skin and gills of freshwater fish (Schäperclaus 1991) and is commonly referred to as Ich or whitespot disease. When juvenile or fingerling channel catfish *Ictalurus punctatus* are raised at high densities, *I. multifiliis* can eradicate an entire fish population unless the parasite's reproductive cycle is interrupted (Tucker and Robinson 1990).

The well-documented life cycle of *I. multifiliis* involves an infective stage (theront) that burrows into the skin or gill of fish to feed on mucus and tissue (Beckert and Allison 1967). Infection is followed by a reproductive phase in which the theront matures into a trophont that dislodges, settles, and attaches to a solid surface. The trophont then forms a cyst that undergoes mitosis and releases theronts (Schäperclaus 1991). Killing the infective theront or the detached trophont with various antiproto-

zoal drugs can stop the reproductive cycle and prevent spread of the disease to other fish (Tucker and Robinson 1990; Schäperclaus 1991).

In February 1993, the U.S. Food and Drug Administration (FDA) defined applications for aquacultural therapeutic use (R. E. Geyer, deputy director, Office of Surveillance and Compliance, FDA, 1993 letter to J. R. MacMillan, Fish Health Section, American Fisheries Society). In May 1993, the Director of the U.S. Fish and Wildlife Service (FWS) issued a memorandum ordering a halt to the use of all nonapproved drugs in fish propagation (J. F. Turner, FWS, 1993 memorandum from the Director to the Service Directorate on compliance with FDA drug regulations for fish propagation) so that the agency would be in compliance with FDA therapeutic regulations. There are presently four options for legal use of chemotherapeutics in the USA: (1) the therapeutic has been approved by the FDA; (2) the therapeutic is the subject of an Investigational New Animal Drug (INAD) exemption; (3) the therapeutic has been determined by the FDA to be of low regulatory priority; (4) the therapeutic is not low regulatory priority, but regulatory action has been deferred pending outcome of research. Currently

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<sup>1</sup> Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Received April 11, 2000; accepted July 15, 2000

only formalin (Formalin-F and Parasite-S), oxy-tetracycline (Terramycin), sulfadimethoxine and ormetoprim (Romet 30), and sulfamerazine (no longer manufactured) are FDA-approved therapeutants; each is approved for specific uses (Greenlees 1997).

Formalin is approved and labeled for use in protozoan parasite control, but this chemical does not have widespread use because of its high cost and problems associated with human handling. Copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and potassium permanganate ( $\text{KMnO}_4$ ) are also used in protozoan parasite control. Neither chemical is approved, although regulatory action has been deferred pending outcome of current research. Copper sulfate is the compound of choice to control ichthyophthiriasis because of its effectiveness (Straus 1993; Schlenk et al. 1998) and low cost, but it can be extremely toxic to fish in water of low alkalinity (Straus and Tucker 1993; Wurts and Perschbacher 1994; Perschbacher and Wurts 1999). As an alternative parasiticide,  $\text{KMnO}_4$  is more expensive to use, but is less toxic to fish (Marking and Bills 1975; Tucker 1987) in low alkalinity waters where use of copper sulfate might be unsafe. To consider a new animal therapeutant for approval, the FDA requires data on efficacy, environmental risk, human food safety, product chemistry, mammalian toxicology, and target animal safety. The current research was designed to satisfy a portion of the efficacy data requirement for  $\text{KMnO}_4$ .

The objective of the present study was to evaluate the acute toxicity of  $\text{KMnO}_4$  to free-swimming *I. multifiliis* theronts and to determine the single-dose concentration necessary to prevent an initial infestation of *I. multifiliis* on juvenile channel catfish and juvenile blue tilapia *Tilapia aurea* in well water. The present study also compared the susceptibility of juvenile channel catfish and blue tilapia to *I. multifiliis*. This information will be useful in formulating safe treatment rates for channel catfish and tilapia culture.

### Methods

A local strain of *I. multifiliis* was obtained, and cultures were maintained by serial infestation on fingerling channel catfish (75–100 g). The fish were held at room temperature (23°C) in a static 38-L aquarium filled with 30 L of well water; the aquarium was fitted with an outside biological filter containing pea gravel. Mature trophonts were allowed to dislodge from the fish and were placed into a small beaker containing water from the aquarium. The trophonts were allowed to adhere

to the beaker for 1 h and were then gently rinsed with well water to remove organic matter. Trophonts were then incubated at room temperature for 24 h to allow for mitotic division. Theront concentrations were estimated by pipetting 5- $\mu\text{L}$  droplets of the theront suspension onto a glass slide and counting the organisms (40 $\times$  magnification); the mean count in 10 droplets was extrapolated to determine the final concentrations (Schlenk et al. 1998).

An in vitro study was conducted to determine the acute toxicity of  $\text{KMnO}_4$  to *I. multifiliis* theronts. Approximately 200 theronts in well water were placed in each well of a 96-well microtiter plate (Falcon 3915, nontissue culture treated) and exposed (at 23°C) to concentrations of  $\text{KMnO}_4$  ranging from 0.1 to 1 mg/L (in 0.1-mg/L increments). Acute toxicity was determined by microscopic examination of each well at various intervals up to 4 h after treatment. Mortality of the parasite was assessed by the absence of motility. Unexposed controls were included with each replication ( $N = 3$ ).

Several in vivo studies were conducted to estimate the effective concentration needed to prevent the initial infestation of *I. multifiliis* theronts in channel catfish and blue tilapia. Treatment containers were 3.8-L glass jars containing 1 L of well water and one fish; after 24 h the water level was increased to 3 L. Water quality was monitored daily with a Hach kit (model FF-1A), and water was exchanged when water quality deteriorated (i.e., increased levels of total ammonia nitrogen and nitrite); an air stone in each container maintained dissolved oxygen levels at more than 75% saturation. In the first study, juvenile channel catfish ( $2.7 \pm 0.4$  g, mean  $\pm$  SD) were exposed to 10,000 theronts/L followed by immediate treatment with 0.25–1.50 mg  $\text{KMnO}_4$ /L (in 0.25 mg/L increments; concentrations were nominal). Unexposed controls were included with each replication ( $N = 3$ ). The study was terminated when control fish developed mature *I. multifiliis* trophonts. In the second study, juvenile blue tilapia ( $2.5 \pm 0.3$  g) were exposed and treated in the same manner as the channel catfish.

An additional experiment was performed to compare species sensitivity of channel catfish and blue tilapia. Juvenile channel catfish ( $3.9 \pm 1.2$  g) and juvenile blue tilapia ( $3.3 \pm 0.5$  g) were exposed to 10,000 theronts/L (as described above), but without any  $\text{KMnO}_4$  treatment; three replications were carried out for each species. The study

TABLE 1.—Mortality of *Ichthyophthirius multifiliis* theronts exposed to potassium permanganate in vitro ( $N = 3$ ). Doses of 0.5 mg  $\text{KMnO}_4/\text{L}$  or less had no effect.

Final concentration (mg/L)	Percent mortality						
	15 min	30 min	45 min	1 h	2 h	3 h	4 h
0.6	0	0	0	0	1	1	1
0.7	0	5	10	10	10	10	10
0.8	0	70	70	70	70	70	70
0.9	0	90	95	95	95	95	95
1.0	0	95	>99	>99	>99	>99	>99

was terminated when mature *I. multifiliis* trophonts were visible.

At the conclusion of all three studies, trophonts on the entire body surface were counted visually and all gill arches were microscopically examined for the presence of trophonts. Temperatures were monitored daily during the channel catfish ( $18 \pm 1^\circ\text{C}$ ), blue tilapia ( $17 \pm 2^\circ\text{C}$ ), and the species sensitivity ( $18 \pm 0.5^\circ\text{C}$ ) studies. Total alkalinity and total hardness (APHA et al. 1998) concentrations (215.5 and 74.6 mg/L as  $\text{CaCO}_3$ , respectively) and pH ( $8.6 \pm 0.1$ ) were also measured.

Well water was analyzed for nonpurgeable organic carbon with a Tekmar-Dohrman high-temperature total organic carbon analyzer (model DC-190). Potassium permanganate demand of the well water used during the studies was determined according to the method of Engstrom-Heg (1971).

An estimate of LC50 (the concentration lethal to 50% of test animals) was calculated at 4 h for the *I. multifiliis* theront acute toxicity experiment using log-probit analysis (SAS Institute, Cary, North Carolina) with three replications per data point.

**Results and Discussion**

Exposure of *I. multifiliis* theronts to 0.9 mg  $\text{KMnO}_4/\text{L}$  resulted in more than 95% mortality by 4 h using well water as the media (Table 1). The 4-h LC50 value was estimated to be 0.77 mg/L

TABLE 2.—Number of trophonts on the body surface and gills (in parentheses) of channel catfish on day 7. Gills were not microscopically observed for fish in the control and 0.25 mg/L treatments. No trophonts were found during examination of fish exposed to 1.0 mg or more  $\text{KMnO}_4/\text{L}$ .

Treatment (mg/L)	Number of trophonts on body (and gills)		
	Replicate 1	Replicate 2	Replicate 3
Control	>100	75–100	75–100
0.25	4	4	11
0.50	4 (3)	1 (0)	0 (0)
0.75	1 (0)	0 (0)	0 (0)

(95% confidence interval = 0.76–0.78). Doses of 0.5 mg or less  $\text{KMnO}_4/\text{L}$  had no effect on *I. multifiliis* theront survival through 4 h; however, theronts were noted to be moving faster than controls in doses of 0.3 mg/L or more. Many theronts in doses of 0.7 mg or more  $\text{KMnO}_4/\text{L}$  had a more spherical or swollen appearance by 30 min, whereas other theronts in the same wells were moving faster; theronts exposed to 0.6 mg  $\text{KMnO}_4/\text{L}$  did not have this appearance until 4 h. The spherical or swollen appearance was a precursor to cell lysis. Upon determination of  $\text{KMnO}_4$  toxicity to the theronts, a range of doses was determined for use in the juvenile channel catfish and blue tilapia infestation studies.

Preliminary studies indicated that the infective dose of *I. multifiliis* theronts used by Straus (1993) and Schlenk et al. (1998) did not produce a consistent and thorough infestation in the control juvenile channel catfish. This may have been due to strain differences in *I. multifiliis* (Nigrelli et al. 1976; Dickerson et al. 1993). Therefore, *I. multifiliis* theronts from a single source were used for the entire study, and the best results were obtained with a dose of 10,000 theronts/L.

In the study of efficacy of  $\text{KMnO}_4$  in preventing *I. multifiliis* infestation of juvenile channel catfish, surficial infestation was observed in control fish 6 d after exposure at  $18 \pm 1^\circ\text{C}$ ; gills were microscopically examined on day 7 for mature trophonts (Table 2). Trophonts on the controls were randomly attached on the entire body surface. No trophonts were found during examination of fish exposed to 1.0 mg or more  $\text{KMnO}_4/\text{L}$ . Treatment with lower concentrations of  $\text{KMnO}_4$  drastically reduced the number of mature trophonts present on the fish.

In juvenile blue tilapia, *I. multifiliis* infestation was observed in control fish 8 d after exposure at  $17 \pm 2^\circ\text{C}$ ; gills were examined on day 9 for mature trophonts (Table 3). The lowest dose of  $\text{KMnO}_4$  that prevented infestation was 0.5 mg/L. The greater length of time to observe infestation on the con-

TABLE 3.—Number of trophonts on the body surface and gills (in parentheses) of blue tilapia on day 9. No trophonts were found during examination of fish exposed to 0.5 mg or more of  $\text{KMnO}_4/\text{L}$ .

Treatment (mg/L)	Number of trophonts on body (and gills)		
	Replicate 1	Replicate 2	Replicate 3
Control	6 (0)	15 (1)	30 (0)
0.25	0 (0)	0 (0)	1 (0)

trots was attributed to cooler temperatures in the laboratory. Trophonts on the control fish were observed only on the head and fins. Fewer trophonts were observed on control blue tilapia (Table 3) than on the control channel catfish (Table 2).

Because of the difference in the numbers of trophonts that developed on the control channel catfish and blue tilapia, a further experiment was performed to confirm these observations by exposing juvenile channel catfish and blue tilapia to 10,000 theronts/L to determine the ability of *I. multifiliis* to initially infest each species. Infestation was observed 5 d after exposure at  $18 \pm 0.5^\circ\text{C}$ , and gills were examined on day 6 for mature trophonts (Table 4). The results showed that channel catfish were infested with 33 times more trophonts than the blue tilapia at the termination of the study. This discrepancy may be due to physiological differences between the species (scales do not provide a good surface for theronts to attach) or innate susceptibility (slime coat properties of the blue tilapia may impede theront attachment).

A temperature of  $18^\circ\text{C}$  was chosen because this is a reliable temperature to grow *I. multifiliis* in our laboratories. Temperature plays an important role in the development of *I. multifiliis* infestations. At  $21\text{--}24^\circ\text{C}$ , the life cycle takes 3–4 d, but it takes 10–14 d at  $15^\circ\text{C}$  and up to 5 weeks at  $10^\circ\text{C}$  (Meyer 1974). Channel catfish tolerate a wide range of temperatures; production ponds may ice over in winter and water temperatures may reach  $35^\circ\text{C}$  in summer. Low or fluctuating water temperatures have a detrimental effect on the immune response of channel catfish (Collins et al. 1976). Tilapias are tolerant to high water temperatures, prefer  $26\text{--}32^\circ\text{C}$ , stop feeding below  $15^\circ\text{C}$ , and die at temperatures below  $12^\circ\text{C}$ ; however, the blue tilapia appears to be the most cold tolerant of the tilapia species cultured in the USA (Shepard and Bromage 1988). The temperature used during the present study is at the lower limit of the blue tilapia's range and could therefore cause some stress to the fish.

TABLE 4.—Number of trophonts on the body surface and gills (in parentheses) of channel catfish and blue tilapia on day 6 of the two-species study to determine the ability of *I. multifiliis* to initially infest each species when exposed to 10,000 theronts/L; no potassium permanganate was used.

Fish	Number of trophonts on body (and gills)		
	Replicate 1	Replicate 2	Replicate 3
Channel catfish	20 (10)	20 (9)	38 (3)
Blue tilapia	1 (1)	1 (0)	0 (0)

Potassium permanganate is one of the most widely used inorganic chemicals in the world and much information is available on its chemistry, manufacture, and various uses (Duncan 1978). It has been used in the past in aquaculture but, as mentioned previously, is not currently approved by the U.S. Environmental Protection Agency or the FDA. Because  $\text{KMnO}_4$  is a strong oxidizer, its effectiveness is dictated by the amount of easily oxidizable material in the water (Marking and Bills 1975). A spectrophotometric method for measuring the  $\text{KMnO}_4$  demand of waters was developed by Engstrom-Heg (1971); Boyd (1979) described a 15-min visual method that gave similar results.

Tucker and Boyd (1977) questioned the general effectiveness of  $\text{KMnO}_4$  applications of 2–4 mg/L (the standard treatment for bacterial infection of fish at the time), based on in vitro tests with waterborne bacteria, and described relationships between  $\text{KMnO}_4$  treatment and water quality. Wellborn (1979) described recommendations for using  $\text{KMnO}_4$  for channel catfish in controlling external protozoan parasites, monogenetic trematodes, and external fungal and bacterial infections. Treatment recommendations varied from 2 to 8 mg/L as an indefinite pond treatment, and the author suggested that the reddish color imparted by the permanganate radical should persist for 12 h or retreatment would be necessary. Jee and Plumb (1981) developed  $\text{KMnO}_4$  treatment rates for ponds containing fish with external *Flexibacter columnaris* infections and concluded that the treatment rate should total 4 mg/L plus the amount indicated from the 15-min  $\text{KMnO}_4$  demand measurement.

Tucker (1984) found that pond application of  $\text{KMnO}_4$  at rates recommended by Jee and Plumb (1981) to treat external bacterial or protozoan diseases of channel catfish would seldom have resulted in an effective treatment and suggested that a 180-min  $\text{KMnO}_4$  demand measurement should be considered as a minimum treatment rate. Tuck-

er's (1984) study suggested that  $\text{KMnO}_4$  preferentially oxidized soluble organic matter in the initial stages of treatment; he found soluble organic matter to be a highly variable proportion of the total organic matter in the pond waters studied. Tucker (1989) further refined this information and proposed a procedure to multiply the 15-min  $\text{KMnO}_4$  demand measurement of pond water by 2.5 to obtain an estimate of the total amount of  $\text{KMnO}_4$  needed to treat the pond.

Nonpurgeable organic carbon content was determined for the well water, and ranged from 0.3 to 0.9 mg/L. Potassium permanganate demand for the well water used during the studies was found to be 0.25 mg/L. According to the 15-min  $\text{KMnO}_4$  demand method proposed by Tucker (1989), the treatment rate suggested for this water would be 0.625 mg  $\text{KMnO}_4$ /L, which is similar to the effective treatment rate determined in our study. Culture ponds rarely have such low organic carbon content. As an example, Tucker (1984) reported the chemical oxygen demand (COD) of pond water samples to be 21–112 mg/L; COD is a commonly used indicator of the organic content of water (Boyd 1979).

The results of our study do not represent  $\text{KMnO}_4$  application rates that will be useful in treating ichthyophthiriasis under field conditions. Two factors will strongly influence effective treatment protocols in the field. First,  $\text{KMnO}_4$  is detoxified by reactions with easily oxidizable substances in the water; the rate of detoxification is dependent on the concentration of these substances and will vary for each pond. Detoxification leaves  $\text{KMnO}_4$  ineffective as a parasiticide. Second, multiple applications are needed for an effective treatment because temperature dictates the duration of the life cycle of *I. multifiliis*. Mitchell (1991) describes treatment regimes ranging from daily to every fourth or fifth day until the infestation is halted.

Future studies will investigate the effect of low concentrations of  $\text{KMnO}_4$  on *I. multifiliis* theront attachment to the host; perhaps  $\text{KMnO}_4$  stimulates secretion of mucus and does not allow the free-swimming theronts to attach. Ongoing research will focus on the efficacy of  $\text{KMnO}_4$  in the treatment of established ichthyophthiriasis in channel catfish.

In summary, exposure of *I. multifiliis* theronts to 0.9 mg  $\text{KMnO}_4$ /L for 4 h resulted in greater than 95% mortality with well water as the media; the estimated 4-h  $\text{LC}_{50}$  was 0.77 mg  $\text{KMnO}_4$ /L. The lowest effective doses of potassium permanganate that prevented initial infestation of *I. multifiliis* on

juvenile channel catfish and blue tilapia in well water were 1.0 and 0.5 mg/L, respectively.

### Acknowledgments

This research was supported in part by a grant from the International Association of Fish and Wildlife Agencies. Special thanks to Jan Simpson for technical help throughout the studies. Ahmed Darwish, Ken Davis, and Craig Tucker provided critical reviews of the manuscript.

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