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Comparison of the Acute Toxicity of Potassium Permanganate to Hybrid Striped Bass in Well Water and Diluted Well Water

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Abstract.—The acute toxicity of potassium permanganate ($KMnO_4$) to many species of fish has been studied; however, there is no data for hybrid striped bass (female white bass *Morone chrysops* × male striped bass *M. saxatilis*). Hybrid striped bass juveniles were exposed to $KMnO_4$ in a series of static toxicity tests in waters composed of filtered (75 μm) well water or filtered well water diluted with deionized water. Estimates of mean 24-h LC50 (median lethal concentration) were 4.47 and 2.97 mg/L $KMnO_4$ in waters having total alkalinities of 109 and 213 mg/L (as $CaCO_3$), respectively. These LC50 values were significantly different ($P < 0.05$) by the Student-Newman-Keuls means comparison test. Data from the present study demonstrate that the acute toxicity of $KMnO_4$ to hybrid striped bass juveniles is lower in waters of lower total alkalinity and total hardness. The results indicate that hybrid striped bass juveniles are tolerant to therapeutically relevant concentrations of $KMnO_4$ and such treatments in low alkalinity waters are safer than treatments at higher alkalinity.

Hybrid striped bass, also known as sunshine bass (female white bass *Morone chrysops* × male striped bass *M. saxatilis*), culture is a growing section of U.S. aquaculture production. The initial hybrid striped bass crossing was accomplished in 1965 with the production of palmetto bass (female white bass × male striped bass; Bishop 1968). As aquaculture continues to expand and find new niches and markets, hybrid striped bass production has become an important part of this expansion. In a survey of state agriculture extension programs and 52 major producers, U.S. production of hybrid striped bass grew from 405,000 lb. in 1987 to 10,903,000 lb. in 2001 (Jim Carlberg, Kent SeaTech Corporation, personal communication).

Potassium permanganate ($KMnO_4$) is a widely used inorganic chemical worldwide and much information is available on its chemistry, manufacture, and uses (Duncan 1978). In aquaculture, $KMnO_4$ has been used as a water/bath treatment for protozoan parasites in commercial and ornamental fish culture; however, it is not approved by the U.S. Food and Drug Administration for therapeutic use in aquaculture. Regulatory action on the use of $KMnO_4$ has been deferred pending the outcome of ongoing research. Straus and Griffin (2002) describe the four legal options for using aquatic therapeutants in the United States.

Potassium permanganate is a strong oxidizing agent that is consumed in reactions with dissolved and particulate organic matter present in fish culture waters (Tucker 1984). The effectiveness of $KMnO_4$ as a parasiticide is dictated by the amount of such easily oxidizable material (Marking and Bills 1975). Hughes (1975) recommended treatment of striped bass production ponds with $KMnO_4$ prior to harvest to reduce parasitic infestations. Other than production ponds, $KMnO_4$ has also been used as a prophylaxis against parasites on hybrid striped bass fingerlings in raceway environments with very low concentrations of oxidizable substances.

The acute toxicity of $KMnO_4$ to various fish species has been studied (Marking and Bills 1975; Tucker 1987), including several studies investigating striped bass (Wellborn 1969; Hughes 1971; Bills et al. 1993; Reardon and Harrell 1994); however, there are no data for hybrid striped bass. The present study determined the acute toxicity of $KMnO_4$ to hybrid striped bass in waters composed of filtered well water or filtered well water diluted with deionized water to adjust its alkalinity and hardness. The study was also designed to determine safe levels of application in these waters.

Materials and Methods

Hybrid striped bass juveniles were obtained from Keo Fish Farm (Keo, Arkansas, USA) and acclimated to 22 ± 1 C and a 12-h light/dark cycle. Mean (± SD) fish weight and total length were 9.4 ± 2.0 g and 9.9 ± 0.6 cm, respectively. Fish were offered a commercial 45% protein, floating salmon diet (Silver Cup Fish Feed, Murray, Utah, USA) every day to maintain body weight during their 2-wk acclimation period. Fish were not fed for 48 h prior to or during toxicity tests.

Tests were conducted in 57-L glass aquaria equipped with air stones to maintain dissolved oxygen levels greater than 75% saturation. Each aquarium contained seven fish (Solbé 1993). The study was conducted under static test conditions (APHA 1998) in a completely randomized design with three replications per treatment.

Aquaria contained 40 L of filtered well water or filtered well water diluted 50% with deionized water; well water was filtered through a 75-μM canister filter. The water was analyzed for total organic carbon (TOC; high temperature combustion method, APHA 1998) with a Dohrman 80, Total Organic Carbon Analyzer (Cincinnati, Ohio, USA). A visual 15-min potassium permanganate demand (PPD) was determined for each dilution (Boyd 1979).

Fish were exposed to a control (no $KMnO_4$ added) and seven concentrations of $KMnO_4$ (0.5, 0.8, 1.1, 1.7, 2.5, 3.8 and 5.7 mg/L). The $KMnO_4$ used was ACS reagent grade $KMnO_4$ (34.75% Mn) purchased from the Sigma Chemical Co. (St. Louis, Missouri, USA). A stock solution was prepared immediately prior to treatment and used to dose the tanks. The concentrations used were chosen to give from 0% to 100% mortality of test animals within 24 h based on the results of preliminary studies. The following water quality characteristics were monitored daily: total ammonia-nitrogen and nitrite-nitrogen (Hach kit FF-1A, Loveland, Colorado, USA); pH (Orion Research 720A Meter, Boston, Massachusetts, USA); dissolved oxygen and temperature (YSI Model 95, Yellow Springs, Ohio, USA). Total alkalinity (standard acid titration method, APHA 1998), total hardness (EDTA titration method, APHA 1998) and pH were measured at the beginning of the study.

Immediately after each $KMnO_4$ treatment was thoroughly dispersed (~ 15 sec), a 15-mL water sample was taken, filtered through a 0.45-μm nylon filter and then acidified with nitric acid. Soluble Mn^{++} concentrations were measured (APHA 1998) with a Perkin-Elmer Optima 2000 DV Inductively Coupled Plasma (ICP) Optical Emission Spectrometer (Norwalk, Connecticut, USA).

The LC50 values (median lethal concentration) and associated 95% confidence intervals for each treatment were determined by the Trimmed Spearman-Karber method (Hamilton et al. 1977) by using the mean fish mortality. Software for the Trimmed Spearman-Karber method (version 1.5) was obtained from the National Exposure Research Laboratory of the U.S. Environmental Protection Agency (http://www.epa.gov/nerleerd). The LC50 values were analyzed by the General Linear Model procedure followed by the Student-Newman-Keuls means comparison test using SAS (SAS Institute, Cary, North Carolina, USA) on a personal computer. A level of $P < 0.05$ was used to conclude a significant difference among means.

Results and Discussion

The present study determined the 24-h LC50 values for $KMnO_4$ to hybrid striped bass juveniles in a series of static toxicity tests; fish were observed for an additional 72 h and no mortalities occurred. Tanks treated with higher concentrations of $KMnO_4$ exhibited high rates of mortality within 12 h. Marking and Bills (1975) de-

TABLE 1. *Water characteristics and 24-h LC50 values (and 95% confidence intervals) for the acute toxicity of potassium permanganate to hybrid striped bass.*

Water	100% filtered well water	50% filtered well water
Initial pH[a]	8.66 ± 0.01	8.18 ± 0.12
Initial Total Alkalinity[a] (mg/L $CaCO_3$)	213.40 ± 3.88	109.13 ± 0.48
Initial Total Hardness[a] (m/L $CaCO_3$)	101.60 ± 2.50	55.06 ± 2.01
Initial Total Organic Carbon[a] (mg/L TOC)	14.63 ± 1.22	8.85 ± 0.64
Water Temperature[a] (C)	22.2 ± 0.3	22.2 ± 0.3
24-h LC50 (mg/L $KMnO_4$)	2.97 (2.75–3.20)	4.47 (4.24–4.72)

[a] Mean ± SD; $N = 3$.

termined the toxicity of $KMnO_4$ to ten species of fish and reported that toxicity changed little after 24 h.

Total ammonia-nitrogen ranged from 0.7–1.2 mg/L (calculated un-ionized ammonia was 0.05–0.21 mg/L) and nitrite-nitrogen ranged from 0.2–0.6 mg/L during the study. Water quality in the study was acceptable for intensive striped bass culture as described by Nicholson et al. (1990). Rapid opercular movement, lethargy and loss of equilibrium were noticed in many fish prior to death in $KMnO_4$-treated waters. No fish died in the control tanks.

Hybrid striped bass mortality rates rapidly decreased with decreased concentrations of $KMnO_4$. In the 100% filtered well water, the 5.7 and 3.8 mg/L $KMnO_4$ treatments resulted in 100% and 95% mortality, respectively, while treatment with 2.5 and 1.7 mg/L $KMnO_4$ resulted in 0.05% and 9% mortality, respectively. Waters of lower alkalinity and hardness demonstrated lower acute toxicity. In the 50% filtered well water, treatment with 5.7 mg/L $KMnO_4$ resulted in 100% mortality, while treatment with 3.8 mg/L $KMnO_4$ resulted in 9% mortality. Estimates of 24-h LC50 values are presented in Table 1 along with initial pH, total alkalinity, total hardness, total organic carbon, and water temperature for the 100% and 50% filtered well water. The LC50 values were significantly different ($P < 0.05$) by the Student-Newman-Keuls means comparison test. Marking and Bills (1975) also reported that the acute toxicity of $KMnO_4$ to rainbow trout *Oncorhynchus mykiss* and channel catfish *Ictalurus punctatus* was greatest in very hard waters, and at 96 h these toxicity differences were also statistically significant. In the study of Marking and Bills (1975), rainbow trout were exposed in waters having identical pHs while channel catfish waters were not pH stabilized, which may have contributed to the differences in toxicity.

Hughes (1971) estimated the 24-h LC50 of $KMnO_4$ to be 5.0 mg/L (96-h LC50 was 4.0 mg/L) for 1-mo-old striped bass fingerlings (3.5–5.1 cm) when total alkalinity was 32 mg/L (approximate), total hardness was 36 mg/L (approximate), pH was 7.6 (approximate), and water temperature was 21 C (water chemistry was determined from water reconstituted according to Hughes 1971). Wellborn (1969) estimated the 96-h LC50 to be 2.5 mg/L $KMnO_4$ for 2.7-g (6-cm total length) striped bass fingerlings in dechlorinated tap water when total alkalinity, total hardness, pH, and water temperature averaged 64 mg/L, 35 mg/L, 8.2, and 21 C, respectively. Wellborn (1969) and Hughes (1971) concluded that striped bass are more sensitive to chemicals than are most freshwater fishes. Bills et al. (1993) determined the 24 and 96-h LC50 values for $KMnO_4$ for 1-g juvenile striped bass to be 3.52 and 1.58 mg/L, respectively, in reconstituted soft water (hardness = 40–48 mg/L as $CaCO_3$, water temperature = 17 C). Reardon and Harrell (1994) estimated the 96-h LC50 value for 1-mo-old striped bass juveniles in ground water (alkalinity = 93 mg/L, hardness = 54 mg/L, pH = 9.1, temp = 23 C) to be 0.96 mg/L $KMnO_4$. The 96-h LC50 values Reardon and Harrell (1994) reported for juvenile striped bass indicated greater $KMnO_4$ toxicity than those cited by Hughes (1971) and Wellborn (1969); differences were contributed to various water quality characteristics and organic content. The high pH of their test water may have resulted in higher toxicity as compared to the other studies. In all of these studies, fish were smaller and alkalinity, hardness, or pHs were lower than those of the present study; therefore, a higher LC50 value was expected in the present study.

In the present study, the highest dose without any mortality was 1.1 mg/L $KMnO_4$ in the 100% filtered well water and 2.5 mg/L $KMnO_4$ in the 50% filtered well water. Bills et al. (1993) indicated that juvenile striped bass can tolerate up to nearly 10 mg/L for 6 h. This margin of safety is obviously dependent on the water chemistry, water quality, and water temperature. The water characteristics reported by Bills et al. (1993) are different than those in the present study.

The lethal effect of exposure to $KMnO_4$ appears to be unrelated to metallic manganese toxicity, but rather the strong oxidative action of the MnO_4^- ion (Griffin et al. 2002). In a study of the effects of varying salinities on the toxicity of $KMnO_4$ to juvenile striped bass, Reardon and Harrell (1994) reported that $KMnO_4$ caused structural gill damage and that tolerance to $KMnO_4$ was lowest in freshwater where the osmotic or ionic imbalance was greatest. While studying the histological effects of $KMnO_4$ exposure on channel catfish, Darwish et al. (2002) noted similar gill lesions, necrosis, spongiosis, epithelial hyperplasia, hypertrophy, and lamellar fusion as noted by Cruz and Tamse (1986) in milkfish *Chanos chanos* Forsskal, by Dureza (1988) in Nile tilapia *Tilapia nilotica*, and by Das and Kaviraj (1994) in common carp *Cyprinus carpio*. Cruz and Tamse (1986) reported that the intensity of tissue damage increased with increased concentration. Gills were found to return to normal in about 6–8 d after exposure (Darwish et al. 2002); Cruz and Tamse (1986) reported that sublethal pathological changes in tissue appear to be temporary, and that fish recover when returned to a $KMnO_4$-free environment. Griffin et al. (2002) suggested that changes observed in plasma chloride, osmolality, and hematocrit of whole blood were consistent with a significant loss of electrolytes. Cardiac function can be compromised by a significant loss of plasma electrolytes, a situation that may lead to death (Wood 1989).

The recommended dose of $KMnO_4$ as an aquaculture therapeutant (bath treatment) is based on the 15-min visual PPD (Boyd 1979). In this study, the 15-min visual PPD was estimated to be 0.75 mg/L in the 100% filtered well water and 0.50 mg/L in the 50% filtered well water. Tucker (1987) found the 15-min PPD was highly correlated with the toxicity of $KMnO_4$ to juvenile channel catfish that were exposed in various pond waters. The TOC content (Table 1) is a measure of easily oxidizable substances in the water. In the present study, the TOC was 14.63 mg/L in the 100% filtered well water and 8.85 mg/L in the 50% filtered well water. As with the other measured water characteristics, TOC was less in the diluted well water. The 15-min PPD and the TOC content are reported here to better define the composition of the test waters.

Soluble Mn^{++} was defined as the amount of Mn^{++} that would pass through a 0.45-μm nylon filter. After the sample was filtered, it was then acidified with nitric acid prior to measurement by ICP. The measured soluble Mn^{++} divided by the calculated Mn^{++} concentration indicates the amount of recovery in each dilution. Note that the amount of soluble Mn^{++} in the 100% filtered well water averaged about 85% of the total calculated Mn^{++} concentration while soluble Mn^{++} in the 50% filtered well water averaged about 84% recovery, shortly after treatment (Table 2); this is probably due to

TABLE 2. *Treatment rates for potassium permanganate ($KMnO_4$), calculated and actual soluble Mn concentrations, and percent Mn recovered immediately after addition to tanks containing hybrid striped bass.*

Percent filtered well water	$KMnO_4$ treatment (mg/L)	Calculated Mn (mg/L)	Measured soluble Mn (mg/L)	Measured/Calculated Mn (%)
100%	0	0	−0.01 ± 0.00[a]	
	0.5	0.17	0.14 ± 0.02	78.9
	0.8	0.28	0.26 ± 0.04	92.7
	1.1	0.38	0.32 ± 0.01	82.8
	1.7	0.59	0.50 ± 0.01	84.8
	2.5	0.87	0.78 ± 0.04	89.8
	3.8	1.32	1.10 ± 0.08	82.9
	5.7	1.98	1.66 ± 0.08	83.9
50%	0	0	−0.01 ± 0.00	
	0.5	0.17	0.13 ± 0.01	76.7
	0.8	0.28	0.24 ± 0.01	84.5
	1.1	0.38	0.32 ± 0.03	84.1
	1.7	0.59	0.50 ± 0.02	83.9
	2.5	0.87	0.76 ± 0.02	88.0
	3.8	1.32	1.17 ± 0.03	88.9
	5.7	1.98	1.68 ± 0.03	84.9

[a] Determined by Inductively Coupled Plasma Optical Emission Spectrometry. Mean ± SD; $N = 3$.

binding to the aquarium walls, fish mucus, or other suspended solids.

The mechanism of action for $KMnO_4$ to aquatic animals is not known. The present study and the study of Marking and Bills (1975) reported statistically higher acute toxicity values in waters of greater alkalinity or hardness; however, a literature search indicated that the mode of toxicity of $KMnO_4$ has not been studied. One possible scenario is that the ion concentration, and therefore the redox potential of the filtered well water would be higher than the 50% filtered well water, and this would be a more oxidizing environment. Future research should address the toxic action of $KMnO_4$ and other oxidizing agents used in aquaculture.

In summary, $KMnO_4$ has been the compound of choice for some producers as a therapeutant for ectoparasites because of its effectiveness and reduced toxicity in waters with low alkalinity and hardness. This information will help extension personnel formulate safer and more effective application rates for $KMnO_4$ in hybrid striped bass production. The results of the present study suggest that a safe application rate of $KMnO_4$ for hybrid striped bass juveniles is 2.5 mg/L in low alkalinity (109 mg/L as $CaCO_3$) waters and 1.1 mg/L in high alkalinity (213 mg/L as $CaCO_3$) waters; these results are applicable to clean water systems with low $KMnO_4$ demands, and do not represent rates that will be useful under field conditions since other factors influence effective treatment protocols in the field. As mentioned previously, $KMnO_4$ is not approved by the U.S. Food and Drug Administration for therapeutic use in aquaculture; however, regulatory action on the use of $KMnO_4$ has been deferred pending the outcome of ongoing research.

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