

Effect of Exposure to Potassium Permanganate on Stress Indicators in Channel Catfish *Ictalurus punctatus*

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Abstract

Juvenile channel catfish *Ictalurus punctatus* were exposed to 1× (0.44 mg/L), 3× (1.32 mg/L), or 5× (2.19 mg/L) the recommended therapeutic concentrations of waterborne potassium permanganate (KMnO₄) for 36 h to determine the toxicity of the chemical. The fish were observed for 14 d after exposure. Gill, liver, and blood samples were collected before exposure, at 12, 24, and 36 h of exposure, and at 48-h intervals for 14 d thereafter. Analysis of homogenized gill tissue showed a transient increase in manganese content that quickly disappeared once exposure was discontinued. Fish exposed to the 3× and 5× concentrations of KMnO₄ experienced 9 and 50.6% mortality, respectively. Plasma cortisol was elevated more than ten-fold at the 5× concentration. Both plasma chloride and osmolality were significantly reduced at the 3× and 5× concentrations but were unchanged at the 1×. Packed cell volumes (PCV) of whole blood rose significantly in response to 3× and 5× concentrations of KMnO₄. Mortality may have been the result of blood electrolyte depletion as indicated by increased PCVs, loss of chloride, and reduced osmolality. All stress indicators measured, except PCV at the 5× concentration, were indistinguishable from unexposed controls within 48 h after exposure was discontinued. At the 1× concentration (the concentration most like that employed in a disease treatment) no changes were observed in any stress indicators measured suggesting that KMnO₄ may be safely used as a disease therapeutant for channel catfish.

Potassium permanganate (KMnO₄) has been used as a therapeutant and prophylactic for fish diseases since 1918 when the first controlled test of its efficacy against myxobacteriosis was performed by Davis (1922). The therapeutic use of KMnO₄ was extended to control invertebrate parasites of fish by Hess (1930) and methods for its application were developed by Kingsbury and Embody (1932). The chemical became popular for treating diseased trout because it could be used in soft waters where other chemicals, such as copper sulfate, were too toxic to use. When channel catfish *Ictalurus punctatus* culture became established in the United States, KMnO₄ was found to be effective for control of some warm-water bacterial, parasitic, or fungal diseases (Wellborn 1985). Users of aquaculture chemicals have been frequently advised to use chemicals sparingly and only when needed to avoid stressing the treated fish

and possibly inducing greater harm than benefit (Wellborn 1985; Tucker and Robinson 1990). A limited amount of information is available about the toxicity of KMnO₄. The acute toxicity of KMnO₄ to channel catfish is greater at lower temperatures, at higher pH, and in harder water (Marking and Bills 1975). Tucker (1987) reported that toxicity to fingerling channel catfish was closely related to the chemical oxygen demand (COD) of the culture water. The 96 h LC₅₀ increased from 4.5 to 17.6 mg/L as the COD increased from 21 to 118 mg/L. While measurement of factors affecting LC₅₀ provides useful information for comparisons with other chemicals, the sublethal effects of exposure to KMnO₄ are more important in evaluating the safety of KMnO₄ for disease control. Little information is available concerning the sublethal effects of exposure to KMnO₄. Schlenk et al. (2000) reported that metallothionein

messenger RNA expression in gills of channel catfish was reduced after 8 wk of exposure to 2 mg KMnO_4/L ; but overall, minimal changes were observed in weight, length, condition index and liver somatic index in catfish exposed to 0.5–2.0 mg KMnO_4/L for 4 wk. Sublethal effects of manganese on carbohydrate metabolism in Mossambique tilapia *Oreochromis mossambicus* (Barnhoorn et al. 1999) and the histopathological response of Milkfish *Chanos chanos* to KMnO_4 (Cruz and Tamse 1986) have been reported but there are no reports of the sublethal effects of KMnO_4 exposure on classical physiological stress indicators in channel catfish (Pickering 1981).

Potassium permanganate is not approved for any use in aquaculture in the United States and the U.S. Food and Drug Administration (FDA) is currently considering approval for its use as a therapeutant for control of waterborne parasitic or fungal diseases of cultured freshwater fishes. The study reported here was done to obtain data needed to establish the margin of safety for use of the chemical, as part of the approval process, by comparing the toxicity to the target species with the effective therapeutic concentration. Standards for testing chemical toxicity are available from FDA and were employed in the tests described here (Anonymous 1999).

Materials and Methods

Experimental Conditions

Channel catfish were produced for the study as described by Griffin et al. (1997). The fish were raised in indoor flow-through tanks supplied with well water to avoid exposures in earthen ponds that contain manganese. The only sources of manganese available to the fish, other than that added during the study, were their food and water supply. Water quality characteristics during the study were as follows: temperature 21.5 ± 0.5 C; pH 7.6 ± 0.2 ; oxygen 7.7 ± 0.2 mg/L; alkalinity 184 ± 3 mg/L (as CaCO_3);

total ammonia 0.91 ± 0.04 mg/L; and unionized ammonia 0.03 ± 0.01 mg/L.

Fish were offered a commercial catfish feed daily at the rate of 2% of body weight, and uneaten feed was removed within 1 h. The manganese content of the feed was 231 ± 18 mg/kg ($N = 7$ samples). Technical grade KMnO_4 used for this study was provided by Carus Chemical Company (Ottawa, Illinois, USA); purity 97%.

Four groups of 50 channel catfish (25 of each sex) weighing 314 ± 52 g were transferred from holding tanks to 735-L cylindrical fiberglass tanks (124×61 cm) and allowed to acclimate to the tanks and the environment for 2 wk before KMnO_4 exposure. Flow rate of water into the experimental tanks was maintained at 6.2 L/min (12 tank volumes/d) and tanks were cleaned twice weekly.

Channel catfish were exposed to 1, 3, or 5 times ($1\times$, $3\times$ or $5\times$) the recommended therapeutic dose of KMnO_4 and for 3 times the recommended treatment time (Anonymous 1999). The recommended concentration for use of KMnO_4 as a therapeutant in channel catfish culture is 2.5 times the 15-min KMnO_4 demand (PPD) of the water to which it is to be applied (Tucker 1989). The recommended treatment time for KMnO_4 is 12 h (Moore et al. 1984; Tucker and Robinson 1990); therefore, the exposure time in this study (3 times the recommended time) was 36 h. The 15-min PPD was determined using the methods of Engstrom-Heg (1971) on water samples taken daily from the tanks of fish during a 14-d acclimation period for the fish. The average PPD during the acclimation period was 0.175 mg KMnO_4/L . The concentrations of KMnO_4 for exposure in this study were calculated accordingly and are presented in Table 1.

Experimental Procedures and Sample Collection

Immediately before beginning KMnO_4 exposures, two fish from each tank (one of each sex as determined by external examination) were removed and a blood sample

TABLE 1. Potassium permanganate ($KMnO_4$) and Manganese (Mn^{+}) in control and treatment tanks expressed as introduced, residual un-reduced, or total concentrations.

KMnO ₄ or Mn ⁺ concentrations ^b	Treatment group ^a			
	Control	1×	3×	5×
Expected concentration of KMnO ₄ introduced during exposure	0	0.44	1.32	2.19
Expected concentration of Mn ⁺ introduced during exposure	0	0.15	0.46	0.76
Residual unreduced Mn ⁺ ± SEM measured ^c at each sampling period (N = 12)	0	0.11 ± 0.04	0.29 ± 0.09	0.55 ± 0.11
Total Mn ⁺ ± SEM on filtered, acidified water samples ^d (N = 24)	0.02 ± 0.01	0.12 ± 0.03	0.43 ± 0.10	0.60 ± 0.11

^a See Methods section for calculations of chemical concentrations based on Potassium Permanganate Demand (PPD).

^b All measurements given as mg/L.

^c Method of Engstrom-Heg (1971).

^d Graphite furnace atomic absorption spectrophotometry.

collected with a heparinized syringe from the caudal sinus. A subsample of blood was transferred to a capillary tube for later determination of packed cell volume. An aliquot of whole blood was diluted 1:10 in calcium-magnesium-phenol red free Hank's balanced salt solution for later total red blood cell counts. The spinal cord was then severed and the second and third gill arches removed from each aspect. Gill arches and liver samples were frozen for later total manganese analysis. Plasma was separated by centrifugation, and stored at -85 C until analyses.

Exposure was initiated by adding a concentrated solution of $KMnO_4$ in deionized water to each tank to bring the concentrations to the specified treatment level (Table 1). Peristaltic pumps delivered potassium permanganate from reservoirs of stock solutions to the tanks to maintain the concentration of each tank throughout the exposure time. The untreated control was dosed similarly with deionized water. Fish were sacrificed and samples collected at 12, 24, and 36 h during the exposure period and at 48-h intervals thereafter for 14 d during recovery. Tanks were observed, mortalities removed and recorded, and flow rates adjusted daily. When fish were removed from

tanks, either for sampling or as mortalities, they were replaced with fin-clipped fish of a similar size. Fin clipping of replacements was done to avoid selecting replacement fish when sampling later in the study and to avoid including replacement fish in mortality counts. Water samples were collected at each sampling period during exposure for total manganese analysis (Griffin et al. 1999). Water samples were also removed to measure residual permanganate by the Engstrom-Heg (1971) method. Fish were not fed for 24 h prior to exposure or during the 36-h exposure period and were returned to the normal feeding regime after the exposure period. The entire procedure was then repeated to give three replications.

Tissue and Plasma Analyses

Gill tissues were freeze-dried in a LabConco Lyph-Lock 6 lyophilizer and then homogenized in a Wiley intermediate mill. Approximate 100-mg samples were accurately weighed and digested in 70% nitric acid in a CEM microwave digestion system (Skelly and Distefano 1988; Van Wyck 1988; CEM Corp. 1994). Digested samples were assayed for manganese content with a Thermo Jarrell Ash (Model Scan 4) graphite furnace atomic absorption spec-

TABLE 2. Manganese (mg/kg dry weight \pm SEM) in homogenized gill tissue and cartilage of channel catfish exposed to $KMnO_4$ for 36 h.

Time	Exposure group ^a			
	Control	1 \times	3 \times	5 \times
Preexposure	26 \pm 2 ^b	18 \pm 5 ^b	23 \pm 13 ^b	40 \pm 17 ^b
Exposed 12 h	13 \pm 1 ^b	364 \pm 159	253 \pm 28	274 \pm 24
Exposed 24 h	14 \pm 5	311 \pm 81 ^c	261 \pm 65 ^c	213 \pm 23
Exposed 36 h	13 \pm 3	311 \pm 42	566 \pm 100 ^c	537 \pm 304 ^b
48 h Post exposure	13 \pm 2 ^b	17 \pm 2 ^b	13 \pm 2 ^b	ND ^d

^a See Methods Section for calculations of chemical concentrations based on Potassium Permanganate Demand (PPD).

^b $N = 4$, otherwise $N = 6$.

^c Values in rows are significantly ($P \leq 0.05$) different from controls by Bonferroni's Means Comparison.

^d No data.

trophotometer (GFAAS). Liver tissue was analyzed for manganese content as previously described (Griffin et al. 1999). Muscle of spiny dogfish *Squalus acanthias* (National Research Council of Canada, Ottawa) with and without added elemental manganese was the certified reference material used for methods validation.

Blood filled capillary tubes were centrifuged on a hematocrit centrifuge and packed cell volumes (PCV) measured. Plasma cortisol was measured by radioimmunoassay using Chiron cortisol RIA kits (Chiron Diagnostics Corporation, Norwood, Massachusetts, USA); plasma glucose was measured using hexokinase determination (Sigma Diagnostics No. 115-A); plasma chloride was measured with a Corning 925 chloride analyzer; plasma osmolality was measured with a Wescor Model 5500 vapor pressure osmometer. Selected plasma samples were diluted with 1% nitric acid and manganese measured with GFAAS. Total erythrocyte counts were performed microscopically with a hemocytometer.

Data were analyzed by analysis of variance and Bonferroni's means comparison (Gulley 1993). Statistical significance was set at $P \leq 0.05$.

Results

The estimated concentrations of $KMnO_4$ and manganese added to the control and experimental tanks and the measured concentrations of residual un-reduced manganese and total manganese in the tanks are presented in Table 1. No mortalities occurred in either the untreated controls or the 1 \times treatment. In the 3 \times treatment cumulative mortalities were 9.3% and were 50.6% in the 5 \times treatment.

Gill-associated manganese increased in the 1 \times and 3 \times treatments after 24-h exposure and in the 3 \times and 5 \times treatments after 36-h exposure (Table 2). Concentrations of manganese returned to levels indistinguishable from untreated controls within 48 h after exposure. Manganese content of liver tissue did not change significantly during the 36-h exposure period or during the 14-d recovery (Control 1.82 mg/kg \pm 0.06 SEM, $N = 42$; Exposed 1.90 mg/kg \pm 0.06 SEM, $N = 26$; Recovery 1.92 mg/kg \pm 0.04 SEM, $N = 56$). Manganese concentrations in selected plasma samples taken before, during, and after exposure were below the lower level of sensitivity of the graphite furnace atomic absorption spectrophotometer (recorded consistently less than 1 μ g/L).

Cortisol concentrations in plasma from

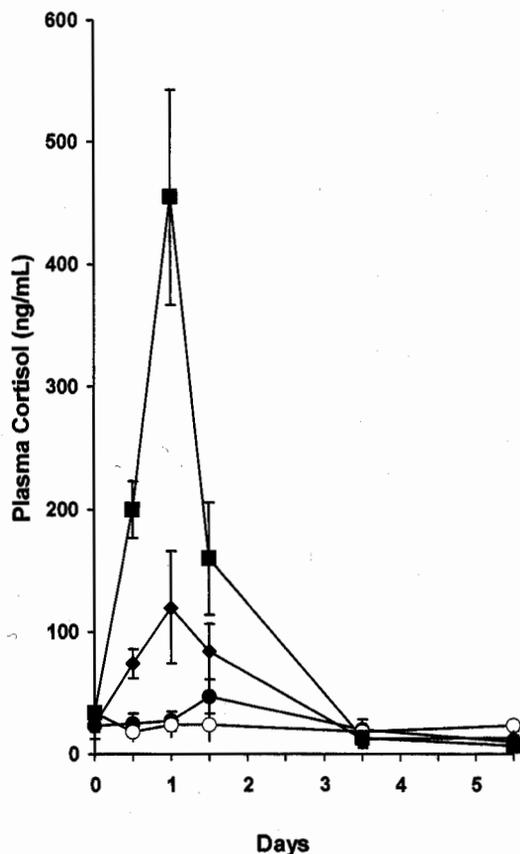


FIGURE 1. Changes in plasma cortisol concentrations of channel catfish exposed to $KMnO_4$ for 36 h; the first 1.5 d on the x axis represent the treatment period, beyond 1.5 d the recovery period is represented. The symbols are: open circles = untreated controls, solid circles = exposure to 0.44 mg/L, solid diamonds = exposure to 1.32 mg/L, solid squares = exposure to 2.19 mg/L. Error bars represent SEM.

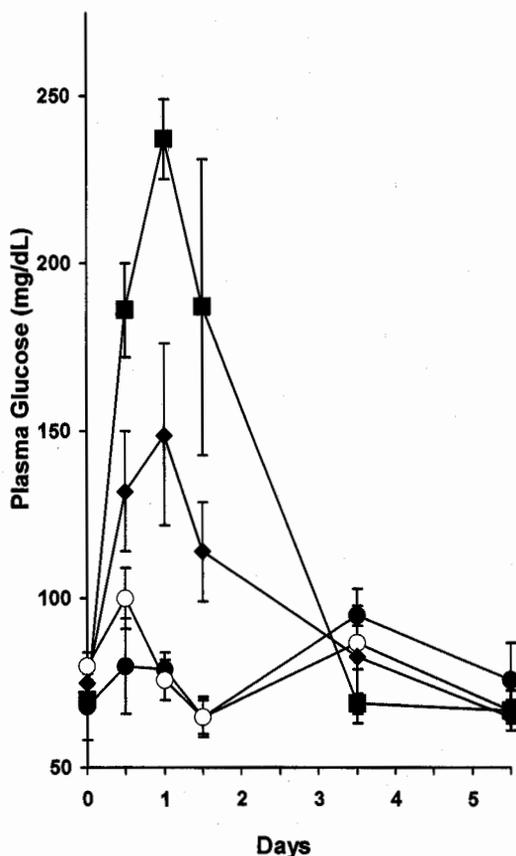


FIGURE 2. Changes in plasma glucose concentrations of channel catfish exposed to $KMnO_4$ for 36 h; the first 1.5 d on the x axis represent the treatment period, beyond 1.5 d the recovery period is represented. The symbols are: open circles = untreated controls, solid circles = exposure to 0.44 mg/L, solid diamonds = exposure to 1.32 mg/L, solid squares = exposure to 2.19 mg/L. Error bars represent SEM.

fish in the 5 \times treatment were significantly higher than controls at 12, 24 and 36 h. Plasma cortisol was elevated in the 3 \times treatment at 12, 24 and 36 h but the difference was not statistically significant. All treatments were indistinguishable from controls within 48 h after exposure (Fig. 1). No significant difference was detected in plasma cortisol levels between controls and the 1 \times or 3 \times treatments.

Plasma glucose concentrations in plasma of fish in the 5 \times treatment were significantly higher than controls at 12, 24 and 36 h of exposure (Fig. 2). The elevations seen

in plasma glucose in the 3 \times treatment at 12, 24 and 36 h were not significantly different from controls. Plasma glucose returned to normal in all groups within 48 h after exposure.

Plasma chloride concentrations were significantly reduced during exposure in the 5 \times treatment at 12, 24 and 36 h of exposure and in the 3 \times treatment at 24 and 36 h of exposure (Fig. 3). Plasma chloride was slightly depressed in the 1 \times treatment but not significantly. Plasma chloride returned to normal in all treatments within 48 h after exposure.

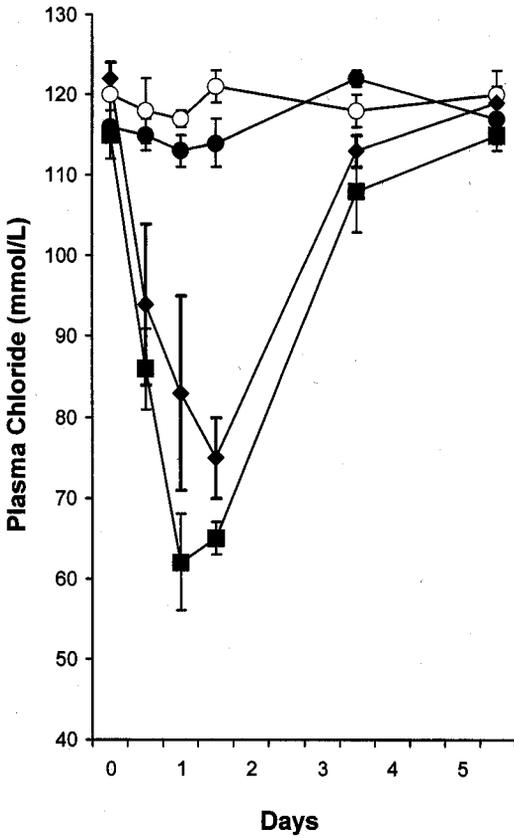


FIGURE 3. Changes in plasma chloride concentrations of channel catfish exposed to $KMnO_4$ for 36 h; the first 1.5 d on the x axis represent the treatment period, beyond 1.5 d the recovery period is represented. The symbols are: open circles = untreated controls, solid circles = exposure to 0.44 mg/L, solid diamonds = exposure to 1.33 mg/L, solid squares = exposure to 2.19 mg/L. Error bars represent SEM.

Plasma osmolality was significantly reduced during exposure in the 3 \times and 5 \times treatments at 24 and 36 h of exposure (Fig. 4). The depression in osmolality seen in the 3 \times and 5 \times treatments at 12 h of exposure and in the 1 \times treatment at 24 and 36 h of exposure was not significant. Plasma osmolality returned to normal in all treatments within 48 h after exposure.

The packed cell volume (PCV) of whole blood was significantly higher than controls in the 3 \times and 5 \times treatments at 24 h of exposure (Fig. 5) and in the 5 \times treatment at 36 h of exposure. The PCV values of

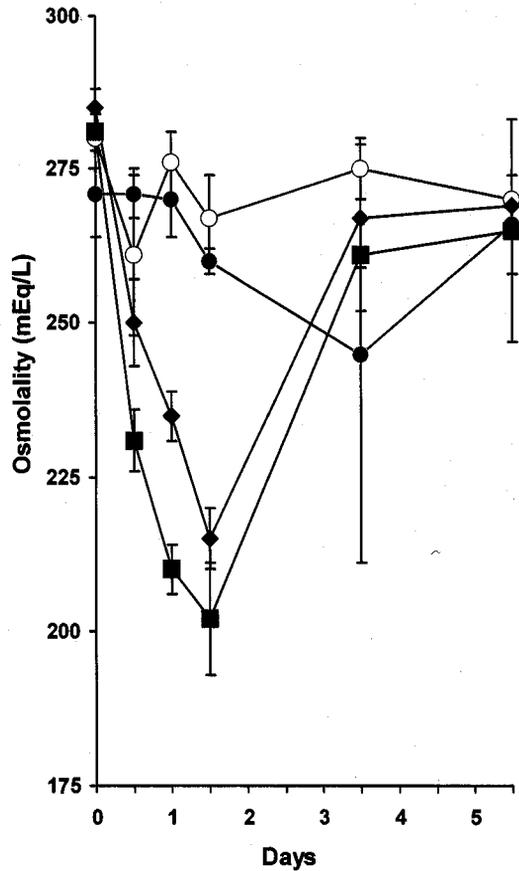


FIGURE 4. Changes in plasma osmolality of channel catfish exposed to $KMnO_4$ for 36 h; the first 1.5 d on the x axis represent the treatment period, beyond 1.5 d the recovery period is represented. The symbols are: open circles = untreated controls, solid circles = exposure to 0.44 mg/L, solid diamonds = exposure to 1.315 mg/L, solid squares = exposure to 2.19 mg/L. Error bars represent SEM.

control fish did not change significantly during the study. The values in fish in the 1 \times treatment were elevated, though not significantly, at 24 and 36 h. The fish in all treatments had PCV values similar to controls by 4 d post exposure. No significant changes were observed in total erythrocyte counts (data not shown).

Discussion

The lethal effect of exposure to $KMnO_4$ appears to be unrelated to metallic manganese toxicity. It has been previously shown

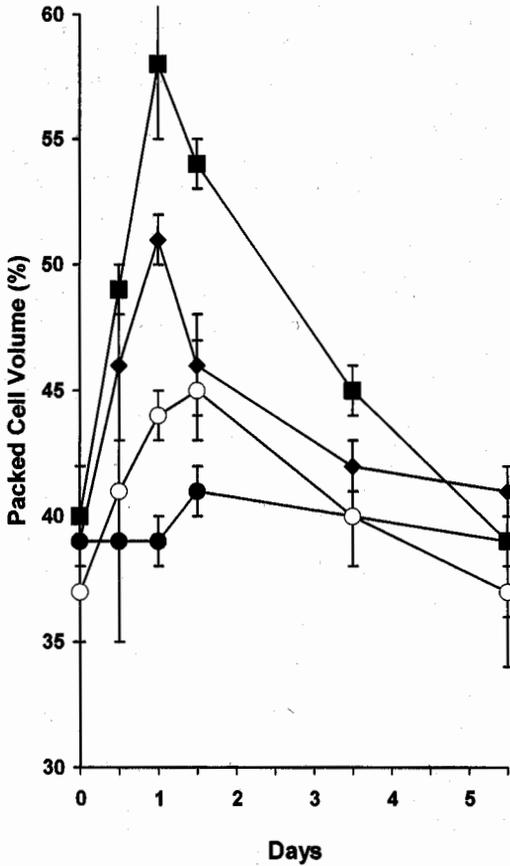


FIGURE 5. Changes in the packed cell volume of whole blood of channel catfish exposed to $KMnO_4$ for 36 h; the first 1.5 d on the x axis represent the treatment period, beyond 1.5 d the recovery period is represented. The symbols are: open circles = untreated controls, solid circles = exposure to 0.44 mg/L, solid diamonds = exposure to 1.32 mg/L, solid squares = exposure to 2.19 mg/L. Error bars represent SEM.

that there is no significant uptake of manganese in muscle and liver (Griffin et al. 1999) as a result of chronic exposure to $KMnO_4$; observations confirmed for short term acute exposure by the results of this study. The absence of a significant increase in concentrations of manganese in liver tissue and in plasma suggests that the manganese detected in homogenized gill preparations is associated with the surface of gill structures and is not internalized. At the time when gill-associated manganese was highest (range of 253 to 566 mg Mn^{++}/kg

dry weight during exposure), the manganese concentration in the plasma remained less than 1 $\mu g/L$. The manganese content of gill tissue returned to pre-exposure levels within 48 h after exposure was discontinued, and there were no changes in manganese concentration of plasma or liver.

While plasma cortisol and plasma glucose concentrations are traditional indicators of stress in fish, they are not usually thought to be directly involved in mortality resulting from highly stressful conditions or treatments. Both indicators were elevated in this study, but high concentrations of both have been reported without accompanying mortalities (Davis et al. 1984). More directly associated with mortality may be the changes observed in plasma chloride and osmolality and in the PCV of whole blood, which all were found to have changes consistent with a significant loss of electrolytes. Plasma chloride loss was measured directly and the more general change in plasma electrolyte content is shown as changes in plasma osmolality. The increase in whole blood PCV could be brought about either by an increase in the number of erythrocytes or by the physical enlargement of the existing cells. The number of erythrocytes/ mm^3 did not change, as indicated by the total erythrocyte counts on diluted whole blood, suggesting that the increases observed in the whole blood PCV are due to erythrocyte enlargement resulting from hydration caused by the osmotic imbalance between the cytosol and the plasma; an imbalance caused by the loss of plasma electrolytes. Significant loss of plasma electrolytes can result in compromise of cardiac function and can lead to death (Wood 1989).

The $1 \times KMnO_4$ dosage is near the maximum concentration that can be tolerated, without mortality, on a continuous basis by channel catfish in the water available at this laboratory (Griffin et al. 1999). This concentration most closely matches the concentration that would constitute a therapeutic treatment for fish in the water available for

this study (Tucker and Robinson 1990). The changes in the stress indicators measured in this study at this concentration were quite small and most of the parameters measured were no different than unexposed controls. The whole blood PCV of the fish in the low exposure group was slightly (though not significantly) elevated after 24-h exposure, but plasma cortisol, glucose, chloride, and osmolality were unchanged throughout the exposure. Given the absence of manganese uptake during potassium permanganate exposure observed here and previously (Griffin et al. 1999) and the mild physiological changes that occurred, $KMnO_4$ would appear to be safe to use for control of surficial diseases of cultured channel catfish.

Acknowledgments

This research was supported in part by a grant to B.R.G. from the International Association of Fish and Wildlife Agencies.

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