

## Histological and Hematological Evaluation of Potassium Permanganate Exposure in Channel Catfish

AHMED M. DARWISH,\* BILLY R. GRIFFIN, DAVID L. STRAUS, AND  
ANDREW J. MITCHELL

Harry K. Dupree—Stuttgart National Aquaculture Research Center,  
United States Department of Agriculture, Agriculture Research Service,  
Post Office Box 1050, Stuttgart, Arkansas 72160, USA

**Abstract.**—A histological and hematological study was performed to evaluate the effect of waterborne exposures of channel catfish *Ictalurus punctatus* to potassium permanganate (KMnO<sub>4</sub>). Three concentrations of KMnO<sub>4</sub> were chosen to represent one, three, and five times the therapeutic concentrations (0.438, 1.315, and 2.190 mg/L, respectively), based on the KMnO<sub>4</sub> demand, for 36 h, which is three times the usual treatment duration. The organs examined were the gill, liver, and trunk kidney. Differential leukocyte counts of neutrophils and monocytes in the blood and plasma enzyme analyses (lactate dehydrogenase and alanine transaminase) were also performed. The gill was the only organ to show microscopic lesions. Fish exposed to the therapeutic concentration of KMnO<sub>4</sub> for 36 h had mild hypertrophy and spongiosis in the gills sampled during exposure, but no lesions were noticed 2 d postexposure. Gills of fish exposed to three and five times the therapeutic dose had extensive hyperplasia, epithelial hypertrophy and necrosis, lamellar fusion, spongiosis, leukocytic infiltration, obliteration of the interlamellar space with an inflammatory exudate containing necrotic epithelial cells, and occasional multifocal hemorrhages. No mortalities were observed in fish not exposed (control) or in fish exposed to the therapeutic dose of KMnO<sub>4</sub>. Mortalities were only observed in fish exposed to three and five times the therapeutic dose of KMnO<sub>4</sub> (9.4% and 49.6%, respectively) with most of these mortalities occurring from exposure to 2 d postexposure. The gills of surviving fish exposed to three and five times the therapeutic dose for 36 h appeared normal at 8 d postexposure. Neutrophil count and plasma alanine transaminase activity increased significantly in fish exposed to five times the therapeutic dose; lactate dehydrogenase activity showed no change. Exposure to the therapeutic dose at three times the therapeutic exposure time caused mild lesions but recovery occurred within 48 h post-exposure.

Potassium permanganate (KMnO<sub>4</sub>), a strong oxidant, has been used for many years to treat numerous skin and gill diseases of cultured fish (Duncan 1978; Wellborn 1985; Noga 1996). Despite its therapeutic value, KMnO<sub>4</sub> is not currently approved for any aquaculture use in the USA. The U.S. Food and Drug Administration (FDA) is currently considering approval of KMnO<sub>4</sub> for use as a therapeutant for certain diseases of channel catfish *Ictalurus punctatus*. As part of its evaluation process, the FDA requires a target animal safety study that includes a histological evaluation of the effects of the therapeutant.

The histological effects of KMnO<sub>4</sub> exposure have been studied in milkfish *Chanos chanos*, common carp *Cyprinus carpio*, and Nile tilapia *Tilapia nilotica* (Cruz and Tamse 1986; Dureza 1988; Das and Kaviraj 1994) but not in channel catfish. Different species of fish differ in the mechanisms, types, and magnitudes of their responses to wa-

terborne irritants (Lauren 1991; Arellano et al. 1999; Ibrahim et al. 2000).

Changes in plasma enzyme activity are used as indicators of tissue injury, environmental stress, or a disease condition. The rate of increase of plasma enzyme activity depends on the concentration of an enzyme in cells, the rate of leakage caused by injury, and the rate of clearance of the enzyme from plasma (Boyd 1983). Hematological changes in fish, such as monocyte and neutrophil counts, occur in response to toxicants, irritants, or inflammatory conditions and can lead to detrimental effects on fish health (Grizzle 1977; Ainsworth et al. 1991). The objective of this study was to evaluate the response of channel catfish to KMnO<sub>4</sub> exposure and their recovery from that exposure by examining (1) the histology of gill, liver, and trunk kidney; (2) changes in plasma enzyme activity concentrations (alanine aminotransaminase [ALT] and lactate dehydrogenase [LDH]); and (3) changes in the numbers of neutrophils and monocytes. To achieve this objective, we selected three concentrations of KMnO<sub>4</sub> representing one, three, and

\* Corresponding author: adarwish@spa.ars.usda.gov

Received June 12, 2001; accepted November 27, 2001

five times the recommended therapeutic dose (based on the potassium permanganate demand [PPD]) and used an exposure time of 36 h (three times the recommended treatment duration).

### Methods

*Experimental fish.*—Channel catfish were supplied from experimental stocks raised at the Harry K. Dupree–Stuttgart National Aquaculture Research Center, Stuttgart, Arkansas. These fish were produced from a single lot, as previously described by Griffin et al. (1997).

*Experimental design.*—Three replicates of the same experimental design were conducted. In each replicate, four tanks were each stocked with 50 randomly selected fish (weighing 200–250 g) and the fish were acclimated to the experimental conditions for 14 d. At the end of the acclimation period, each tank was randomly assigned to one of four treatments: Three tanks were dosed with either one, three, or five times the therapeutic concentration of  $\text{KMnO}_4$  (1 $\times$ , 3 $\times$ , or 5 $\times$ ), and one tank received no  $\text{KMnO}_4$  (control).

*Experimental conditions.*—The experimental tanks were flow-through fiberglass tanks (735 L) with a turnover time of 4 h (flow rate, about 3.1 L/min). Fish were fed daily at 2% of their body weight a ration having a manganese content of  $231 \pm 18$  mg/kg (mean  $\pm$  SE). The manganese content was determined by atomic absorption spectrophotometry (Thermo Jarrell Ash Corporation, Franklin, Massachusetts) in seven feed samples (Griffin et al. 1999). Water quality parameters were measured at every fish sampling time (Hach Company, Loveland, Colorado) and were as follows: temperature,  $21 \pm 0.5^\circ\text{C}$ ; dissolved oxygen,  $7.7 \pm 0.2$  mg/L; total alkalinity (as  $\text{CaCO}_3$ ),  $184 \pm 3$  mg/L; total ammonia,  $0.91 \pm 0.04$  mg/L; pH,  $7 \pm 0.2$ ; and unionized ammonia,  $0.03 \pm 0.01$  mg/L.

*Determination of dosage and exposure time.*—The therapeutic dosage (1 $\times$ ) is defined as a concentration 2.5 times the 15-min PPD, as described by Tucker (1989). In this study the PPD was calculated by averaging the PPD measured daily during the 14-d acclimation period in water samples collected 5 h after feeding. Based on the PPD average of 0.175 mg  $\text{KMnO}_4$ /L, the 1 $\times$ , 3 $\times$ , and 5 $\times$  dosages of  $\text{KMnO}_4$  were established as 0.438, 1.315, and 2.190 mg/L, respectively. For the purpose of this target animal safety study, exposure for 12 h was considered the therapeutic exposure time (Wellborn 1985; Noga 1996); we used three times that exposure, or 36 h.

*Dosing method.*—After the acclimation period, concentrated solutions of  $\text{KMnO}_4$  were added to all tanks except the control tank to achieve the predetermined concentrations.  $\text{KMnO}_4$  dissolved in deionized water was pumped to the experimental tanks by peristaltic pumps to maintain the exposure concentrations (1 $\times$ , 3 $\times$ , and 5 $\times$ ). The concentrations of manganese and residual  $\text{KMnO}_4$  in the tanks were confirmed in water samples collected before and after the initial addition of  $\text{KMnO}_4$ , again at 12-h intervals for 36 h (the exposure time), and then at 48-h intervals after the end of the exposure for 14 d. Manganese was measured by graphite furnace atomic absorption spectrometry according to Griffin et al. (1999) and residual  $\text{KMnO}_4$  by the method of Engstrom-Heg (1971). These data are reported in Griffin et al. (2002).

*Fish sampling.*—Two fish were randomly selected from each tank at each sampling time. We conducted the experiment three times, which yielded six fish for each treatment at each sampling time. The sampling was done before the initial addition of  $\text{KMnO}_4$ , then at 12-h intervals for 36 h (the exposure time), and then at 48-h intervals after the end of the exposure for 14 d. In all three experiments, 248 fish were sampled, 66 fish from each treatment, except for the 5 $\times$  treatment, where only 50 fish were sampled because of mortality within the group. Blood from the caudal vessels was collected in heparin-containing syringes for plasma enzyme analyses and preparation of blood smears. Fish were then double-pithed, after which a 10–15-mm segment from the central portion of the second left gill arch and representative samples of liver and trunk kidney were removed and immediately fixed in Bouin's solution for 24–48 h.

*Histology processing.*—Fixed tissues were rinsed twice with 50% ethanol and stored in 70% ethanol. Stored tissues were dehydrated in isopropanol, cleared in Hemo-De (Fisher Scientific, Pittsburgh, Pennsylvania), and embedded in Paraplast Plus (Oxford Labware, St. Louis, Missouri). Gill filaments were embedded at a 30–45° angle to the horizontal to show the lamellae, the central portion of filaments, and the leading and trailing edges of each filament. Tissues were sectioned (4–6  $\mu\text{m}$  thick) and stained with hematoxylin and eosin (Luna 1968).

*Hematology and enzyme assay.*—For differential leukocyte counts of monocytes and neutrophils, blood smears were stained with Sudan black and for nonspecific esterase (procedures 380 and 91, respectively; Sigma Diagnostics, St. Louis, Mis-

TABLE 1.—Severity code for leukocyte infiltration in gills.

| Severity code | Description  |
|---------------|--|
| 0             | Lymphocytes were scattered and scarce, usually found only in the epithelium of the basal portion of both the filament and lamellae.  |
| 1             | Number of lymphocytes was about 25% of the number of filament epithelial cells; lymphocytes were commonly present in the basal and middle portion of the filament epithelium and the epithelium of the base of lamellae.   |
| 2             | Number of lymphocytes was about 25–50% of the number of filament epithelial cells; lymphocytes were commonly present in the basal and middle portions of the filament and sometimes the superficial portion of the filament epithelium and scattered throughout the lamellar epithelium.                                 |
| 3             | Number of lymphocytes was about equal to or greater than 50% of the number of filament epithelial cells; lymphocytes were commonly present in all areas of the filament and lamellar epithelium; scarce scattered macrophages and neutrophils were also present in the filament epithelium and the central venous sinus. |

souri). In channel catfish, neutrophils are Sudan black—positive and monocytes are esterase-positive (Petrie-Hanson and Ainsworth 2000). Counts of 200 leukocytes per slide were differentiated as neutrophils or monocytes and used to calculate the percentage of neutrophils and monocytes.

Plasma was removed from whole blood after centrifugation and stored at  $-80^{\circ}\text{C}$ . Plasma LDH activity was measured by monitoring the reduction of  $\text{NAD}^{+}$  at 340 nm (procedure 228-10; Sigma Diagnostics), and plasma ALT was measured colorimetrically (procedure 505-P; Sigma Diagnostics).

*Histologic observation.*—Three well-oriented representative filaments of the second gill arch from each fish were assessed. In addition to subjective lesion evaluation, severity codes were used to quantify branchial epithelium hypertrophy, lamellar fusion, epithelial spongiosis, hyperplasia, necrosis, and leukocytic infiltration.

Hypertrophy of branchial epithelium was defined as cells having an abnormal increase in size and swollen nuclei ( $\geq 5 \mu\text{m}$  in diameter). The percentage of cells showing hypertrophy in the basal, middle, and superficial portions in the filament and lamellar epithelium was used to determine the severity code. A severity code of 0 indicates no pronounced epithelial hypertrophy, whereas severity codes of 1, 2, and 3 indicate hypertrophy in 20–30%, 40–60%, and 70% or more, respectively, of the epithelial cells.

Lamellar fusion is the joining of adjacent la-

TABLE 2.—Severity code for spongiosis of gill epithelium.

| Severity code | Description  |
|---------------|--|
| 0             | No significant intracellular spaces were present in the epithelium of lamellae and filament.   |
| 1             | Intercellular spaces of about 2–11 $\mu\text{m}$ were occasionally seen between the two layers of lamellar epithelial cells, scattered spaces (2–6 $\mu\text{m}$ ) were present in filament epithelium, or specimens had both characteristics. |
| 2             | Both intercellular spaces larger than 11 $\mu\text{m}$ between layers of lamellar epithelial cells and spaces larger than 6 $\mu\text{m}$ in the filament epithelium were occasionally present.  |
| 3             | Both intracellular spaces larger than 11 $\mu\text{m}$ between layers of lamellar epithelial cells and spaces larger than 6 $\mu\text{m}$ in the filament epithelium were commonly present.  |

mellae as a result of epithelial hypertrophy and hyperplasia in the interlamellar space or the binding of lamellar epithelium of one lamella to another. A severity code of 0 was designated for filaments showing no noticeable fusion. Filaments showing up to one-third of the lamellae fused were assigned a severity code of 1; having one-third to two-thirds of lamellae fused was coded 2; and those with more than two-thirds of lamellae fused were coded 3. The severity code for leukocyte infiltration of gills and epithelium spongiosis was adopted from Grizzle and Kiryu (1993) with few modifications (Tables 1, 2). The severity codes for hyperplasia and necrosis are defined later (see Figures 7 and 8).

*Statistics.*—Results for the replicates from all three experiments were combined and analyzed by one-way analysis of variance (ANOVA) and Tukey's multiple means comparison. When there was an unequal number of samples within a treatment—as was observed in the  $5\times$  treatment fish, which had 49.6% mortality (Griffin et al., in press)—Fisher's test was used (Minitab, Inc., State College, Pennsylvania). We used the same analysis for the hematology and plasma enzymes assay results, after logarithmically transforming the data to account for the wide distribution of the means. Results were considered significant at the 95% confidence level.

## Results

### *Histological Evaluation of Experimental Fish*

The liver and trunk kidney from the fish in the control treatment and all the other treatments exposed to  $\text{KMnO}_4$  showed no abnormal microscopic changes (Grizzle and Rogers 1976). Also, gills of

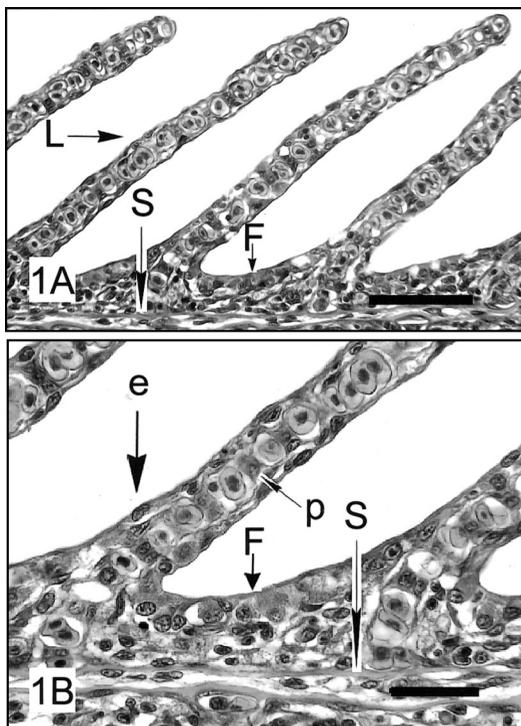


FIGURE 1.—(A) Control gill from channel catfish not exposed to  $\text{KMnO}_4$  and showing normal filament (F), lamellae (L), and central venous sinus (S). Hematoxylin and eosin (H&E) stain; bar = 50  $\mu\text{m}$ . (B) Enlargement of (A) showing the filament epithelium (F), central venous sinus (S) and lamella with epithelial cells (e) and pillar cells (p). H&E stain; bar = 20  $\mu\text{m}$ .

fish sampled before exposure to  $\text{KMnO}_4$  and those of fish from the control groups were normal (Figures 1A, B; Grizzle and Rogers 1976). Microscopic lesions were observed only in gills of fish exposed to  $\text{KMnO}_4$ . Gills exposed to the therapeutic dose (1 $\times$ ) of  $\text{KMnO}_4$  had mild hypertrophy and spongiosis of the epithelium of the filaments and lamellae. The gills in both the 3 $\times$  and 5 $\times$  treatments had extensive epithelial hyperplasia, lamellar fusion, and obliteration of the interlamellar spaces with inflammatory exudate containing necrotic epithelial cells (Figures 2A, B and 3A, B). Hyperplasia was multifocal or diffuse, causing partial or complete obliteration of the interlamellar spaces. Inflammatory exudate was characterized by leukocytic infiltration—predominantly lymphocytes, a few scattered macrophages and neutrophils, eosinophilic cellular debris, and proteinaceous material. Multifocal or multifocal coalescing necrosis involved all epithelial layers of the lamellae and filaments (Figures 3A, B). Pillar

cells did not show the necrosis seen in the epithelial cells. Multifocal hemorrhages in the lamellae and filaments were occasionally seen in the 3 $\times$  and 5 $\times$  treatments. The central venous sinus and lamellar sinusoids were dilated and engorged with blood (Figure 3A). There was no increase in the number of mucous cells in the lamellar epithelium of treated fish relative to the control.

The severity of hypertrophy was significantly greater in all treated fish than in the control (Figure 2B). The difference between the severity of hypertrophy observed in the 3 $\times$  and 5 $\times$  exposed gills was not significant, but in both treatments the gills were characterized by significantly more severe hypertrophy than in the control and the 1 $\times$  treated fish. The severity of epithelial hypertrophy declined significantly postexposure, none being observed in the 1 $\times$ , 3 $\times$ , and 5 $\times$  exposed gills at 4, 6, and 8 d postexposure (PE), respectively. Severe lamellar fusion was seen only in the 3 $\times$  and 5 $\times$  levels of exposure, with differences between the two levels being significant (Figure 4). Lamellar fusion was not noticeable in the 3 $\times$  and 5 $\times$  treated gills at 2 and 6 d PE, respectively.

Leukocytic infiltration was observed during  $\text{KMnO}_4$  exposure only in the 3 $\times$  and 5 $\times$  exposed gills. The severity of infiltration was significantly higher at 5 $\times$  than 3 $\times$  at 12 and 36 h of exposure (Figure 5), but no significant leukocytic infiltration was noticed at 2 d PE (Figure 6). Spongiosis of gill epithelium during exposure to  $\text{KMnO}_4$  was significantly greater in all treatment groups compared with the controls. After 12 h of exposure to  $\text{KMnO}_4$ , spongiosis in the 5 $\times$  gills was significantly more severe than in the 1 $\times$  and 3 $\times$  treated gills, whereas after 24 and 36 h of exposure spongiosis severity was indistinguishable between the 3 $\times$  and 5 $\times$  treatments. Gills of fish in the 1 $\times$  treatment had no spongiosis at 2 d PE; the 3 $\times$  and the 5 $\times$  treatments were indistinguishable from controls at 6 d PE.

Significant hyperplasia of the lamellae and filaments epithelium was present in fish exposed to the 3 $\times$  and 5 $\times$  doses (Figure 7). This hyperplasia after 12 h of exposure was observed until 4 d PE (Figure 2A). Significant gill necrosis was observed during  $\text{KMnO}_4$  exposure only in the 3 $\times$  and 5 $\times$  treatments (Figures 3A, B). The difference between 3 $\times$  and 5 $\times$  treatments was not significant (Figure 8) except at 12 h of exposure, where scattered necrotic cells were observed in the 3 $\times$  treated gills and severe multifocal and multifocal coalescing areas of necrosis were seen in gills of fish treated with the 5 $\times$  dose. Hemorrhages in lamellae

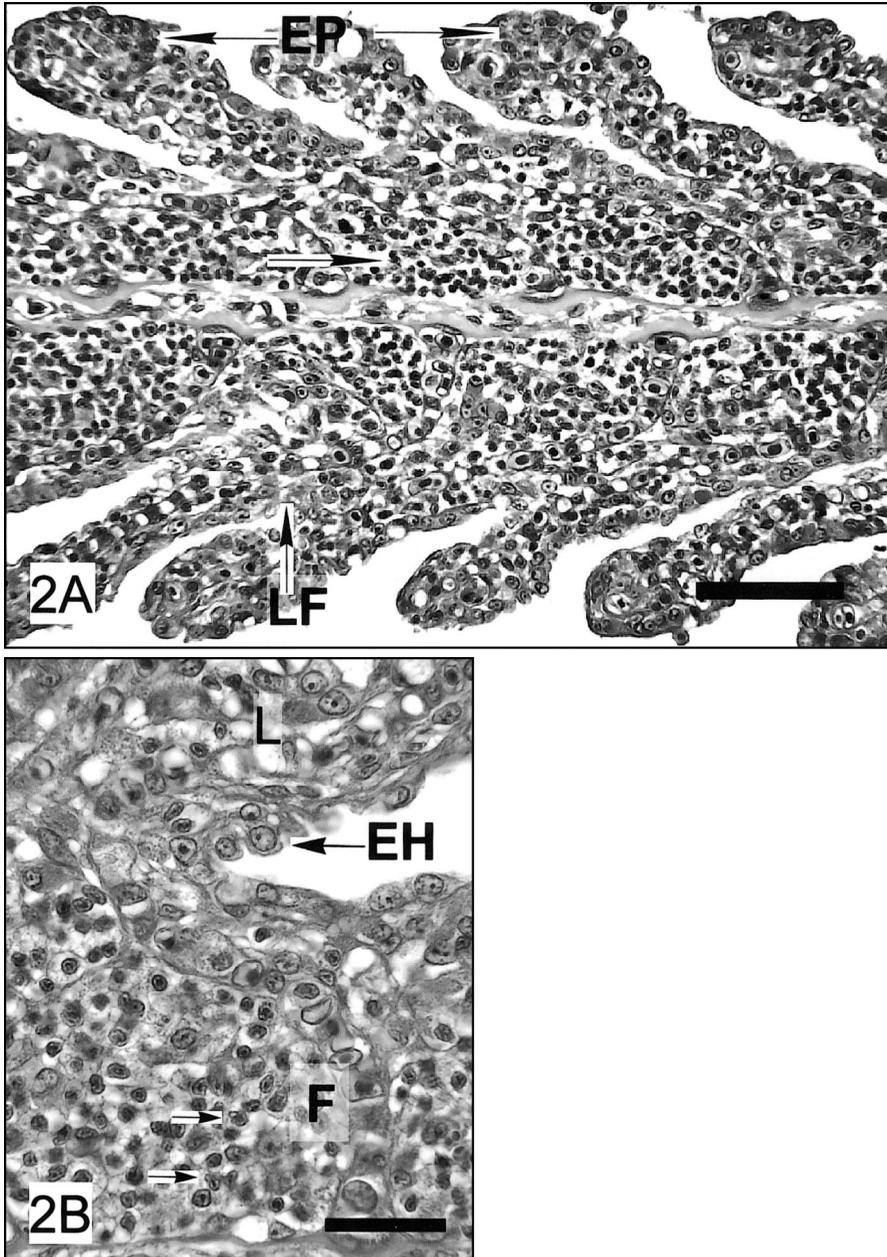


FIGURE 2.—(A) Severe multifocal branchitis in a gill from channel catfish exposed to 3× the therapeutic dose for 12 h showing lamellar fusion (LF), epithelial hyperplasia (EP), leukocytic infiltration (unlabeled arrow), and spongiosis. Hematoxylin and eosin (H&E) stain; bar = 50  $\mu\text{m}$ . (B) Enlargement of (A) showing epithelial hypertrophy (EH) and leukocytic infiltration (unlabeled arrows). H&E stain; filament = F, lamella = L; bar = 20  $\mu\text{m}$ .

and filaments epithelium were seen in one-sixth of the gills 3× and 5× treated at 24 and 36 h of exposure. The gills appeared normal at 2 d PE in the 1× exposed gills and at 8 d PE in the 3× and 5× exposed gills. Mortality data have been reported by Griffin et al. (2002) and were 9.4% in

the 3× treated fish and 49.6% at 5× treatment, most of the mortalities occurring from exposure to the first 2 d PE (92% at 3× and 95% at 5×). No mortalities occurred in the fish not exposed to  $\text{KMnO}_4$  or in the fish exposed to 1× dose of  $\text{KMnO}_4$ .

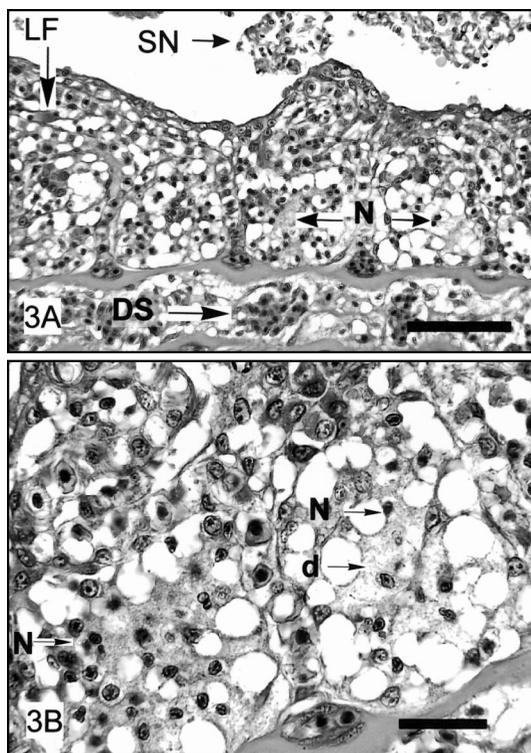


FIGURE 3.—(A) Severe multifocal necrotizing branchitis in a gill from channel catfish exposed to 5× the therapeutic dose for 12 h and showing multifocal necrosis (N), lamellar fusion (LF), sloughing of necrotic cells (SN), and dilated central venous sinus (DS) engorged with red blood cells. Hematoxylin and eosin (H&E) stain; bar = 50 μm. (B) Enlargement of (A) showing necrotic cells (N), cellular debris and proteinaceous material (d), and leukocytic cells. H&E stain; bar = 20 μm.

*Neutrophils and Monocytes Counts*

The fish exposed to the 3× and 5× treatment concentrations of KMnO<sub>4</sub> had a significant increase in the percentage of neutrophils during exposure, which declined to approximately preexposure values at 48 h PE (Figure 9). The percentage of neutrophils in 1× treated fish and control fish did not differ significantly, nor did that in the 3× and 5× exposed fish. The percentage of monocytes did not change during the course of the experiment.

*Plasma Enzymes Activities*

Plasma ALT activity was significantly greater in 5× exposed fish than in all the other treatments at 24 and 36 h of exposure and declined to the preexposure value at 2 d PE (Figure 10). The activity

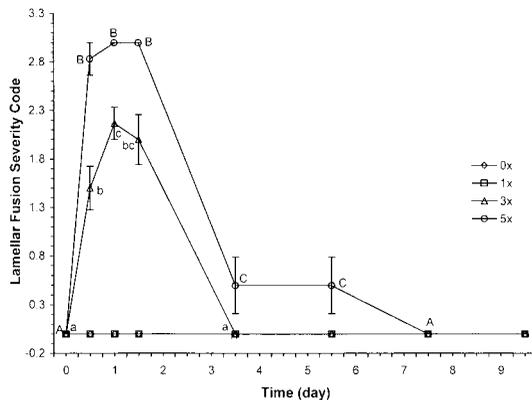


FIGURE 4.—Mean severity code (±SE) for lamellar fusion in gills of channel catfish exposed to no KMnO<sub>4</sub> or to one, three, or five times the therapeutic dose (0×, 1×, 3×, or 5×). The first 36 h (1.5 d) is the KMnO<sub>4</sub> exposure time, which is followed by an 8-d postexposure period. Within each KMnO<sub>4</sub> exposure condition, means with different letters are significantly different (*P* < 0.05); lowercase letters are used with the 3× means and uppercase letters with the 5× means.

of LDH in KMnO<sub>4</sub>exposed fish was unchanged during and after the exposure.

**Discussion**

This is the first study in fish to evaluate the safety margin of KMnO<sub>4</sub> as a therapeutic agent. This study is also the first study in channel catfish to take into consideration the relevance of the PPD, which is crucial in determining the toxicity and the therapeutic value of KMnO<sub>4</sub> (Tucker and Boyd 1977; Jee and Plumb 1981). Fish exposed to the therapeutic dose had mild lesions in the gill, whereas those receiving the 3× and 5× treatments had severe necrotizing gill branchitis. Earlier studies of KMnO<sub>4</sub> exposure in milkfish, common carp, and Nile tilapia examined the gills primarily and reported similar lesions, necrosis, spongiosis, epithelial hyperplasia, hypertrophy, and lamellar fusion (Cruz and Tamse 1986; Dureza 1988; Das and Kaviraj 1994).

The sampling of gills, liver, and kidney was done because of the physiological importance of these organs in absorption, metabolism, and elimination of chemicals (Roberts 1989). The toxic mechanism of KMnO<sub>4</sub> is not well understood; however, the principal effect of KMnO<sub>4</sub> is known to be due to the strong oxidative power of the MnO<sub>4</sub><sup>-</sup> ion. Elemental manganese can also cause heavy metal poisoning (Boyd 1984; Dureza 1988). The finding of gill lesions was expected, given the external location of the gills and their sensitivity

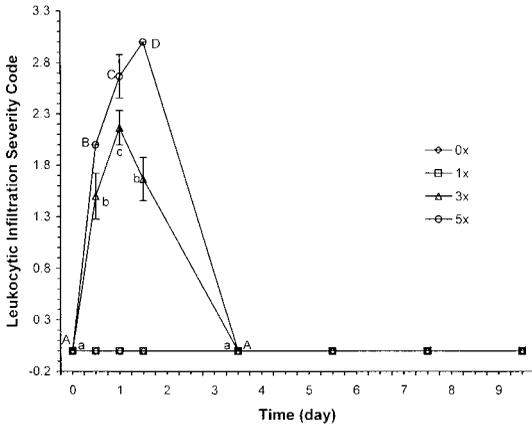


FIGURE 5.—Mean severity code ( $\pm$ SE) for leukocytic infiltration into branchial tissue of channel catfish exposed to no  $\text{KMnO}_4$  or to one, three, or five times the therapeutic dose (0 $\times$ , 1 $\times$ , 3 $\times$ , or 5 $\times$ ). The first 36 h (1.5 d) is the  $\text{KMnO}_4$  exposure time, which is followed by an 8-d postexposure period. Within each  $\text{KMnO}_4$  exposure condition, means with different letters are significantly different ( $P < 0.05$ ); lowercase letters are used with the 3 $\times$  means and uppercase letters with the 5 $\times$  means. Branchial tissue in the control fish and in fish exposed to the 1 $\times$  dose showed no significant leukocytic infiltration.

to heavy metals and oxidants (Lauren 1991; Arrellano et al. 1999; Speare et al. 1999).

The mild nature of the gill lesions and the absence of change in differential leukocytic counts and plasma enzyme activities in the fish exposed to  $\text{KMnO}_4$  at the therapeutic concentration for three times the usual treatment duration (36 h) suggest a low toxicity for this agent when used at the recommended concentration. Only mild hypertrophy, spongiosis, and hyperplasia were found in the gills sampled during exposure at the therapeutic dose of  $\text{KMnO}_4$ , and recovery from these was seen 2 d PE. However,  $\text{KMnO}_4$  appears to be toxic when used at 3 $\times$  and 5 $\times$  the therapeutic dose for 3 times the normal therapeutic duration. This toxicity is indicated by the severe gill lesions, the neutrophilia, the significant increase in ALT at the 5 $\times$  concentration, and most importantly, the 9.4% and 49.6% mortalities in the 3 $\times$  and 5 $\times$  treatments, respectively. Moreover, toxicity apparently increases with the increase in the dose of  $\text{KMnO}_4$  from 3 $\times$  to 5 $\times$  as indicated by more severe gill lesions, neutrophilia, increased plasma ALT activity, and increased mortalities.

The gill lesions in this study—spongiosis, hypertrophy, hyperplasia, and lamellar fusion—are all alterations that would serve as a defense mech-

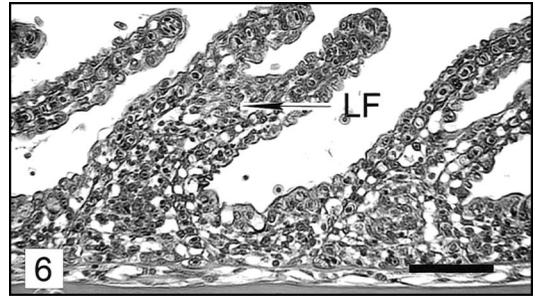


FIGURE 6.—Resolution progress at 2 d postexposure in gill tissue from a channel catfish exposed to 5 $\times$  the therapeutic dose. Epithelial hypertrophy, hyperplasia, and lamellar fusion (LF) are still evident but not necrosis or significant leukocytic infiltration (compare with Figures 3A, B). Hematoxylin and eosin stain; bar = 50  $\mu\text{m}$ .

anism (Mallatt 1985). Spongiosis, hypertrophy, and hyperplasia increase the distance between the waterborne irritant ( $\text{KMnO}_4$  in this study) and the bloodstream, whereas lamellar fusion reduces the amount of vulnerable gill surface area (Mallatt

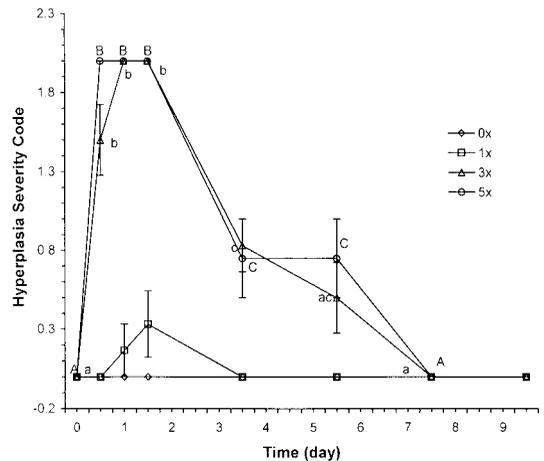


FIGURE 7.—Mean severity code ( $\pm$ SE) for epithelial hyperplasia in branchial tissue of channel catfish exposed to no  $\text{KMnO}_4$  or to one, three, or five times the therapeutic dose (0 $\times$ , 1 $\times$ , 3 $\times$ , or 5 $\times$ ). The first 36 h (1.5 d) is the  $\text{KMnO}_4$  exposure time, which is followed by an 8-d postexposure period. Within each  $\text{KMnO}_4$  exposure condition, means with different letters are significantly different ( $P < 0.05$ ); lowercase letters are used with the 3 $\times$  means and uppercase letters with the 5 $\times$  means. The hyperplasia seen in the gills exposed to the 1 $\times$  dose was statistically insignificant. The severity code assigned was as follows: 0 = hyperplasia was not detected; 1 = hyperplasia was commonly multifocal and commonly causing partial obliteration of the interlamellar space; 2 = hyperplasia of the water contact epithelium was consistently diffuse and commonly causing complete obliteration of the interlamellar space.

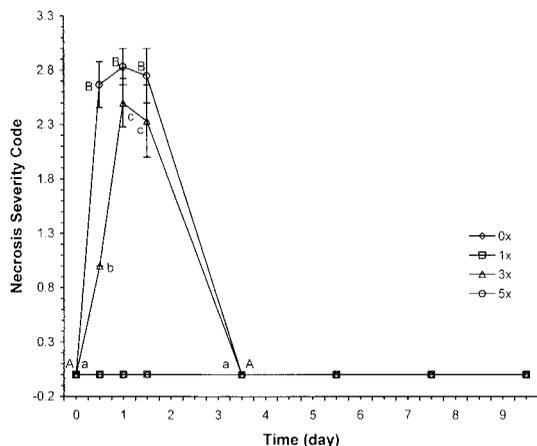


FIGURE 8.—Mean severity code ( $\pm$ SE) for epithelial necrosis in branchial tissue of channel catfish exposed to no  $\text{KMnO}_4$  or to one, three, or five times the therapeutic dose (0 $\times$ , 1 $\times$ , 3 $\times$ , or 5 $\times$ ). The first 36 h (1.5 d) is the  $\text{KMnO}_4$  exposure time, which is followed by an 8-d postexposure period. Within each  $\text{KMnO}_4$  exposure condition, means with different letters are significantly different ( $P < 0.05$ ); lowercase letters are used with the 3 $\times$  means, uppercase letters with the 5 $\times$  means. Branchial tissue in the control fish and in the fish exposed to the 1 $\times$  dose showed no necrosis. The severity code assigned was as follows: 0 = no necrosis was observed; 1 = necrotic epithelial cells in the filament and lamellae were scarce and scattered; 2 = necrosis involving all the epithelial cell-layers of the filaments and lamellae was commonly multifocal and occasionally coalescing; 3 = necrosis involved all epithelial cell layers of the filaments and lamellae and was commonly diffuse.

1985). These changes are commonly reported after exposure to various heavy metals or oxidants (Daoust et al. 1984; Mallatt 1985; Kierner and Black 1997; Speare et al. 1999).

Spongiosis and hypertrophy of epithelial cells were consistent in all the  $\text{KMnO}_4$ -exposed fish. Although the exact mechanism causing spongiosis or hypertrophy of the epithelial cells is not known, Roberts (1989) has proposed that the two lesions could be caused by the alteration in membrane permeability at the cellular and tissue level, leading to swelling of lamellar epithelial cells or edema in the interepithelial spaces. (Daoust et al. (1984) also suggested that hypertrophy could be caused by an increase in cellular metabolism possibly directed toward repair of cellular damage or detoxification. Lamellar fusion such as that observed in the 3 $\times$  and 5 $\times$  treatments can be caused by exposure to heavy metal cations, which can alter the negative charges on the epithelial cells of the lamellae and thus cause attraction between adjacent

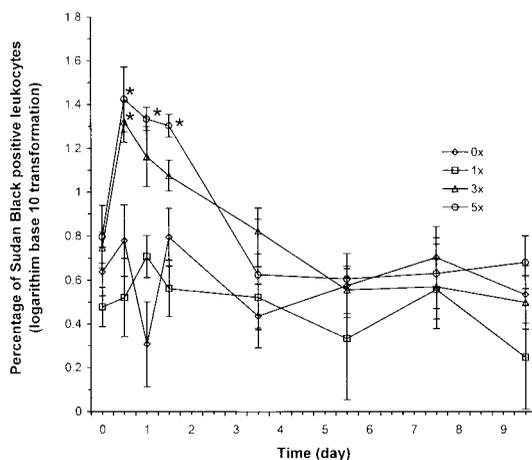


FIGURE 9.—Mean percentage of Sudan Black positive leukocytes ( $\pm$ SE) in channel catfish exposed to no  $\text{KMnO}_4$  or to one, three, or five times the therapeutic dose (0 $\times$ , 1 $\times$ , 3 $\times$ , or 5 $\times$ ). The first 36 h (1.5 d) is the  $\text{KMnO}_4$  exposure time, which is followed by an 8-d postexposure period. Means with an asterisk are significantly different from the means for the controls ( $P < 0.05$ ).

lamellae. The negative charges present under physiological pH are from the carbohydrate moiety of the glycoproteins on the outside of the plasma membrane of the epithelial cells (Daoust et al. 1984).

The mechanism of gill epithelial necrosis caused by  $\text{KMnO}_4$  is unknown, but necrosis has been fre-

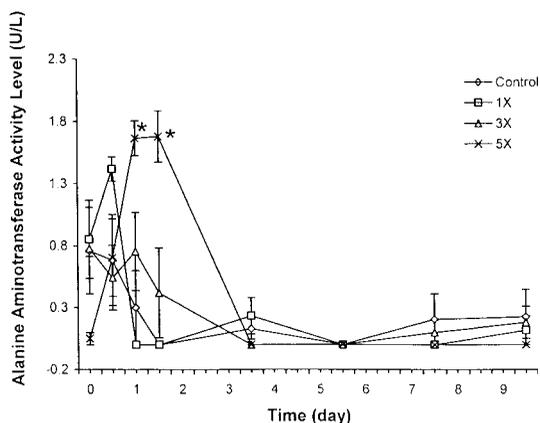


FIGURE 10.—Alanine transaminase (ALT) activity means ( $\pm$ SE) in channel catfish exposed to no  $\text{KMnO}_4$  or to one, three, or five times the therapeutic dose (0 $\times$ , 1 $\times$ , 3 $\times$ , or 5 $\times$ ). The first 36 h (1.5 d) is the  $\text{KMnO}_4$  exposure time, which is followed by an 8-d postexposure period. At 24 and 36 h of  $\text{KMnO}_4$  exposure, ALT activity in the 5 $\times$  treatment was significantly higher than the control (\*,  $P < 0.05$ ).

quently associated with heavy metal toxicity and oxidant exposure and is accompanied by mortalities (Mallatt 1985; Speare et al. 1999). In the 3× and 5× treatments, necrosis of the gill epithelium was associated with mortalities. Despite the necrosis seen in the lamellar epithelium layers, the pillar cells did not appear to be necrotic. Perhaps pillar cells are more resistant to metals than are other kinds of cells (Mallatt 1985). The leukocytic infiltration in this study can be recognized as an integral part of the inflammatory process (Mallatt 1985).

The absence of lesions in the liver is consistent with the report that exposure to  $\text{KMnO}_4$  does not lead to any significant accumulation of manganese in the liver of channel catfish (Griffin et al. 1999). This absence of manganese accumulation in the liver could be related to the fact that the  $\text{MnO}_4^-$  ion responsible for the strong oxidative power of  $\text{KMnO}_4$  can cause gross destruction of cells but in the process is reduced to  $\text{MnO}_2$ , which is relatively nontoxic, insoluble, and biologically unavailable (Boyd 1984; Lasier et al. 2000). The presence of gill lesions with no internal lesions has also been reported in fish exposed to cadmium and mercury (Versteeg and Giesy 1986; Sorensen 1991).

Plasma enzymes such as ALT and LDH are tissue enzymes that may increase in blood as a result of leakage from cells in injured tissue and hence are used as an indicator of specific or multiple organ dysfunction. These enzymes are commonly used to distinguish between healthy and diseased animals (Boyd 1983). In mammals, the relative distribution of ALT in different tissues differs from one species to another and hence so does the clinical interpretation of an ALT increase in plasma. For example, ALT increase in plasma of humans, dogs, and cats indicates liver damage, given the high concentration of this enzyme in the liver of these species; such is not the case in other species (Pratt 1997).

The same species differences in the distribution of ALT in tissues seem to exist in fish (Bell 1968; D'Apollonia and Anderson 1980; Casillas et al. 1982). In channel catfish, the relative distribution of ALT is unknown and hence the complete clinical value of plasma ALT in the species remains to be demonstrated. In this study, a significant increase in plasma ALT in catfish exposed to the 5× dose was associated with massive gill necrosis. Despite this association, using ALT increase to indicate gill damage would not necessarily be warranted because ALT-specific activity might be relatively low in channel catfish gill, but the gill ne-

crosis was massive enough to reflect an increase in the plasma activity (Casillas et al. 1982). The lack of change in plasma ALT in the 3× treated fish could reflect a slow release of ALT, the result of a gradual onset of gill damage, and the ability of the clearance mechanism to keep the enzyme level in check. The 2-d PE decline of ALT activity to the control value would be indicative of recovery, which agrees with the absence of necrosis. The absence of change in the plasma LDH complements the observance of no lesions in the trunk kidney and the liver (Racicot et al. 1975). In mammals, LDH has a wide distribution in different tissues and is therefore considered a nonspecific indicator of tissue damage (Pratt 1997).

The neutrophilia in the 3× and 5× treated fish could have been induced by stress or branchitis (Grizzle 1977; Ellsaesser and Clem 1987; Ainsworth et al. 1991; Duncan et al. 1994; Jandl 1996). In cases of inflammation, products of tissue injury stimulate a variety of cells to release cytokines and other mediators that will increase production and release of neutrophils into the bloodstream (Duncan et al. 1994; Jandl 1996). The absence of neutrophilia 48 h after the end of exposure to the  $\text{KMnO}_4$  corresponds to the decrease in the branchitis severity or stress and the return of plasma enzyme activities to control values.

The ability of severely damaged gills to return to normal in about 6–8 d PE is remarkable but not surprising (Ferguson 1989; Speare et al. 1999). Also no fibroplasia was observed in this study, which is consistent with reports indicating that fibroplasia is rare in fish (Mallatt 1985; Speare et al. 1999). The disappearance of necrosis in the 3× and 5× exposed fish 48 h PE is probably explained by rapid elimination of necrotic cells through extrusion into the external environment, digestion by lysosomal enzymes within phagocytic cells, or both (Daoust et al. 1984).

In pond treatments, a minimum effective concentration of  $\text{KMnO}_4$  is 2 mg/L (Wellborn 1985; Noga 1996) and is usually applied once or twice per disease incident, depending on the organic load. This application will result in high concentrations for brief intervals (because of the half-life of  $\text{KMnO}_4$  in ponds), but during much of the 12-h exposure time the  $\text{KMnO}_4$  concentration will be below the therapeutic concentration (Wellborn 1985; Noga 1996). Simulating  $\text{KMnO}_4$  treatment in ponds is very challenging because ponds have different loads of organic matter, which consequently affects the half-life of  $\text{KMnO}_4$ . In the present experiment, the well water used had a very

low organic load, and the therapeutic  $\text{KMnO}_4$  concentration had to be based on this organic load (Tucker 1989). Having calculated the therapeutic  $\text{KMnO}_4$  concentration based on the well water conditions, our objective was to create a repeatable extreme condition by applying a constant concentration for a long period. Given this dosing regimen, we think it reasonable to assume that the lesions in a field treatment should not be more severe than the ones observed in this study.

These results indicate that  $\text{KMnO}_4$  used at the therapeutic dose at three times the usual exposure period will cause mild gill lesions that resolve by 48 h after the exposure is discontinued. Exposure to  $3\times$  and  $5\times$  dosage for 36 h, however, can cause fatalities, although the surviving fish are capable of recovery within 6–8 d PE.

### Acknowledgments

We acknowledge the valuable help of Andy Goodwin; the technical review of John Grizzle, Renate Reimschuessel, and Ken Davis; and the help of Steven Massa in processing the tissues. Mention of trade names of commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

### References

- Ainsworth, A. J., C. Dexiang, and P. R. Waterstrat. 1991. Changes in peripheral blood leukocyte percentage and function of neutrophils in stressed channel catfish. *Journal of Aquatic Animal Health* 3:41–47.
- Arellano, J. M., V. Storch, and C. Sarasquete. 1999. Histological changes and copper accumulation in liver and gills of senegales sole, *Solea senegalensis*. *Ecotoxicology and Environmental Safety* 44:62–72.
- Bell, G. R. 1968. Distribution of transaminase (aminotransferases) in the tissue of Pacific salmon (*Oncorhynchus*), with emphasis on the properties and diagnostic use of glutamic-oxalacetic transaminase. *Journal of Fisheries Research Board of Canada* 25: 1247–1268.
- Boyd, C. E. 1984. Water quality in warmwater fish ponds. Alabama Agricultural Experiment Station, Auburn University, Auburn.
- Boyd, J. W. 1983. The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. *Veterinary Clinical Pathology* 12:9–24.
- Casillas, E., J. Sundquist, and W. E. Ames. 1982. Optimization of assay for, and the selected tissue distribution of, alanine aminotransferase and aspartate aminotransferase of English sole, *Parophrys vetulus* Girard. *Journal of Fish Biology* 21:197–204.
- Cruz, E. R., and C. T. Tamse. 1986. Histopathological response of milkfish *Chanos chanos* forsskal fingerlings to potassium permanganate. *Fish Pathology* 21:151–159.
- Daoust, P. Y., G. Wobeser, and J. D. Newstead. 1984. Acute pathological effects of inorganic mercury and copper in gills of rainbow trout. *Veterinary Pathology* 21:93–101.
- D'Apollonia, S., and P. D. Anderson. 1980. Optimal assay conditions for serum and liver glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and sorbitol dehydrogenase from rainbow trout, *Salmo gairdneri*. *Canadian Journal of Fisheries and Aquatic Sciences* 37:163–169.
- Das, B. K., and A. Kaviraj. 1994. Influence of potassium permanganate, cobalt chloride, and dietary supplement of vitamin B complex on the histopathological changes in gill epithelium of common carp exposed to cadmium. *Progressive Fish-Culturist* 56:265–268.
- Duncan, J. R., K. W. Prasse, and E. A. Mahaffey. 1994. *Veterinary laboratory medicine: clinical pathology*, 3rd edition. Iowa State University Press, Ames.
- Duncan, T. O. 1978. The use of potassium permanganate ( $\text{KMnO}_4$ ) in fisheries: a literature review. U.S. Department of Commerce, Document PB275397, Washington, D.C.
- Dureza, L. A. 1988. Toxicity and lesions of the gills of *Tilapia nilotica* fry and fingerlings exposed to formalin, furanace, potassium permanganate and malachite green. Doctoral dissertation. Auburn University, Auburn, Alabama.
- Ellsaesser, C. F., and L. W. Clem. 1987. Cortisol-induced hematologic and immunologic changes in channel catfish (*Ictalurus punctatus*). *Comparative Biochemistry and Physiology* 87A:405–408.
- Engstrom-Heg, R. 1971. Direct measurement of potassium permanganate demand and residual potassium permanganate. *New York Fish and Game* 18:117–122.
- Ferguson, H. W. 1989. *Systemic pathology of fish*, 1st edition. Iowa State University Press, Ames.
- Griffin, B. R., K. B. Davis, A. Darwish, and D. L. Straus. 2002. Effect of exposure to waterborne permanganate on stress indicator in channel catfish. *Journal of the World Aquaculture Society* 33:1–9.
- Griffin, B. R., J. L. Gollon, M. S. Hobbs, F. F. Kadlubar, D. Schlenk, and C. D. Brand. 1999. Effect of waterborne potassium permanganate exposure on manganese content in liver and axial muscle of channel catfish. *Journal of Aquatic Animal Health* 11:305–309.
- Griffin, B. R., M. S. Hobbs, J. L. Gollon, D. Schlenk, F. F. Kadlubar, and C. D. Brand. 1997. Effect of waterborne copper sulfate exposure on copper content in liver and axial muscle of channel catfish. *Journal of Aquatic Animal Health* 9:144–150.
- Grizzle J. M. 1977. Hematological changes in fingerling channel catfish exposed to malachite green. *Progressive Fish-Culturist* 39:90–93.
- Grizzle J. M., and Y. Kiryu. 1993. Histopathology of gill, liver, and pancreas, and serum enzyme levels of channel catfish infected with *Aeromonas hydro-*

- phila* complex. Journal of Aquatic Animal Health 5:36–50.
- Grizzle J. M., and W. A. Rogers. 1976. Anatomy and histology of the channel catfish. Alabama Agricultural Experiment Station, Auburn University, Auburn.
- Ibrahim A., B. M. Mackinnon, and M. D. B. Burt. 2000. The influence of sub-lethal levels of zinc on smoltifying Atlantic salmon *Salmo salar* and on their subsequent susceptibility to infection with *Lepeophtheirus salmonis*. Contributions to Zoology 69: 119–128.
- Jandl, J. 1996. Blood: text book of hematology, 2nd edition. Little, Brown and Company, Boston.
- Jee L. K., and J. A. Plumb. 1981. Effects of organic load on potassium permanganate as a treatment of *Flexibacter columnaris*. Transactions of the American Fisheries Society 110:86–89.
- Kiemer, M. C. B., and K. D. Black. 1997. The effect of hydrogen peroxide on the gill tissues of Atlantic salmon, *Salmo salar* L. Aquaculture 153:181–189.
- Lasier, P. J., P. V. Winger, and K. J. Bogenrieder. 2000. Toxicity of manganese to *Ceriodaphnia dubia* and *Hyalella azteca*. Archives of Environmental Contamination and Toxicology 38:289–304.
- Lauren, D. J. 1991. The fish gill: a sensitive target for waterborne pollutants. Pages 223–244 in M. A. Mayes and M. G. Barron, editors. Aquatic toxicology and risk assessment, 14th volume. American Society for Testing and Materials, Philadelphia.
- Luna, L. G., editor. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology, 3rd edition. McGraw-Hill, New York.
- Mallatt, J. 1985. Fish gill structural changes induced by toxicants and other irritants: a statistical review. Canadian Journal of Fisheries and Aquatic Sciences 42:630–648.
- Noga, E. J. 1996. Fish diseases diagnosis and treatment. Mosby, New York.
- Petrie-Hanson, L., and A. J. Ainsworth. 2000. Differential cytochemical staining characteristics of channel catfish leukocytes identify cell populations in lymphoid organs. Veterinary Immunology and Immunopathology 73:129–144.
- Pratt, P. W., editor. 1997. Laboratory procedures for veterinary technicians, 3rd edition. Mosby, St. Louis.
- Racicot, J. G., M. Gaudet, and C. Leray. 1975. Blood and liver enzymes in rainbow trout (*Salmo gairdneri* Rich.) with emphasis on their diagnostic use: study of CCl<sub>4</sub> toxicity and a case of *Aeromonas* infection. Journal of Fish Biology 7:825–835.
- Roberts, R. J. 1989. Fish pathology, 2nd edition. Bailliere Tindall, London.
- Sorensen, E. M. 1991. Mercury. Pages 312–318 in Metal poisoning in fish. CRC Press, Boca Raton, Florida.
- Speare D. J., V. Carvajal, and B. S. Horney. 1999. Growth suppression and branchitis in trout exposed to hydrogen peroxide. Journal of Comparative Pathology 120:391–402.
- Tucker, C. S. 1989. Method of estimating potassium permanganate disease treatment rates for channel catfish in ponds. Progressive Fish-Culturist 51:24–26.
- Tucker, C. S., and C. E. Boyd. 1977. Relationship between potassium permanganate treatment and water quality. Transaction of the American Fisheries Society 106:481–488.
- Versteeg, D. J., and J. P. Giesy. 1986. The histological and biochemical effects of cadmium exposure in bluegill sunfish (*Lepomis macrochirus*). Ecotoxicology and Environmental Safety 11:31–43.
- Wellborn, T. L. 1985. Control and therapy. Page 84 in J. A. Plumb, editor. Principal diseases of farm raised catfish. Auburn University, Auburn, Alabama.