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Marine Environmental Research 50 (2000) 267–271

MARINE ENVIRONMENTAL RESEARCH

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Pathology of fathead minnows (*Pimephales promelas*) exposed to chlorine dioxide and chlorite

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Abstract

Fathead minnows (*Pimephales promelas*) were exposed to the biocide chlorine dioxide (0.13 and 0.19 mg l^{-1}) for up to 12 h and to its primary decomposition product, chlorite (177 and 304 mg l^{-1}), for up to 96 h followed by recovery periods of up to 14 days. Chlorine dioxide exposure produced dose-dependent gill pathology including epithelial lifting, hypertrophy, hyperplasia, lamellar fusion, and necrosis. Complete recovery, even in fish with severe hypertrophy and lamellar fusion, was achieved within 4 days. Chlorite did not produce gill pathology even at a lethal exposure level (304 mg l^{-1} for 96 h) but did elicit a chronic inflammatory response with a marked increase in circulating and fixed phagocytes within hematopoietic and vascular tissues. This study indicates that chlorine dioxide is approximately 1000 times more toxic to fathead minnows than chlorite. Further, exposure of fathead minnows to these distinct but related compounds is consistently associated with very different pathologies. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Fathead minnow; Chlorine dioxide; Chlorite; Histopathology; Lesions; Fish

This study is a preliminary investigation into the mechanisms of toxicity of chlorine dioxide (ClO_2) and its primary decomposition product, chlorite (ClO_2^-). Chlorine dioxide is under consideration as a substitute for chlorine in biocidal applications because of its efficacy as a disinfecting agent (Berg, Aieta & Roberts,

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PII: S0141-1136(00)00048-9

1980). Further, chlorine dioxide does not generate toxic nitrogenous residuals such as chloramines (Rosenblatt, 1978) or carcinogenic organic residuals such as trihalomethanes (Michael et al., 1981). Chlorine dioxide is rapidly reduced to chlorite when applied to natural waters. Because the chlorite residual is persistent, the toxic effects of both compounds on aquatic organisms are of interest. The fathead minnow, *Pimephales promelas*, was used as a model to investigate the pathologic effects of exposure to chlorine dioxide and chlorite. Histopathology was selected because of its usefulness as a diagnostic tool in the search for target tissues and mechanisms of action of environmental contaminants (Hinton & Laurén, 1990).

Laboratory-acclimated adult fathead minnows were exposed at 25°C in a flow-through delivery system (modified from Fisher et al., 1994) to two concentrations each of chlorine dioxide and chlorite. The exposure apparatus was designed to be representative of a steam electric power plant once-through cooling water system. Exposure concentrations were selected based on preliminary studies. Unchlorinated deep-well water served as control water and test diluent. Chlorine dioxide and chlorite stock solutions were prepared by dilution of concentrated master stocks in reverse-osmosis filtered well water. The chlorine dioxide master stock was generated by acidification of sodium chlorite in hexane and extraction of the resulting chlorine dioxide into aqueous solution. A 25% (w/v) sodium chlorite solution served as chlorite master stock. Chlorine dioxide and chlorite concentrations were measured by amperometric titration with phenylarsene oxide and reported as total residual oxidant as chlorine equivalents (APHA, 1995). Temperature, pH, dissolved oxygen, hardness, alkalinity, and conductivity were monitored throughout exposures to ensure adequate water quality. Daily feedings of Tetramin® tropical fish flake food were discontinued 24 h prior to, and throughout, exposures but resumed during the 14-day post-exposure recovery period.

Fish were sampled from treatments several times during exposure intervals and following 4- and 14-day recovery periods in oxidant-free water. Sufficient specimens were taken at each interval ($n = 10$) to allow comparisons of effects within treatments over time and between treatments at fixed times. Fish were sacrificed by overdose of buffered MS222, necropsied, prepared for histology, and stained with hematoxylin and eosin (H&E) (Kane, 1996). Lesions observed in individuals by light microscopy were ranked using a semi-quantitative classification system: (0) no effect, (1) minimal, (2) mild, (3) moderate, (4) marked, and (5) severe (Reimschuessel, Bennett & Lipsky, 1992). Histopathology was assessed blindly to avoid bias.

Histopathologic results indicated that gill epithelium was the primary target tissue effected by chlorine dioxide exposure (Table 1). Other tissues in contact with the aqueous environment (e.g. epidermis, oral mucosa, cornea) showed no pathology. A chlorine dioxide concentration of 0.13 mg l^{-1} (± 0.008 S.D.) caused gill pathology in 80% of the fish sampled following both 12 and 48 h exposures. Lesions most frequently observed were epithelial hypertrophy and hyperplasia resulting in moderate fusion of secondary lamellae. Necrosis was observed in one of 10 specimen sampled at 12 h and in five of 10 specimens sampled at 48 h.

All fish sampled following 6 h exposure to 0.19 mg l^{-1} (± 0.011 S.D.) chlorine dioxide displayed moderate to marked epithelial hypertrophy with 80% exhibiting partial to complete fusion of secondary lamellae. Cellular debris from necrotic epithelial

Table 1
Incidence and severity of histological alterations in fathead minnow *Pimephales promelas* gill epithelium following exposure to chlorine dioxide

Treatment	Gill lesion categories				
	Lifting and edema	Epithelial hypertrophy	Epithelial hyperplasia	Fusion of secondary lamellae	Necrosis
Control	10[a] (3.0)[b]	20 (1.0)	0	0	0
0.13 mg l^{-1}					
12 h	10 (2.0)	70 (3.0)	20 (2.0)	60 (3.2)	10 (3.0)
48 h	0	80 (2.7)	20 (2.0)	60 (4.0)	50 (2.8)
0.19 mg l^{-1}					
6 h	40 (4.0)	100 (3.3)	0	80 (3.6)	50 (3.2)
12 h	30 (4.0)	100 (3.9)	20 (2.0)	100 (4.5)	50 (4.0)
4 days[c]	0	10 (2.0)	0	0	0
14 days[c]	10 (2.0)	0	0	0	0

[a] Percent of specimens exhibiting gill lesions within each categories ($n = 10$).
[b] Lesion severity calculated as the mean of ranks from individuals in which lesions were observed (range: 0–5).
[c] Duration of recovery under control conditions following 12 h exposure to 0.19 mg l^{-1} chlorine dioxide.

cells was observed in 50% of the specimens. All 10 fish sampled from the 0.19 mg l^{-1} treatment after a 12 h exposure exhibited moderate to severe epithelial hypertrophy with epithelial hyperplasia evident in several specimens (Fig. 1). Complete fusion of secondary lamellae occurred in all specimens with necrosis evident in half.

Chlorine dioxide-induced lesions resembled those reported by Middaugh, Crane and Couch (1977) in juvenile spot (*Leiostomus xanthurus*) exposed to total residual chlorine and by Richardson, Burton, Block and Stavola (1981) in white perch (*Morone americana*) exposed to ozone-produced oxidants. These authors attributed the occurrence of gill lesions to the oxidizing capabilities of the compounds they were studying.

Despite the severity of gill pathology, fish displayed a remarkable capacity for recovery. Of 23 fish remaining in the 0.19 mg l^{-1} treatment after the 12 h exposure only three died during the recovery period, one at 12 h and two at 48 h post-exposure. The 20 remaining fish were processed for histology at 4 and 14 days post-exposure, and in all cases exhibited fully restored gill architecture. Rapid and complete recovery of severe gill epithelial hyperplasia has been reported in white perch exposed to ozone-produced oxidants (Richardson et al., 1981) and in brown bullhead (*Ameiurus nebulosus*) exposed to copper (Reimschuessel, Kane, Muhvich & Lipsky, 1993).

Chlorite did not produce gill pathology even following exposure to an ultimately lethal concentration of 304 mg l^{-1} ($\pm$10.4 S.D.) for 96 h. H&E sections of fish exposed to 177 mg l^{-1} ($\pm$5.6 S.D.) and 304 mg l^{-1} treatments revealed a dose-dependent proliferation of lightly eosinophilic golden/brown cells occurring individually in gills, spleen, and head kidney, and in clusters in the bulbus arteriosus. Immunostaining with diaminobenzidine revealed the presence of peroxidases found principally within

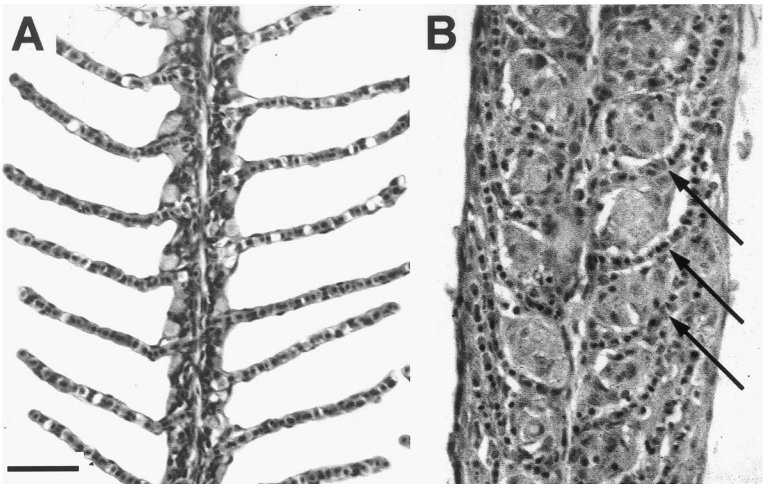

Fig. 1. Sagittal sections of primary and secondary gill lamellae from fathead minnows exposed to (A) control conditions and (B) 0.19 mg l^{-1} chlorine dioxide for 12 h (B). Epithelial hypertrophy and hyperplasia have resulted in complete fusion of secondary lamellae (arrows) in the chlorine dioxide exposed fish (H&E; Bar = 38 μm).

phagocytic macrophages. Recovery following chlorite exposure was indicated by incremental decreases in the abundance of these presumptive macrophages in tissues examined after 4 and 14 day recovery periods.

Chlorine dioxide-associated gill pathology in fathead minnows resembles a typical irritant response beginning with epithelial lifting, hypertrophy, and hyperplasia and resulting in lamellar fusion and necrosis. Acute mortality occurs within 24 h at chlorine dioxide concentrations as low as 0.19 mg l^{-1}. Chlorite-induced acute mortality occurs at 1000-fold higher concentrations (>177 mg l^{-1}) with longer exposure (>48 h). Chlorite appears to produce a chronic inflammatory response characterized by a systemic proliferation of macrophages without gill pathology. Under laboratory conditions fathead minnows recover rapidly from acute exposures to both compounds.

Effective biocidal application of chlorine dioxide by power plants would require concentrations similar to those used for chlorine (i.e. 0.5 – 2.0 mgl^{-1}). In instances of low oxidant demand, acutely toxic chlorine dioxide residuals (i.e. $\geqslant 0.19$ mg l^{-1}) could reach receiving waters. Since chlorite concentration would not exceed the initial chlorine dioxide concentration (i.e. $\leqslant 2.0$ mg l^{-1}) it is unlikely that chlorite resulting from power plant biofouling control would be acutely toxic to fish.

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