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Comparative toxicity of sodium carbonate peroxyhydrate to freshwater organisms

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ABSTRACT

Sodium carbonate peroxyhydrate (SCP) is a granular algaecide containing H_2O_2 as an active ingredient to control growth of noxious algae. Measurements of sensitivities of target and non-target species to hydrogen peroxide are necessary for water resource managers to make informed decisions and minimize risks for non-target species when treating noxious algae. The objective of this study was to measure and compare responses among a target noxious alga (cyanobacterium *Microcystis aeruginosa*) and non-target organisms including a eukaryotic alga (chlorophyte *Pseudokirchneriella subcapitata*), microcrustacean (*Ceriodaphnia dubia*), benthic amphipod (*Hyalella azteca*), and fathead minnow (*Pimephales promelas*) to exposures of hydrogen peroxide as SCP. Hydrogen peroxide exposures were confirmed using the I_3^- method. SCP margins of safety for these organisms were compared with published toxicity data to provide context for other commonly used algaecides and herbicides (e.g. copper formulations, endothall, and diquat dibromide). Algal responses (cell density and chlorophyll *a* concentrations) and animal mortality were measured after 96 h aqueous exposures to SCP in laboratory-formulated water to estimate EC_{50} and LC_{50} values, as well as potency slopes. Despite a shorter test duration, *M. aeruginosa* was more sensitive to hydrogen peroxide as SCP (96 h EC_{50}:0.9–1.0 mg L^{-1} H_2O_2) than the eukaryotic alga *P. subcapitata* (7-d EC_{50}:5.2–9.2 mg L^{-1} H_2O_2), indicating potential for selective control of prokaryotic algae. For the three non-target animals evaluated, measured 96-h LC_{50} values ranged from 1.0 to 19.7 mg L^{-1} H_2O_2. *C. dubia* was the most sensitive species, and the least sensitive species was *P. promelas,* which is not likely to be affected by concentrations of hydrogen peroxide as SCP that would be used to control noxious algae (e.g. *M. aeruginosa*). Based on information from peer-reviewed literature, other algaecides could be similarly selective for cyanobacteria. Of the algaecides compared, SCP can selectively mitigate risks associated with noxious cyanobacterial growths (e.g. *M. aeruginosa*), with an enhanced margin of safety for non-target species (e.g. *P. promelas*).

1. Introduction

Sodium carbonate peroxyhydrate (SCP) is a relatively new, hydrogen peroxide-based active ingredient (USEPA, 2004) used by water resource managers in algaecide formulations to control growths of noxious algae that interfere with critical uses of water resources (Gettys et al., 2014). As a granular algaecide, SCP is applied by broadcast over a treatment area from a boat or the shore (Bishop and Rodgers, 2011), dissolved in site water and sprayed, or mixed and injected into the water column, depending on the specific location and distribution of target algae. A treatment goal for using an algaecide to control the growth of noxious algae is to maximize efficacy for target algal species while minimizing risks for non-target species. To use SCP effectively and efficiently for controlling noxious algal growths in aquatic systems, comparative toxicity data are needed for both target and non-target species, which are anticipated to have different sensitivities. Comparative toxicity studies often evaluate the toxicity of a single constituent to an array of organisms or contrast the toxicity of an array of constituents to a select organism(s). Laboratory experiments involve exposing organisms in relatively unconfounded situations in order to discern innate sensitivity. Ranking organisms in terms of their sensitivity to SCP provides information about types of algae (e.g. prokaryotic vs. eukaryotic) that can be effectively controlled by SCP, and can be used to calculate potential margins of safety for non-target organisms. After relative sensitivity information is measured for a range of target and non-target organisms exposed to SCP, comparisons of toxicity data for SCP and other algaecides can provide context for the relative toxicity of different active ingredients that are available for use (i.e. hydrogen peroxide, copper formulations, endothal, and diquat dibromide). These comparative toxicity data provide information necessary for making scientifically defensible algal management decisions (Fitzgerald 1964; Fitzgerald and Jackson 1979;

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Mastin et al., 2002; Osgood 2007).

Efficacy for target algal populations is central to a successful algaecide treatment design. Drabkova et al. (2007) suggested that prokaryotic cyanobacteria were more sensitive than eukaryotic green algae to exposures of hydrogen peroxide. This indicates a potential benefit of SCP as a selective algaecide for treating target cyanobacteria, while minimizing effects for non-target eukaryotic algal species. Exposure to a series of hydrogen peroxide concentrations from SCP can identify the relative sensitivities (i.e. EC_{50s}) of and potency (i.e. exposure-response slopes) to *Microcystis aeruginosa* Kützing, a prokaryotic cyanobacterium that can produce toxins (e.g. microcystins and nodularins; WHO, 1993, Falconer 1999; Carmichael et al., 2001; Zurawell et al., 2005), and *Pseudokirchneriella subcapitata* Gomont, a eukaryotic green alga that can benefit some water resources as a source of food for aquatic animals (USEPA, 2002). Management decisions to minimize risks for non-target organisms can be supported by comparative toxicity information (i.e. LC_{50} values and exposure-response slopes) for a taxonomic range of animals (i.e. invertebrates and vertebrates). *Ceriodaphnia dubia* Richard (micro crustacean) and *Hyalella azteca* Saussure (amphipod) are invertebrates that inhabit water columns and sediment-water interfaces of North American water bodies, respectively (USEPA, 2000; 2002; APHA, 2005). *Pimephales promelas* Rafinesque (fathead minnow) inhabits water columns of North America and provide a means for contrasting the responses of a vertebrate with responses of invertebrates test species (USEPA, 2002; APHA, 2005) in the present context of exposures to an SCP algaecide. The non-target animal species included in this study are common test organisms for evaluating the potencies of pesticides and other toxic materials (USEPA, 2000; 2002; APHA, 2005). This taxonomic range of target and non-target species improves the confidence with which toxicity data for SCP can be used for risk assessments.

Algaecide exposure concentrations must be confirmed to evaluate toxicity to target and non-target organisms (USEPA, 2002). The analytical method used to confirm H_2O_2 from SCP exposures must be sensitive enough to measure concentrations of hydrogen peroxide within the manufacturer's recommended application rates. Klassen et al. (1994) demonstrated a simple and sensitive as well as accurate method for measuring H_2O_2 concentrations as low as $\sim$0.1 mg L^{-1} by reacting samples with acidified potassium iodide (KI) and using visible wavelength spectrometry to measure the optical absorbance of the triiodide (I_3) formed. Kinley et al. (2015) indicated that the attributes of the I_3^- method (i.e. detection limit and storage stability) are sufficient to confirm concentrations of H_2O_2 from SCP exposures in these laboratory toxicity tests.

The overall objective of this study was to compare responses of an array of freshwater organisms following exposures to hydrogen peroxide as SCP in laboratory formulated water. To achieve this overall objective, specific objectives were to (i) measure and compare responses of a prokaryotic alga (*M. aeruginosa*) and a eukaryotic alga (*P. subcapitata*) in terms of cell density and chlorophyll *a* concentrations to 96 h exposures of hydrogen peroxide as SCP, (ii) measure and compare responses of a vertebrate (*P. promelas*) and invertebrates (*C. dubia* and *H. azteca*) in terms of mortality to 96 hr exposures of hydrogen peroxide as SCP, (iii) confirm exposures of hydrogen peroxide resulting from additions of SCP, and (iv) compare measured toxicity of SCP to vertebrates, invertebrates, and algae with published toxicity data for copper algaecide formulations, endothall, and diquat dibromide.

Table 1
Physical properties and fate characteristics of Phycomycin® SCP.

Active ingredient	85% SCP
Maximum application	36.9 mg L^{-1} (10.2 mg L^{-1} H_2O_2)[a]
Formulation	SCP and inert ingredients
Physical state	Coarse white grains[a]
Water solubility	140 g/L at 24 °C[a]
Boiling Point	Not applicable[a]
pH	10.4–10.6 (1% solution)[a]
CAS number	497-19-8[a]

[a] AB (2007).

2. Materials and methods

2.1. Preparation of SCP exposures

The algaecide Phycomycin® SCP (Applied Biochemists Inc., Germantown, WI 53022; Table 1), was used as the source of SCP. Exposures were accomplished by dissolving SCP (active ingredient 27.6% H_2O_2) granules in moderately hard water or algal growth medium (AB, 2007). Hydrogen peroxide concentrations were measured spectrophotometrically immediately after complete dissolution of SCP using the I_3^- method with a 1 cm cuvette and SpectraMax®M2 Microplate Reader (Molecular Devices Corp. Sunnyvale, CA 94089; Klassen et al., 1994; Kinley et al., 2015).

2.2. Toxicity testing procedures

Static, non-renewal exposures were conducted in 250 mL borosilicate beakers (USEPA, 1996a, 1996b). All organisms were exposed to a range of hydrogen peroxide concentrations as SCP to elicit responses ranging from no response to complete mortality or inhibition of growth.

P. subcapitata was obtained from the University of Texas culture collection (UTEX 1648, Austin, TX), and *M. aeruginosa* from the Canadian Phycological Culture Center (CPCC 300) at the University of Waterloo in Ontario, Canada. Prior to testing, both algae were grown in COMBO medium (Kilham et al., 1998) with an 18:6 h light: dark photoperiod at 23 ± 2 °C, illuminated by cool-white fluorescent bulbs (Residential Ecolux 40 W, GE) at 2660 lx. Algae were exposed in 250 mL beakers containing 200 mL of COMBO medium with a cell density of $\sim 10^6$ cells mL^{-1}. Responses of algae to exposures (i.e. cell densities and chlorophyll *a* concentrations) were measured initially and after 96 h. Cell densities were measured using light microscopy and an improved Neubauer hemocytometer (with a gridded sample chamber; Hausser Scientific Co. Horsham, PA 19044) and chlorophyll *a* concentrations were measured fluorometrically (APHA, 2005) using a SpectraMax®M2 Microplate Reader (Molecular Devices Corp. Sunnyvale, CA 94089; APHA 2005).

Freshwater animals (*P. promelas, C. dubia,* and *H. azteca*) were cultured at Clemson University's Aquatic Animal Research Laboratory (AARL) according to methods of the United States Environmental Protection Agency (USEPA, 2002), and in compliance with Clemson University's Institutional Animal Care and Use Committee (IACUC) protocols. All toxicity tests for *P. promelas* were conducted by exposing 30 organisms (< 24-h old) per concentration (10 organisms per replicate for 3 replicates) in 250 mL borosilicate beakers. Toxicity tests for *C. dubia* were conducted by exposing 20 organisms (< 24-h old) per concentration (5 organisms per replicate for 4 replicates) in 15 mL borosilicate vials. During exposures, *C. dubia* were fed once daily with 200 µL of a 1:1 mixture of *P. subcapitata* and YCT (yeast, cerophyll, trout chow). Toxicity tests for *H. azteca* were conducted by exposing 30 organisms (2–3 weeks old) per concentration (10 organisms per