



Identification and Initial Screening of New Compounds to Control Harmful Algal Blooms

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PURPOSE: This technical note identifies, prioritizes, and evaluates “reduced risk” chemical compounds that have the potential to selectively control harmful algal blooms (HAB). Results of these evaluations are shared with key regulatory agencies to expedite development of new algacide registrations for use in lakes and reservoirs.

BACKGROUND: The proliferation of freshwater HAB in lakes and reservoirs around the world (Hallegraeff 1993) represents a direct threat to human and animal health (Billings 1981, Galey et al. 1987, Mahmood et al. 1988, Carmichael and Falconer 1993, Henriksen et al. 1997, Saker et al. 1999, Carmichael et al. 2001, Annadotter et al. 2005). Toxins and secondary metabolites produced by these algae are neurotoxins (i.e. endotoxins, anatoxins, and saxitoxins) and hepatoxins (i.e. microcystins and cylindrospermopsins). The health risk possibilities for HAB have been expanded by new discoveries that cyanobacteria produce a neurodegenerative amino acid toxin that can be linked to amyotrophic lateral sclerosis/Parkinson dementia (Carmichael 2005).

Toxins produced by HAB degrade water quality and jeopardize aquatic ecosystem health by causing fish and wildlife mortality (Penaloza et al. 1990, Lindholm et al. 1999, Birrenkott et al. 2004, Wilde et al. 2005). HAB also negatively impact threatened and endangered species (Wilde et al. 2005) and impede growth of aquatic flora and fauna (Casanova et al. 1999, Oberemm et al. 1999, LeBlanc et al. 2005). In addition, nuisance algal mats can interfere with reservoir operations such as drinking water intakes and hydroelectric generation, cause taste and odor problems in drinking water supplies (reviewed by Carmichael and Falconer (1993)), and produce mortality or off-flavor in farm-raised channel catfish (Martin et al. 1991, Schrader and Blevins 1993, Zimba et al. 2001) at a cost to producers of up to \$60 million annually (Tucker 2000).

HAB are expanding from tropical to temperate waters, and the factors that contribute to their bloom formation, toxin production, and toxin release have proven to be multifaceted and poorly understood (Dokulil and Teubner 2000). Prudent HAB management requires monitoring the water column for algal abundance, nutrient concentrations, temperature, oxygen, and pH. More often, however, current management of HAB has been in a reactive mode, after the bloom has formed. Copper is typically applied as the chemical control option. Copper sulfate and chelated copper products are widely used because they are efficacious and there are no water use restrictions on drinking, swimming, or fishing.

Sensitivity of algae to ionic copper has been found to vary considerably for different algal species (McKnight et al. 1983, Lembi 2000). Nonselective control of HAB can remove beneficial phytoplankton algae, and this may accelerate the growth of targeted HAB due to a lack of competition. Copper treatments at the height of a bloom may temporarily control the nuisance condition (Hullebusch et al. 2002), but often result in release of toxins to the water column (Jones and Orr 1994, Lam et al. 1995, Lam and Prepas 1997). Moreover, repeated use of one chemical for control increases the probability that an organism will become resistant to that product. For

example, resistance to copper has been observed in the cyanobacteria *Microcystis aeruginosa* (Garcia-Villada et al. 2004).

Despite the rise of HAB and recognition of their negative impacts in U.S. water bodies, there have been no coordinated Federal, state, or private efforts towards evaluating potential new algaecides. Current management approaches tend to be reactive (rather than pro-active), inconsistent, and nonselective. Control of the HAB threat requires an innovative and proactive approach to adequately protect the Nation's water resources (Netherland et al. 2005). Development and registration of cost-effective and selective algaecides benefit Corps of Engineer (CE) flood control reservoirs and other aquatic systems throughout the country that are plagued by HAB.

APPROACH: The first step in developing new chemistries for control of HAB is cooperation with industry to identify potential compounds. These compounds should have toxicology profiles that are “reduced-risk” with limited or no water use restrictions on drinking, swimming, or fishing in chemically treated water.

Once compounds are identified and prioritized, small-scale and field evaluations will be undertaken to determine herbicide efficacy and selectivity. The process of developing selective control techniques for aquatic herbicide use is summarized by Poovey and Getsinger (2005). Although these techniques have been tested and are routinely employed in operations for aquatic macrophyte removal (Getsinger et al. 1997, 2001, 2002; Parsons et al. 2001, 2004; Poovey et al. 2004), they have not yet been adopted for control of HAB.

Proactive and predictable control of harmful algae may be achieved using low application rates (1 to 25 $\mu\text{g L}^{-1}$) of algaecides that are specific to plant enzyme systems, if the target algal species is detected before bloom formation. Such chemicals would immediately terminate growth and cause slow, incremental lysis of existing cells. Selective control of HAB would encourage desirable algal population expansion and further deter growth of harmful algae. Low algaecide use rates would reduce environmental loading as well as lower the cost of HAB management. This proactive strategy will require sampling of nuisance algal populations and nutrient concentrations in the water column to monitor conditions that may induce bloom formation.

ERDC scientists will work with U.S. Environmental Protection Agency Office of Pesticide Programs (EPA-OPP) to determine regulatory criteria necessary for achieving aquatic registrations and development of labels for use in lakes and reservoirs. This “laboratory-to-label” approach has proven successful for the registration of three new herbicides used to control invasive aquatic macrophytes (Netherland et al. 2005, Poovey and Getsinger 2005).

PROGRESS: The ERDC Chemical Control Technology Team convened a group of cooperators, including the chemical industry, the U.S. Department of Agriculture (USDA) IR-4 Project (minor use pesticides), and the USEPA-OPP to discuss the lack of alternatives for controlling toxic and nuisance algae blooms. This group identified several compounds that target plant-specific enzyme sites, such as phytoene desaturase (PDS), acetolactate synthase (ALS), and protoporphyrinogen oxidase (PPO), which typically have very low mammalian and non-target toxicity profiles. Other candidate compounds included hydrogen peroxide, acetic acid, and natural products, such as derivatives from 9,10-anthraquinone (Schrader et al. 2003).

Evaluations of candidate compounds in replicated small-scale studies with potential HAB organisms are in progress. Research collaborators include academic and Federal research groups that have algal culture and control expertise, including Purdue University, the University of South Carolina, and the USDA Natural Products Utilization Research Unit at the University of Mississippi. Target algal species that have been cultured are *Lyngbya wollei*, *Stigonematales* sp., *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *Oscillatoria perornata* and *Prymnesium parvum*. Brief descriptions of these organisms and their negative impacts in aquatic systems are presented below.

Lyngbya wollei is a filamentous cyanobacterium that forms thick black mats in rivers and lakes, shading out beneficial native aquatic plants and algae (Beer et al. 1986), degrading water quality, and emitting foul odors. It also has been documented to produce toxins (Yin et al. 1997) and paralytic shellfish poisons (Carmichael et al. 1997). Unlike other algae, *L. wollei* persists throughout the year, overwintering as thick benthic mats that partially rise to the surface during the warmer months (Speziale et al. 1991). Infestations are becoming more common throughout the southeastern United States. It is a nuisance in the Everglades, Rainbow and Crystal Rivers in Florida, Guntersville Reservoir in Alabama, and the lower Rio Grande, Texas. *Lyngbya wollei* survived treatments of diquat (Phlips et al. 1992), chelated copper formulations (Glomski and Netherland, unpublished data), and the amine salt formulation of endothall in laboratory studies (Glomski and Netherland, unpublished data). Control efforts of this species have been hampered by the thick sheath of mucilage that surrounds the algal cells, and the presence of both surface and benthic mats in the spring and summer.

Stigonematales sp. is an epiphytic cyanobacterium found on invasive submersed aquatic plants, such as *Hydrilla verticillata*, which produces a toxin that has been linked to bald eagle deaths from avian vacuolar myelinopathy (AVM; Wilde et al. 2005). Birds that contract AVM have been reported to prey on coots that have consumed contaminated hydrilla. The toxin produced by *Stigonematales* sp. is still unknown, as are water quality characteristics that promote the presence of these algal species. Although present on 95 percent of the hydrilla surface area in some instances, there is no other information about this epiphyte and its relationship with hydrilla or its ability to colonize native vegetation. *Stigonematales* sp. occurs with hydrilla in CE reservoirs in Arkansas, Texas, and South Carolina, and other sites where AVM has been diagnosed (Wilde et al. 2005).

Cylindrospermopsis raciborskii is a nitrogen-fixing cyanobacterium that forms blooms in nutrient-enriched waterbodies of the Southeast (Williams et al. 2005). This species is able to dominate more than 90 percent of the water column in shallow subtropical lakes and may be found throughout the lake system rather than in isolated bloom areas. Originally thought to be a tropical and subtropical species, it was found blooming in an Indiana lake in 2001, and has now spread across the Midwest (Lembi 2005). Although the toxin associated with this species, cylindrospermopsin, affects the human liver (Hawkins et al. 1985), adverse human health effects have not been reported in the United States. Chlorination has been investigated as a control measure in drinking water reservoirs in Australia (Senogles et al. 2000). In laboratory studies, Schrader et al. (2003) found that derivatives from the natural compound 9, 10-anthraquinone were effective against *Cylindrospermopsis* spp., and this chemical could have potential as a selective control tool.

Microcystis aeruginosa is unicellular colonial cyanobacterium that often appears reddish brown when in bloom, instead of the bluish color typical of many cyanobacteria. Widespread in freshwaters, blooms of *M. aeruginosa* have occurred in several large estuarine systems, such as Chesapeake Bay in Maryland, Albemarle-Pamlico Sound in North Carolina, and Florida Bay in Florida. An extensive and serious bloom recently occurred in the St. Johns River, Florida. Because more than 60 variants of microcystin hepatotoxins have been described (Schneider et al. 2005), these blooms represent national economic and environmental threats. Diquat (Phlips et al. 1992, Peterson et al. 1997) and the amine formulation of endothall (Ruzycki et al. 1998) have been investigated for control of *M. aeruginosa* in laboratory studies. As an alternative, the amino acid lysine has been reported to selectively control *Microcystis* spp (Hehmann et al. 2002).

Oscillatoria perornata is a filamentous cyanobacterium that produces metabolites (2-methylisoborneol or MIB), which cause off-flavor in farm-raised channel catfish. Off-flavors result in fish that is unpalatable and may increase production costs by 10 to 20 percent in the United States (Paerl and Tucker 1995). Copper sulfate, chelated copper compounds and diuron are the only compounds currently approved for use in catfish ponds; however, these products are nonselective, and therefore, lethal to many phytoplankton that are needed for fish production. Through a series of bioassays (Schrader et al. 1998, 2003, 2004; Schrader and Harries 2001), a derivative from the natural compound 9,10-anthraquinone has been identified and reported successful for selective control of *O. perornata*.

Prymnesium parvum is a golden alga that releases chemical compounds which combine with cations, such as magnesium and calcium, in the water column to create toxins. *P. parvum* toxins are a major cause of fish kills throughout Texas, including Lake Texoma, a CE reservoir. Although some Texas fish kills have occurred in summer months, the majority of fish kills have occurred in winter months when water temperatures are cooler (Glass 1991). Fish kills associated with *P. parvum* also have been identified in Alabama, Arkansas, Georgia, New Mexico, North Carolina, Oklahoma, South Carolina, and Wyoming. Chelated copper compounds (Duke et al. 2005) and acetic acid (Glass et al. 1991) have been effective against *P. parvum*; however, these are nonselective treatments. An alternative may be a compound that targets the carotenoid pathway, such as a PDS inhibitor. Because *P. parvum* has high concentrations of carotenoid and xanthophyll pigments, it may be more susceptible to this type of algacide than beneficial diatom and green algae.

FUTURE WORK: Culture and screening methods for *Prymnesium parvum* will be undertaken. Outdoor mesocosm studies and field verification trials will be the next step for further evaluating new chemicals against *Lyngbya wollei*, *Stigonematales* sp., *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, and *Oscillatoria perornata*. Middle- and large-scale research will support results from small-scale studies that yielded selective control of these harmful algae. Data collected from the laboratory, mesocosm, and field are needed to determine regulatory criteria necessary for achieving aquatic registrations for successful use of algacides in lakes and reservoirs.

CONCLUSIONS: The recent proliferation of harmful algal blooms (HAB) caused by *Lyngbya*, *Prymnesium*, *Stigonematales*, *Cylindrospermopsis*, *Microcystis*, and others in lakes and reservoirs represents a direct threat to human and animal health, water quality, and overall aquatic ecosystem health, including impacts on threatened and endangered species.

Despite the rise of HAB, and recognition of their negative impacts in U.S. water bodies, there have been no coordinated Federal, state, or private efforts towards evaluating potential new algaecides. Current management strategies are reactive, inconsistent, and nonselective. Control of these noxious algae requires an innovative and proactive approach to adequately protect the Nation's water resources and CE projects.

Several reduced-risk products that are good candidates for evaluation have been identified. Collaborative small-scale research is in progress and should be continued. ERDC scientists will work with USEPA-OPP to determine regulatory criteria necessary for achieving algaecide registrations for use in lakes and reservoirs.

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