



## Screening of Various Herbicide Modes of Action for Selective Control of Algae Responsible for Harmful Blooms

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**PURPOSE:** This research seeks to identify “reduced risk” chemical compounds that have selective activity against harmful algal blooms (HAB). The U.S. Army Corps of Engineers is responsible for managing numerous large reservoirs throughout the country, and the recent proliferation of HAB and the limited tools available for controlling these nuisance algae at a large scale have caused many Corps resource managers to seek non-conventional control methods. Numerous herbicides that target plant-specific enzyme sites, such as phytoene desaturase (PDS) and acetolactate synthase (ALS), are currently under consideration for registration and use in aquatic sites by the U.S. Environmental Protection Agency (USEPA). These compounds exhibit a wide margin of safety for fish and wildlife, and have proven to be highly selective herbicides against numerous aquatic macrophyte species. Because algae, including cyanobacteria, and higher plants have many of the same enzyme systems, some of the enzyme-inhibiting herbicides may be active against algal species responsible for harmful blooms. The U.S. Army Engineer Research and Development Center, Aquatic Nuisance Species Research Program (ANSRP) has encouraged research into innovative methods for managing HAB. While selective control of harmful algae remains an elusive goal, herbicides with modes of action that target the PDS and ALS enzyme systems are being screened to determine if these compounds have the potential to selectively control or inhibit the growth of bloom-forming algae.

**BACKGROUND:** 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). The proliferation of freshwater HAB in lakes and reservoirs around the world (Hallegraeff 1993) represents a potential threat to human and animal health (Billings 1981; Mahmood et al. 1988; Saker et al. 1999; Carmichael et al. 2001). Several species recognized as being particularly problematic bloom formers include *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *Oscillatoria perornata*, *Prymnesium parvum*, and *Pseudanabaena limnetica* (Poovey and Netherland 2006). These algae are known to produce neurotoxins (i.e., endotoxins, anatoxins, and saxitoxins) and hepatoxins (i.e., microcystins and cylindrospermopsins). HAB have been associated with degraded water quality and blooms can jeopardize aquatic ecosystem health by causing fish and wildlife mortality (Penaloza et al. 1990; Henriksen et al. 1997; Lindholm et al. 1999; Birrenkott et al. 2004; Wilde et al. 2005). HAB can also impede growth of aquatic flora and fauna (Casanova et al. 1999; Oberemm et al. 1998; LeBlanc et al. 2005).

Despite the rise of HAB and recognition of their negative impacts in U.S. water bodies, efforts to identify and evaluate potential new algaecides have received minimal research attention. Given the large number of reservoirs under Corps of Engineers’ management, research to identify new cost-effective and selective algaecides for HAB control would benefit both the Corps of Engineers as well as other natural resource managers responsible for maintaining water quality in large reservoirs.

Algal control efforts have largely relied on the use of copper-based compounds, which are broad-spectrum, to target algae after they have grown to nuisance levels. While there are many benefits to copper use (cost-effective and no restrictions on water use), non-selective and reactive control of HAB can remove beneficial algae. Removal of these beneficial algae leaves open niches, which may accelerate the recovery of targeted HAB due to a lack of competition. There are also concerns of the potential for large-scale release of toxins following the large-scale use of contact algaecides such as copper (Jones and Orr 1994). The inability to identify cost-effective and selective alternatives to copper has contributed to skepticism regarding the development of new algaecides and application strategies in the past; nonetheless, research on copper continues with studies to evaluate copper formulations and water quality on efficacy (Murray-Gulde et al. 2002) as well as studies to determine the potential for algal species to become copper-tolerant (Lembi 2000).

Photosynthetic-inhibiting herbicides, such as diquat, diuron, and simazine, also have been investigated for management of HAB. Although diquat, which is registered for aquatic use, has been tested against various cyanobacteria species (Philips et al. 1992), it is not widely used in operational control efforts. Alternatively, diuron and simazine have been used for broad-spectrum algae control in smaller water bodies in the past; however, neither of these compounds is nationally registered for aquatic use today. Diuron has been granted a Section 18 emergency use label issued by USEPA to control algae that cause taste and odor problems in catfish ponds (Schrader et al. 1998; Schrader and Harries 2001; Schrader et al. 2003, 2004; Tucker 2000). Because diuron is a broad-spectrum product, U.S. Department of Agriculture scientists are involved in research to find and evaluate natural and reduced-risk compounds for effective selective algae control in catfish farming (Schrader 2005; Schrader and Harries 2001; Schrader et al. 2003, 2004).

Within the field of aquatic plant management, there are submersed plants, such as hydrilla (*Hydrilla verticillata* L.f. Royle), Eurasian watermilfoil (*Myriophyllum spicatum* L), and egeria (*Egeria densa* Planch.) that are both invasive and problematic. Efforts to control these plants with herbicides focus on both efficacy and selectivity (Netherland et al. 2005). While no approach is completely selective, management efforts seek to minimize damage to native vegetation through the choice of herbicide, use rate, and timing of application (Poovey and Getsinger 2005). At present, the concept of selectivity, in which specific algal species are targeted for control, has not been applied to HAB management.

The recent development and registration of highly selective new herbicides with different modes of action presents a unique opportunity to investigate the algaecide properties of these compounds. Unlike diuron or simazine, which are broad-spectrum, these enzyme-specific inhibitors may be highly specific for certain species of problem algae. Moreover, they have reduced-risk toxicology profiles. As such, they likely will support aquatic labels that do not have water use restrictions, where one may use the water for drinking, swimming, and fishing immediately after application. This study evaluated herbicides with different modes of action: one PDS inhibitor and four ALS inhibitors. One of the ALS inhibitors evaluated, penoxsulam, has been granted USEPA Section 3 registration for national use as an aquatic herbicide, while two other products, imazamox and bispyribac-sodium, are in the process of seeking Section 3 registrations. This technical note describes small-scale flask studies to determine if these new chemistries are active against organisms responsible for HAB and if they show potential for selective control of HAB.

**METHODS:** Two studies were conducted in laboratories at Purdue University, West Lafayette, IN. For both studies, all algae were maintained in unialgal culture in an inorganic liquid medium. Efficacy studies were conducted in 500-mL Erlenmeyer flasks filled with 200 mL of an appropriate culture medium, the alga of interest, and a known concentration of herbicide. Flasks were placed in a controlled-environment growth chamber at 65 to 120  $\mu\text{mol}/\text{m}^2/\text{sec}$  (depending on species), a temperature of 25 °C, and a 16:8 h light:dark photoperiod.

The first study tested a PDS inhibitor (CE-PDS-111) and an ALS inhibitor (CE-ALS-112) against four nuisance algae: planktonic blue-green algae (Cyanobacteria) *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, and *Pseudanabaena limnetica*, and the planktonic green alga (Chlorophyta) *Ankistrodesmus falcatus*. These compounds were screened at rates of 0, 5, 10, and 25  $\mu\text{g ai L}^{-1}$ .

The second study screened three ALS inhibitors (bispyribac, imazamox, and penoxsulam) at use rates of 100, 200, and 500  $\mu\text{g ai L}^{-1}$  for activity against the problematic planktonic blue-green algae *Anabaena* sp, *C. raciborskii*, *M. aeruginosa*, and *P. limnetica*, and the planktonic green alga *A. falcatus*. Two beneficial green algal species were also included, *Scenedesmus quadricauda* and *Selenastrum* sp.

After a two-week exposure period, all flasks were filtered. The planktonic algae were measured for chlorophyll *a* content prior to treatment (referred to as initials) and at the conclusion of a 2-week exposure period. Methanol controls were included for those compounds (CE-PDS-111) dissolved in methanol prior to application.

All treatments consisted of four replicate flasks. Treatments were compared via analysis of variance (ANOVA). Means were separated using the Student Newman Keul's test (0.05 level of significance) for CE-PDS-111 and CE-ALS-112. Efficacy between compounds or algal species was not compared, only various rates within a compound were compared. For the bispyribac, imazamox, and penoxsulam trials, data were subjected to ANOVA and treatments were compared to untreated controls via a Dunnett's test at the 0.05 level of significance.

**RESULTS AND DISCUSSION:** All of the algal species evaluated grew readily during the course of the treatment, evidenced by the increase in chlorophyll content between initial and untreated reference flasks (Table 1). Results indicate that CE-PDS-111 and CE-ALS-112 demonstrate selective properties for algae control. Compound CE-ALS-112 reduced *Cylindrospermopsis* cells, while CE-PDS-111 reduced *Ankistrodesmus*. Both herbicides were effective in controlling *Microcystis*; however, neither impacted *Pseudanabaena* at the concentrations and exposure time evaluated (Table 1).

This high level of specificity for certain algal species is consistent with the selective activity of ALS herbicides against both emergent and submersed aquatic macrophytes (Getsinger et al. 1994; Chiconela et al. 2004; Koschnick et al. 2007; Glomski and Netherland 2008). Although use patterns for ALS and PDS chemistries for aquatic plant control currently focus on low use rates ( $<100 \mu\text{g ai L}^{-1}$ ) with long-term exposures of 60 to 100 days, phytotoxicity was observed here after 14 days with doses as low as 5 and 10  $\mu\text{g ai L}^{-1}$ . Higher doses and/or longer exposure times might result in complete control, which would reduce the threat of bloom formation and toxin release as

toxin production has been associated with low as well as high algal colony counts for *M. aeruginosa* (Boyer 2007).

<b>Table 1</b>				
<b>The Impact of CE-PDS-111 and CE-ALS-112 as Measured by Chlorophyll <i>a</i> (<math>\mu\text{g L}^{-1}</math>) on Four Species of Algae Following a 2-week Exposure in the Laboratory.</b>				
	Chlorophyll <i>a</i> $\mu\text{g L}^{-1}$			
	<i>Microcystis</i>	<i>Cylindrospermopsis</i>	<i>Pseudanabaena</i>	<i>Ankistrodesmus</i>
Initial	41.4	25.8	55.2	178.6
CE-PDS-111 $\mu\text{g ai L}^{-1}$				
Control	1341.3 <sup>a</sup>	369.8 <sup>a</sup>	1199.9 <sup>a</sup>	4902.6 <sup>a</sup>
Methanol Control	980.4 <sup>b</sup>	414.1 <sup>a</sup>	1399.8 <sup>a</sup>	4652.8 <sup>a</sup>
5	24.4 <sup>c</sup>	331.8 <sup>a</sup>	1123.6 <sup>a</sup>	149.2 <sup>b</sup>
10	64.8 <sup>c</sup>	393.8 <sup>a</sup>	989.6 <sup>a</sup>	89.4 <sup>b</sup>
25	58.9 <sup>c</sup>	509.1 <sup>a</sup>	1429.8 <sup>a</sup>	121.4 <sup>b</sup>
CE-ALS-112 $\mu\text{g ai L}^{-1}$				
Control	961.6 <sup>a</sup>	349.9 <sup>a</sup>	1019.0 <sup>a</sup>	5403.9 <sup>a</sup>
5	153.8 <sup>b,c</sup>	106.5 <sup>b</sup>	1345.7 <sup>a</sup>	4588.1 <sup>a</sup>
10	25.4 <sup>c</sup>	49.5 <sup>b</sup>	1084.9 <sup>a</sup>	4064.2 <sup>a</sup>
25	398.0 <sup>b</sup>	64.6 <sup>b</sup>	889.0 <sup>a</sup>	4280.0 <sup>a</sup>
Note: Each treatment was replicated four times. Effects of each compound were analyzed separately for each algal species. Different letters in a column signify significant differences (S-N-K, $p \leq 0.05$ ). Initial chlorophyll <i>a</i> measurement not included in analyses.				

Initial results of screening with bispyribac and imazamox suggest that these products had limited activity against the various algal species tested (Figures 1 through 7). Aside from the reduction in biomass of the green alga *Scenedesmus* by bispyribac, neither compound showed a rate response nor indicated species selectivity; however, a longer exposure time may increase algal phytoxicity to these herbicides. The increase in chlorophyll *a* content between the initial reading and untreated controls indicates that all algae were actively growing during herbicide exposure.

In contrast, many of the penoxsulam treatments significantly reduced chlorophyll *a* levels compared to untreated controls (Figures 1 through 7). Penoxsulam was highly active against the blue-greens *Cylindrospermopsis* and *Anabaena*, and the green alga *Scenedesmus*, with the lowest dose of  $100 \mu\text{g ai L}^{-1}$  providing >90 percent control. A  $100\text{-}\mu\text{g ai L}^{-1}$  controlled *Pseudanabaena* by only 58 percent; however, as penoxsulam concentrations increased to 200 and  $500 \mu\text{g ai L}^{-1}$ , chlorophyll *a* levels decreased by 85 and 90 percent, respectively. Penoxsulam did not reduce chlorophyll *a* for the noxious algae *Microcystis* and *Ankistrodesmus*, or the beneficial green alga *Selenastrum*.

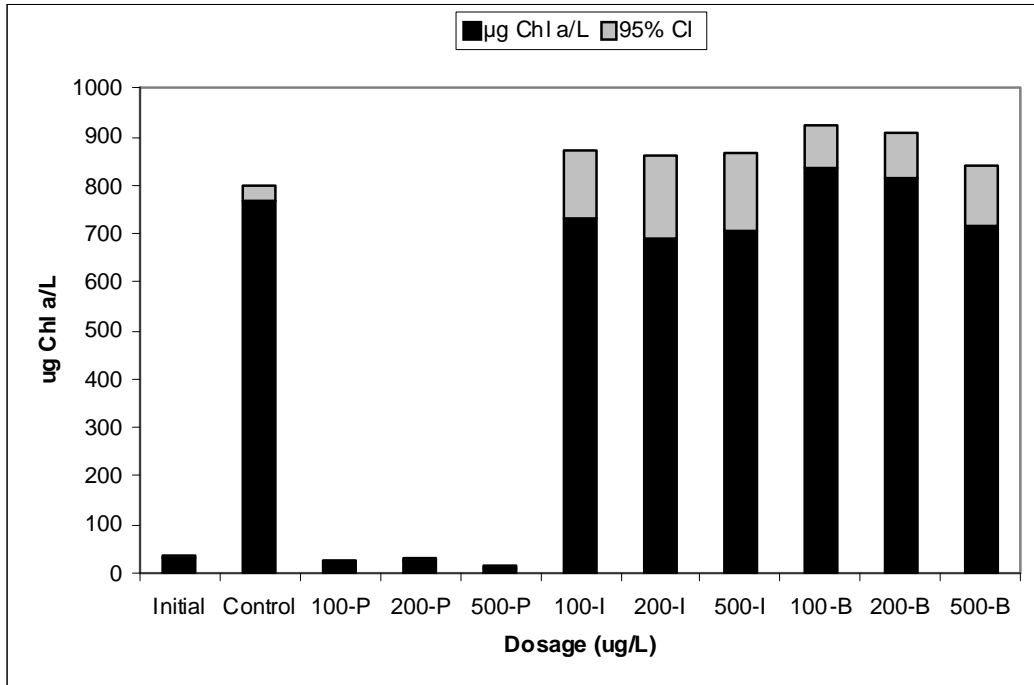


Figure 1. Chlorophyll a response of a unialgal culture of *Cylandrospermopsis raciborskii* to the ALS-inhibiting herbicides penoxsulam (P), Imazamox (I) and Bispyribac (B). Each bar represents the average value of four replicate treatments with a 95-percent confidence interval.

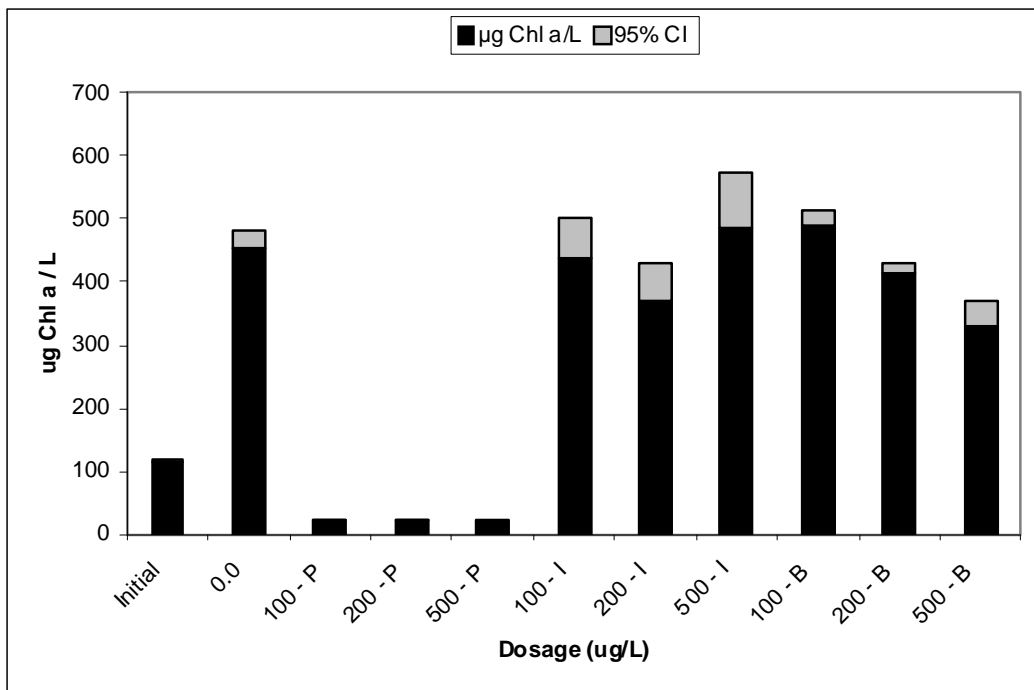


Figure 2. Chlorophyll a response of a unialgal culture of *Anabaena* to the ALS-inhibiting herbicides penoxsulam (P), Imazamox (I) and Bispyribac (B). Each bar represents the average value of four replicate treatments with a 95-percent confidence interval.

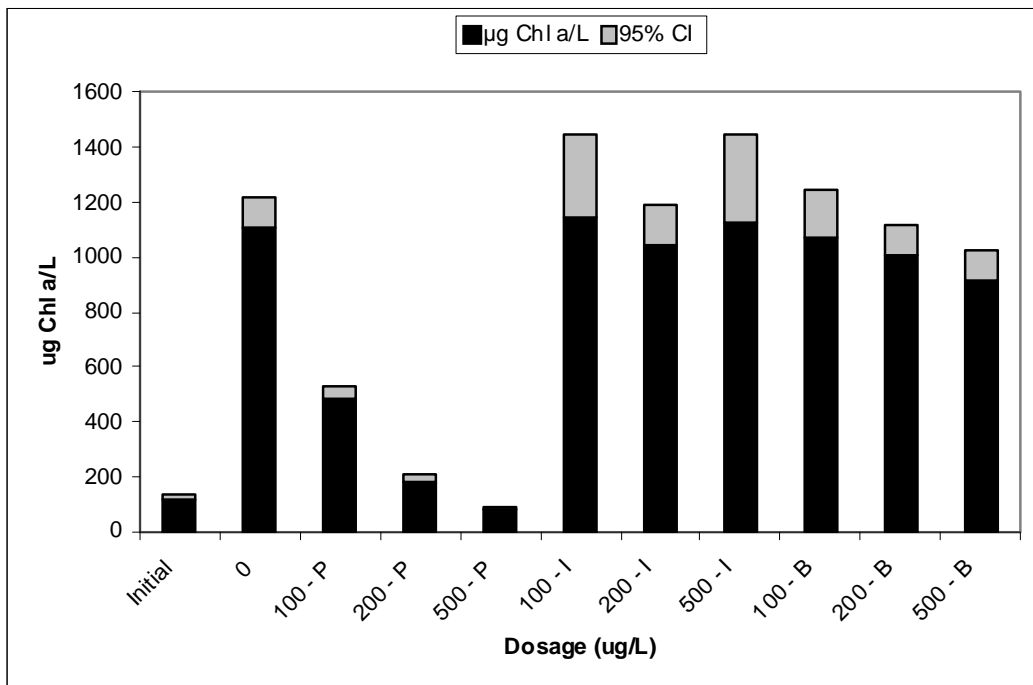


Figure 3. Chlorophyll a response of a unialgal culture of *Pseudanabaena* to the ALS-inhibiting herbicides penoxsulam (P), Imazamox (I) and Bispyribac (B). Each bar represents the average value of four replicate treatments with a 95-percent confidence interval.

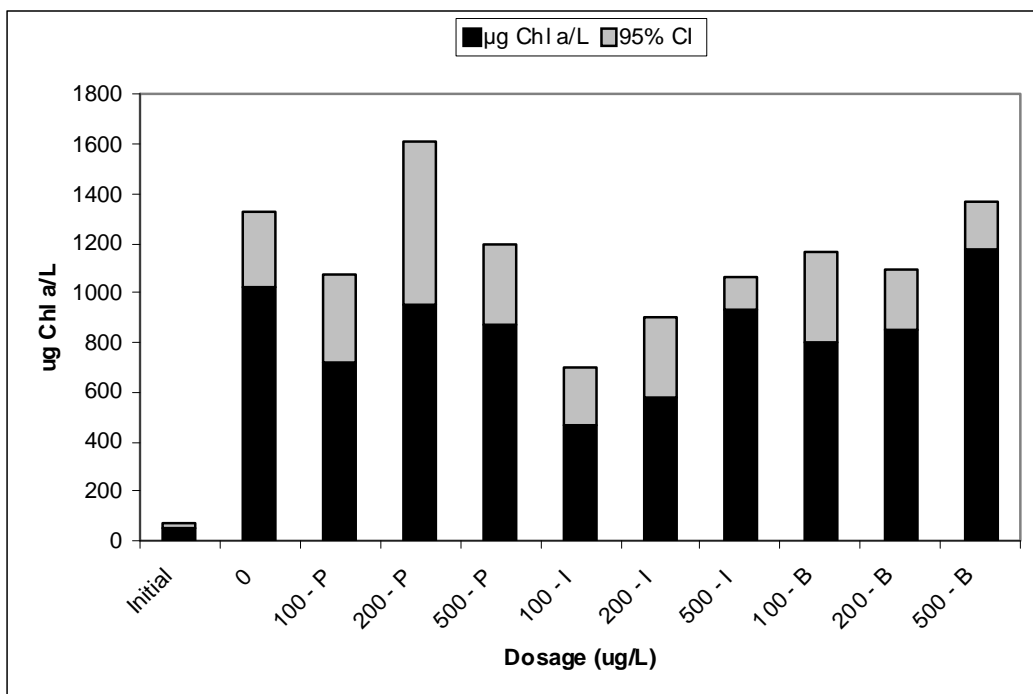


Figure 4. Chlorophyll a response of a unialgal culture of *Microcystis* to the ALS-inhibiting herbicides penoxsulam (P), Imazamox (I) and Bispyribac (B). Each bar represents the average value of four replicate treatments with a 95-percent confidence interval.

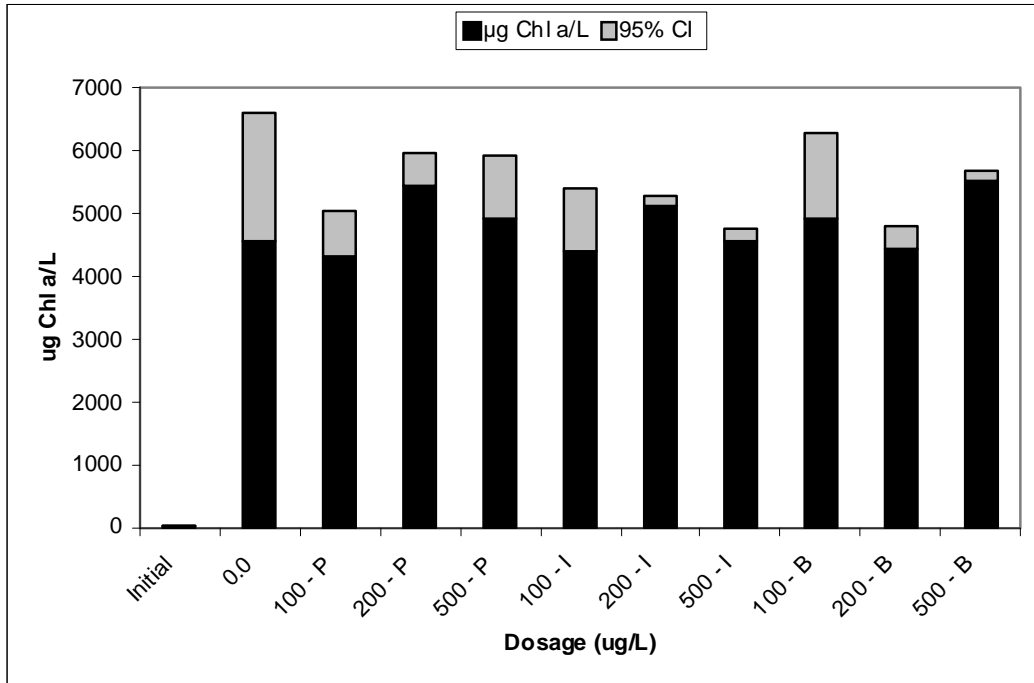


Figure 5. Chlorophyll a response of a unialgal culture of the green alga *Selenastrum* to the ALS-inhibiting herbicides penoxsulam (P), Imazamox (I) and Bispyribac (B). Each bar represents the average value of four replicate treatments with a 95-percent confidence interval.

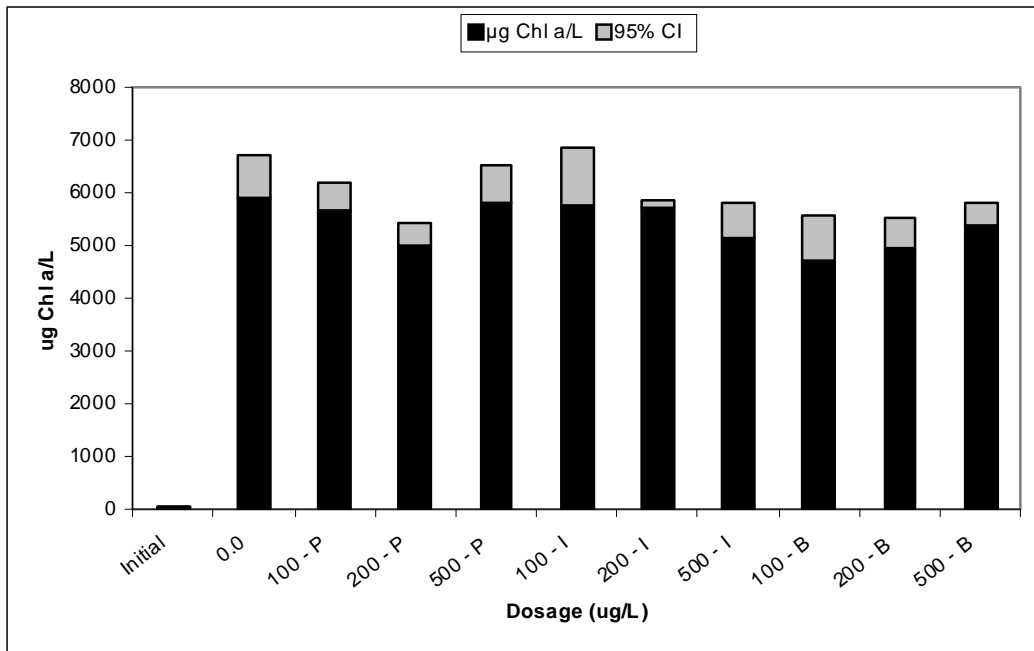


Figure 6. Chlorophyll a response of a unialgal culture of the green alga *Ankistrodesmus* to the ALS-inhibiting herbicides penoxsulam (P), Imazamox (I) and Bispyribac (B). Each bar represents the average of four replicate treatments with a 95-percent confidence interval.

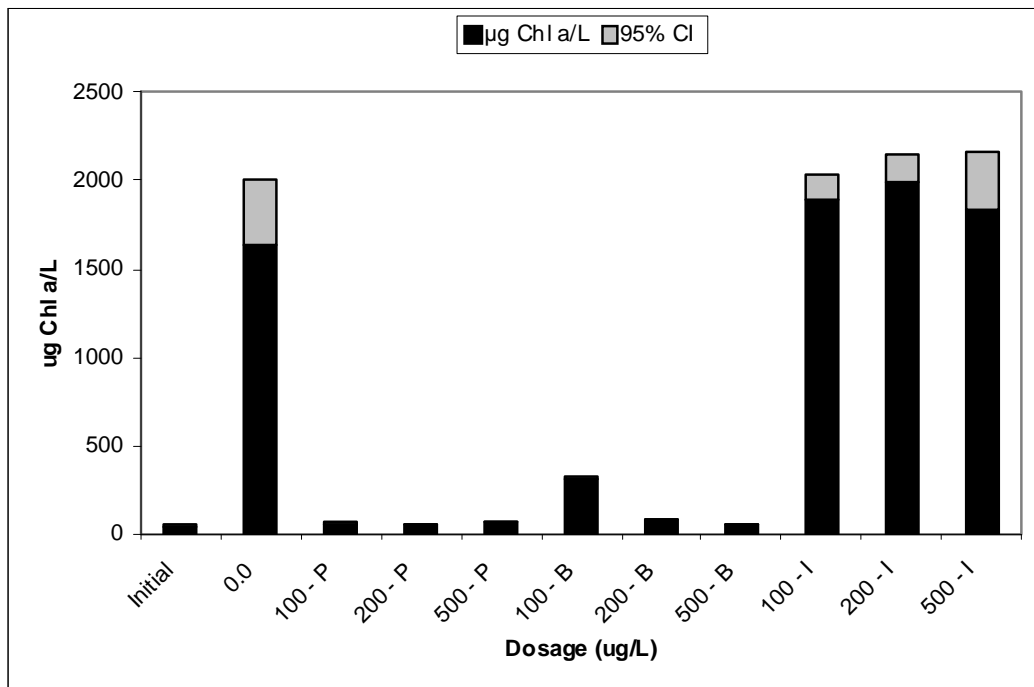


Figure 7. Chlorophyll a response of a unialgal culture of the green alga *Scenedesmus* to the ALS-inhibiting herbicides penoxsulam (P), Imazamox (I) and Bispyribac (B). Each bar represents the average of four replicate treatments with a 95-percent confidence interval.

Results with CE-PDS-111, CE-ALS-112, and penoxsulam treatments indicate that some ALS- and PDS-inhibiting compounds can be effective against organisms responsible for HAB. Short-term exposures used in these studies should be extended to determine the longer-term impact of treatment. Previous work with bensulfuron methyl, another ALS-inhibitor, against submersed aquatic plants indicated that increasing exposure time was more efficacious than increasing herbicide concentration (Nelson et al. 1993; Getsinger et al. 1994).

While penoxsulam did reduce cells of a beneficial green algal species, there was no indication that all these compounds would be active against a broad range of green algae. Differential response of green algae to ALS-inhibiting herbicides has been documented in the laboratory and field (Thompson et al. 1993; Wei et al. 1998; Nyström and Blanck 1998). Further testing with various target and beneficial algal species is needed to determine direct impacts of these herbicides on different algal classes that might be present in conjunction with bloom-forming or noxious algae at different times in the growing season.

**FUTURE WORK:** Further testing of penoxsulam, CE-PDS-111, and CE-ALS-112 is recommended to refine use rates and exposure times for target algae. ALS and PDS compounds are currently being screened against *Prymnesium parvum* and *Lyngbya wollei*. The protoporphyrinogen oxidase (protox) inhibitors flumioxazin and carfentrazone are also being screened for algal activity. The protox inhibitors are rapid-acting contact herbicides that have very short half-lives in aquatic systems. Future studies will evaluate herbicides in mixed cultures of nuisance blue-green and



beneficial green algae to demonstrate how aquatic herbicides may selectively remove strains responsible for HAB without harming beneficial algae.

**CONCLUSIONS:** Current HAB management strategies are reactive, inconsistent, and non-selective. Control of noxious algae requires an innovative and proactive approach to adequately protect the Nation's water resources and Corps projects. Many of the newer classes of herbicides that target specific plant enzyme systems have toxicology packages that are compatible for supporting aquatic use patterns, in which one may use the water for drinking, swimming, and fishing immediately after application.

Initial laboratory screens of the new herbicides suggest that some chemistries have the potential to selectively control organisms responsible for HAB. Contrary to copper and other contact herbicides, these chemistries would be expected to prevent cell division, then slowly kill existing algal cells over a period of several days. Given the relatively long aqueous half-lives of PDS and ALS chemistries in comparison to copper, these compounds could be used to control cell division prior to bloom formation or manage a bloom by preventing further cell division. Further testing of penoxsulam, CE-PDS-111, and CE-ALS-112 is recommended to refine use rates and exposure times for target algae and determine effects on beneficial algae. Results from laboratory and mesocosm studies are needed to determine if larger scale field testing is warranted.

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