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February 26, 2010

Mr. Chris Riddle
Ohio Lake Erie Commission
One Maritime Plaza, 4th Floor
Toledo, OH 43604

Dear Mr. Riddle:

Attached is my final report for LEPF award 09-357, "Glyphosate loadings in Lake Erie watersheds." We believe we have generated data that will allow us to assess potential environmental impacts of glyphosate use in the agricultural lands surrounding the lake. We are grateful for your support, and we will send future publications from this award to you for your records and review.

Once again, please accept my thanks for your assistance with our research.

Sincerely yours,

George S. Bullerjahn, Ph.D.
Professor of Biological Sciences

Final report for LEPF award 09-357, "Glyphosate loadings in Lake Erie watersheds," George S. Bullerjahn, PI

This project was funded in part through the Lake Erie Protection Fund. The LEPF is supported by the voluntary contributions of Ohioans who purchase the *Erie…Our Great Lake* license plate featuring the Marblehead lighthouse.

lakeerie.ohio.gov

Abstract

Phosphonates are organic phosphorus compounds that contain a C-P bond instead of a phosphomonoester bond. Typically viewed as a recalcitrant, less bioavailable form of phosphorus, it has been recently shown that many microorganisms can utilize naturally occurring phosphonates as P sources, where the are hydrolyzed to phosphates. The herbicide glyphosate (Roundup®) is a synthetic phosphonate, and our studies indicate that glyphosate is detectable by ELISA in the Maumee River and nearshore samples from Lake Erie and Sandusky Bay. Detection coincides with the springtime application of glyphosate. Total levels of soluble glyphosate in the nanomolar range suggest that the majority of the glyphosate is quickly metabolized by microbes in the watershed. Thus, the endemic microbes serve to convert P as phosphonate into phosphate, thereby providing a novel route for phosphate delivery into the system. This raises the possibility that loadings of glyphosate yield a chemically distinct bioavailable form of P not accounted for by the Great Lakes Water Quality Agreement. In additional to glyphosate, the total phosphonate pool (derived in part from glyphosate metabolism) has been monitored by ^{31}P NMR of lyophilized DOP. Last, growth assays reveal that endemic cyanobacteria from Lake Erie can utilize glyphosate as a sole source of phosphorus.

Technical report for LEPF award 09-357, "Glyphosate loadings in Lake Erie watersheds," George S. Bullerjahn, PI

We have initiated a thorough assessment of glyphosate concentrations in the Lake Erie watershed, focusing largely on the Maumee River and the Lake Erie western basin. Glyphosate is the active ingredient in the phosphonate herbicide Roundup®, and we hypothesized that glyphosate levels would increase following spring planting of Roundup-Ready® crops in the region. Furthermore, despite the high loadings of herbicide (1000 metric tons in the watershed), microbial degradation and transformation of glyphosate to other phosphorus compounds (likely phosphates) could increase total bioavailable P in the system. Thus, we would see detectable glyphosate in the spring, but these levels in the watershed would decrease as we sampled the nearshore lake. Water samples we have analyzed have included weekly water samples from the Napoleon OH water treatment plant, along with samples from several Maumee River upstream and downstream stations provided by Joe Conroy, Ohio State University (as part of his sampling scheme funded by Ohio Sea Grant). Lake Erie samples have been collected by Dr. Tom Bridgeman (University of Toledo Lake Erie Center).

Our data are summarized in the following figures. In addition to using an ELISA kit to measure glyphosate, we have measured total chlorophyll, alkaline phosphatase activity and particulate organic phosphorus to assess the phosphorus status of the plankton in the water samples. Overall we see the following trends: first, glyphosate is detected only after spring planting, suggesting that microbial activity over the winter had completely depleted the soil of the herbicide (Figure 1). Samples obtained on April 21, May 1 and May 7 were below the detection limit, whereas all subsequent samples yielded detectable glyphosate. The detection of glyphosate in the May 12 sample reflects the onset of spring Roundup® application.

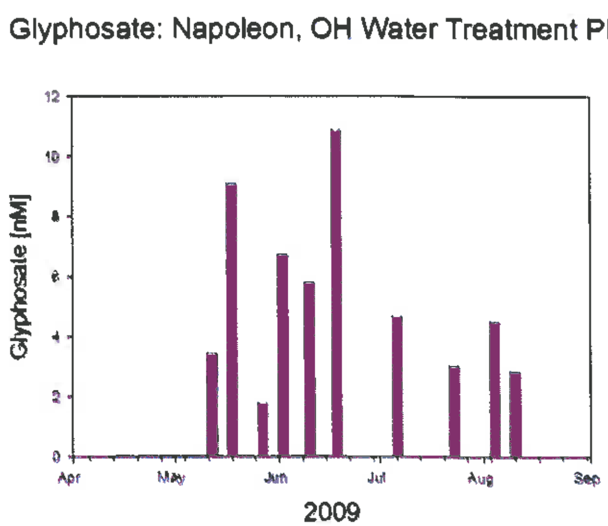

Figure 1. Glyphosate levels detected in the Maumee River (Napoleon, OH) during spring/summer 2009.

Secondly, levels of glyphosate in the watershed during time of peak glyphosate use increase as samples are drawn downstream, most likely a result of accumulating herbicide loadings into the Maumee River as it traverses agricultural lands upstream from Toledo (Figure 2).

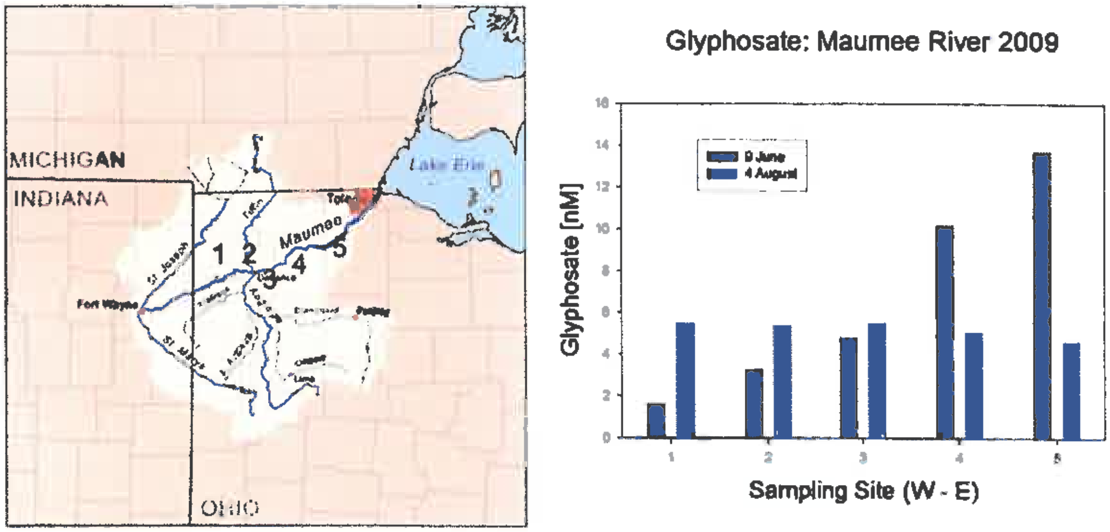

Figure 2. Location of Maumee River sampling sites (1-5) and levels of glyphosate on two dates during spring/summer 2009. The June date corresponds roughly to the period in which glyphosate levels are highest (see Figure 1).

Third, glyphosate levels decrease as the Maumee empties into Lake Erie (Table 1). Given that ca. 1000 metric tons of glyphosate is applied yearly to the Lake Erie watershed (corresponding to 166,000 kilograms P), the levels of glyphosate in the Lake indicate that most of the herbicide has been metabolized by microbes during transit in the Maumee to the lake.

Table 1. Glyphosate concentrations at Maumee Bay stations M3, M4, and 7M, and at the mouth of the Maumee River (station MB20).

Maumee Bay	Date	ppb glyphosate	S.D.	Mol L^{-1} glyphosate	nM
Maumee Bay (M3)	6/1/09	0.18	0.05	1.08E-09	1.08
	7/14/09	0.58	0.22	3.43E-09	3.43
Western Basin (M4)	6/1/09	0.08	0.02	5.02E-10	0.50
	7/14/09	0.40	0.02	2.39E-09	2.39
7M (Bridgeman)	6/15/09	0.07	0.00	4.29E-10	0.43

	6/23/2009	nd	nd	nd	nd
	7/1/09	nd	nd	nd	nd
	7/13/09	0.25	0.33	1.48E-09	1.48
	8/6/09	0.57	0.05	3.37E-09	3.37
MB20 (Bridgeman)	6/15/09	0.30	0.08	1.75E-09	1.75
	6/23/2009	0.29	nd	1.74E-09	1.74
	7/1/09	1.57	nd	9.30E-09	9.30
	7/13/09	1.02	0.14	6.03E-09	6.03
	8/6/09	0.56	0.01	3.31E-09	3.31

Additionally, we have shown that many endemic cyanobacteria in our Lake Erie culture collection can utilize glyphosate as a sole source of phosphorus, indicating that microbes in the watershed are capable of metabolizing phosphonate herbicides (Figure 3).

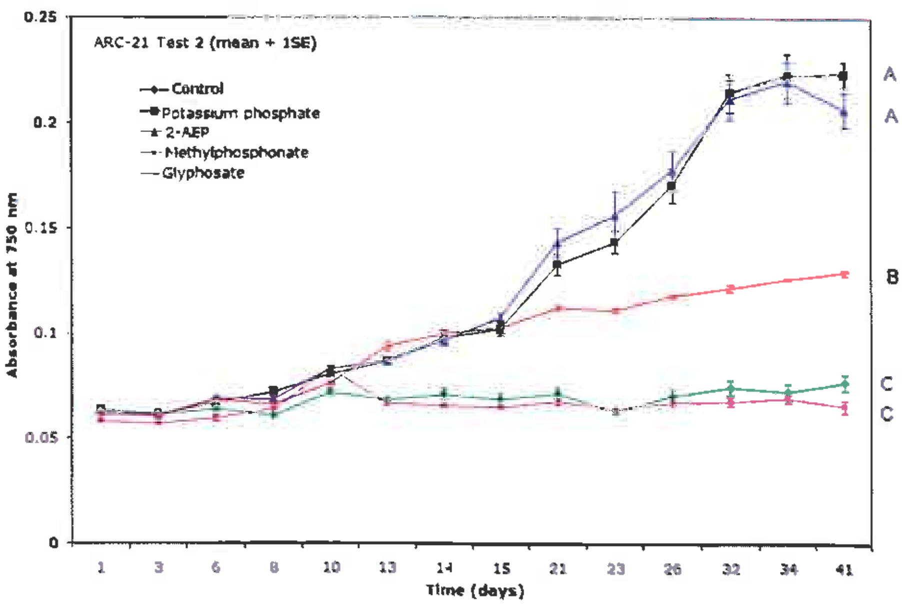

Figure 3. Growth of *Synechococcus* sp. strain ARC-21 (Lake Erie isolate) in phosphate and phosphonate compounds 2-aminoethyl phosphonate (2-AEP), methyl phosphonate and glyphosate.

Last, we have been working on an assay aimed at detecting total phosphonates in the environment that can provide a measure phosphonates other than glyphosate. The technique employs ^{31}P NMR spectroscopy to detect phosphonates distinct from the total phosphates present. Filtered water samples are lyophilized and the resulting organic residue (DOP) analyzed by solid state NMR. We envision this method to allow speciation of phosphonate pools into glyphosate and other compounds that include naturally-occurring phosphonates and metabolites of glyphosate. Indeed, some samples reveal a significant presence of phosphonate componds over total phosphates (Figure 4).

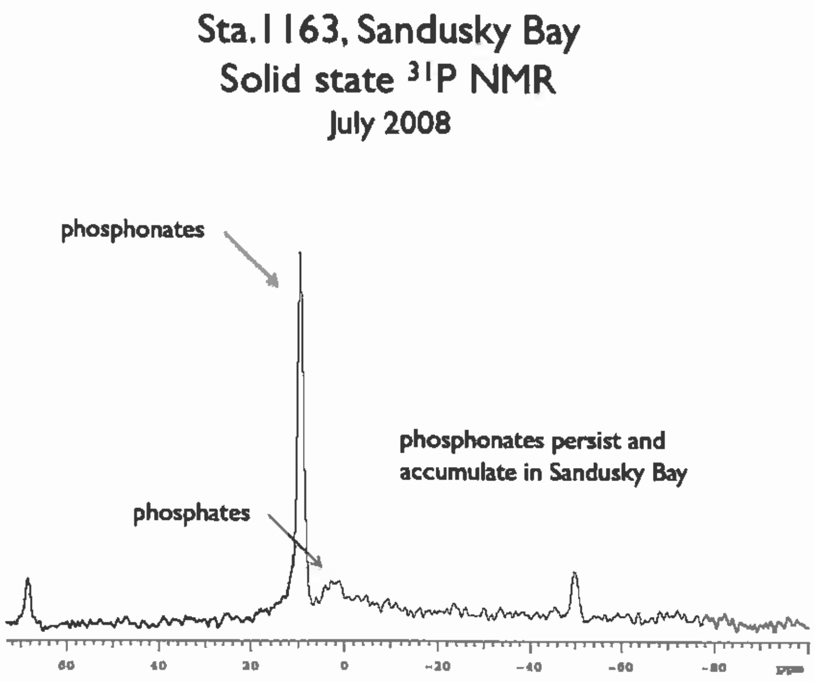

Figure 4. 31-P NMR of Sandusky Bay DOP, yielding resonances associated with the C-P phosphonate bond (left peak, see arrow) and the C-O-P monoester bond (right peak). Sandusky Bay is the main location where a stable pool of phosphonates is detected.

We have presented our first dataset from spring/summer 2008, along with the initial (LEPF-funded) samples from 2009 at the IAGLR annual meeting in May. An update will also be presented at the 2010 meeting. Our complete dataset will be included in a paper modeling the impact of glyphosate use in the Lake Erie watershed. We will write the paper in collaboration with colleagues at Environment Canada (a group led by Susan Watson), who will provide information on glyphosate loadings into Erie from the Ontario side of the lake.

Concluding remarks

Our data indicate that glyphosate is rapidly metabolized in the watershed, yielding low steady-state concentrations of total glyphosate during the spring and summer. We suggest that this rapid metabolism is the result of the assimilation of phosphonates by soil and aquatic microbes, which in turn hydrolyze the phosphonates to phosphates. The year-by-year accumulation of glyphosate could lead to a gradual increase in total dissolved P (as phosphates) in the Erie watershed over time.

Relationship of the project to Lake management

This dataset will be useful to watershed managers as they assess the impact of herbicide use in the region. Whereas glyphosate is a phosphorus-containing compound, it is a form of P (a phosphonate) distinct from the readily bioavailable phosphates. Phosphonates are not counted in the loadings of P into the watershed as mandated by the GLWQA.

Nonetheless, some microbes (bacteria and cyanobacteria) can utilize phosphonates (and hence glyphosate) as a P source. In doing so, they convert the phosphonates to phosphates that are readily available to all biota. Thus, microbial transformation of glyphosate to phosphates results in an increase in total phosphate (TP) in the system. Given the large amount of Roundup® applied to fields in the watershed, we estimate that if all the TP ran into the western basin from the watershed, it would increase TP in the basin by 1 micromolar per year. Thus, the increase in Lake Erie TP since 1996 can be attributable in part to increased use of glyphosate since the advent of Roundup-Ready® crops that same year. Certainly a one micromolar per year increase is both a small and an upper limit, but our data suggest there is an environmental impact of herbicide use over decades of widespread herbicide use. Our data will hopefully be incorporated into future models of P loading into the Lake Erie watershed.

LAKE ERIE PROTECTION FUND

SMALL GRANT - FINAL ACCOUNTING

Grant Number: SG 357-09

v2010

Budget Categories	Original Budget	Funds Spent	Current Balance	Matching Funds
A Salaries & Wages				
				5343.00
B Fringe Benefits				
				1923.00
C Total Salaries & Benefits (A+B)	$0 00	$0 00	$0.00	$7,266.00
D Non-expendable Equipment				
E Expendable Materials & Supplies				
	7182 23	7182 33	0 00	
F Travel				
G Services or Consultants				
	2957.77	2957.77	0.00	
H. Computer Costs				
I. Publications/Presentations				
J All other direct costs				
K Total Direct Costs (C thru J)	$10,140.00	$10,140.10	$0.00	$7,266 00
L Indirect Costs				
	1014.00	1014.00	0 00	2834.00
Total Costs (K + L)	$11,154.00	$11,154 10	$0.00	$10,100 00

I certify that the grant expenditures listed and descriptions of the charges are true and accurate to the best of my knowledge. These expenditures represent approved grant costs that have been previously paid for and for which complete documentation is on file.

		Date
Project Director	[signature]	11/12/10
Authorizing Agent	[signature]	11/24/10
Fiscal Agent	[signature]	11/18/10

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