Advisory Committee Feedback
Material submitted

1. Cover e-mail and comments from Paula Maccabee representing Water Legacy
2. Cover e-mail and comments from Dr. Sara Barsel (Note that Dr. Barsel is referencing the same Water Legacy comments as above)
3. Comments from Len Anderson
4. Cover email and comments from the Minnesota Center for Environmental Advocacy (MCEA)
5. Cover e-mail from Nancy Schuldt with comments from Fond du Lac Band and 1854 Treaty Authority
6. Cover e-mail from Mike Hansel with Minnesota Chamber of Commerce letter and comments
   a. previous Chamber of Commerce comments that are reference in the comment letter
Dear Ms. Engelking:

Attached, with this email, please find the following documents pertaining to the Wild Rice Sulfate Standard Studies which are submitted on behalf of WaterLegacy for consideration by the Scientific Peer Reviewers as well as by Agency staff:


**WaterLegacy Comments and Proposed Charge Questions for Peer Review of the Wild Rice Sulfate Standard Studies**

Please feel free to contact me if you have any questions regarding these materials. We would request an email response informing us of your receipt of these documents and informing us when they have been submitted to the Scientific Peer Reviewers.

Thank you.

Best regards,

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Comments on Wild Rice Sulfate Standard Studies and MPCA Draft Analysis of these Studies

WaterLegacy is a Minnesota non-profit organization formed to protect Minnesota water resources and the communities that rely on them. Along with the comments below, we have submitted Comments and Proposed Charge Questions for the Wild Rice Sulfate Standard Studies peer review.

WaterLegacy appreciates the work done by the Minnesota Pollution Control Agency (MPCA) in its June 2014 Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review (MPCA Draft Analysis) to summarize the wild rice sulfate research. However, our review of the research identifies several important omissions in the MPCA Draft Analysis and areas where the MPCA appears to overstate or inaccurately characterize the research findings. These areas of concern are highlighted in the discussion below.

Our Comments here, like those on the Charge Questions, reflect the study objectives set by the Minnesota Legislature: to determine whether the existing sulfate standard of 10 milligrams per liter in waters producing wild rice is appropriate and whether there should be a temporal or seasonal limitation to the application of the sulfate standard. WaterLegacy notes that official decision-makers in the upcoming rulemaking process will assume that the peer review committee has examined all pertinent data and issues related to these underlying policy questions.

Field Studies
WaterLegacy believes that data should have been supplied from the initial and subsequent Field Survey sampling showing the prevalence of wild rice in low and high sulfate waters. The rarity of finding wild rice in high sulfate waters is significant in itself as well as a factor that should be considered in analyzing Field Survey correlations.

The MPCA Draft Analysis cites 2008 Minnesota Department of Natural Resources (MDNR) surveys showing wild rice is rarely found in higher sulfate waters. (MPCA Draft Analysis, p. 9, lines (ll) 231-233). The Draft Analysis does not directly state that Field Surveys in the MPCA Wild Rice Sulfate Standard Studies also rarely found wild rice beds in waters with more than 10 milligrams per liter (mg/L) of sulfates.¹ This ecological finding is obliquely referenced in the statements, “After the 2011 season, MPCA identified a need to identify and sample sites with elevated sulfate concentrations (above 10 mg/L) that conceivably could host wild rice based on suitable wild rice habitat.” (Id., p. 17, ll 429-431) and, “If wild rice habitats had been sampled probabilistically, most of the sites would have had very low sulfate concentrations and little would have been learned about the effect of elevated sulfate.” (Id., p. 21, ll 497-498).

Even with Field Surveys intentionally biased to find wild rice in high sulfate locations, it appears that the vast majority of wild rice beds in lakes were found in waters with sulfate below 10 mg/L and very few wild rice beds were found in any waters with sulfate above 20-50 mg/L. (Id., p. 35, Figure 15).

The Draft Analysis did note the large difference in the 75th percentile of sulfate in lakes used for the MPCA’s wild rice Field Survey and the sulfate levels in MDNR wild rice lakes. MPCA wild rice Field Survey

¹ Characterization of Field Surveys made in personal communication with Amy Myrbo, July 2, 2014.
lakes have more than three times the sulfate of Minnesota wild rice lakes as a whole. (Id., p. 21, ll 505-513). No stream or river data was provided for a similar comparison.

WaterLegacy does not disagree with MPCA that a biased sample was more efficient to determine under what circumstances wild rice might survive elevated sulfate. However, regulatory limits must protect a resource in its natural environment, so results based on biased sampling must be viewed with caution. WaterLegacy believes that the implications of intentionally biased sampling should be explicitly discussed in terms of the way in which Field Survey data may or may not predict impacts of sulfate and sulfide wild rice under prevalent rather than anomalous conditions.

Hydroponic Experiments
WaterLegacy appreciates that, despite their short-term and limited scope, hydroponic experiments may be useful to confirm the toxic effects of chemicals. However, the converse is not necessarily true. Even if a chemical or a tested concentration of a chemical is not toxic after a few days in a test tube, this doesn't prove that the chemical or concentration would not be toxic in the natural environment.

The Hydroponic Experiments performed on wild rice under MPCA auspices lasted 11 days and measured rates of germination or the growth (as measured by plant length and dry weight change) of emergent seedlings. (MPCA Draft Analysis, p. 13). What we find striking about these results is that, even under the very limited conditions of the hydroponic environment and the few endpoints studied, sulfide was found to have significant effects on seedling growth at 309 micrograms per liter (µg/L) of sulfides. (Id., p. 15).

However, these data are insufficient to demonstrate that sulfide below 300 µg/L or even below 134 µg/L is not also toxic to wild rice, as suggested in the MPCA Draft Analysis. (Id., pp. 15-16). The limitations of wild rice survival in the hydroponic environment prevented assessment of adverse effects of lower sulfide levels at different parts of the wild rice life cycle or over a longer duration.

The insufficiency of hydroponic testing to postulate a level below which sulfide would not be toxic to wild rice is underscored by the Field Survey data. As explained in the Draft Analysis, the Field Survey data demonstrated wild rice sensitivity to sulfide at any concentration above 75 µg/L: “69 to 80% of the sites had wild rice present above the presence threshold when porewater sulfide was less than 75 µg/L, and b) a more-or-less continuous decline in the percent of sites with wild rice present occurred above 75 µg/L.” (Id., p. 36, l. 821 to p. 37, l. 823). Unless a more-or-less continuous decline in the presence of wild rice is desirable, sulfide levels above 75 µg/L should be deemed levels of concern.

Mesocosm Experiments
WaterLegacy believes the Mesocosm Experiments provide critical evidence to demonstrate the adverse effects of increased sulfates on wild rice under more natural conditions and between generations.

The MPCA Draft Analysis noted that seedling emergence from sediment decreased significantly (p < 0.01) with increased sulfate levels, that survival of those seedlings that remained after thinning declined with increasing sulfate concentration, and that, even with poor overall seedling survival in the 2013 season, the trend of less survival with increasing sulfate test concentration remained. (MPCA Draft Analysis, p. 30, ll. 647-651).

The Draft Analysis found “the proportion of viable seeds (those determined to be able to germinate and grow) from each plant remained relatively constant during all three years in the controls (55 – 60%) but
decreased to 48% in 2011, 40% in 2012, and 31% in 2013 at the 300 mg SO₄/L treatment level. These decreases were statistically significant for all three years.” (Id., p. 27, ll. 637-640). Seed weights also remained constant in the control tanks, but progressively decreased in high sulfate treatments. As compared to the controls seed weights in the 300 mg SO₄/L treatment level decreased by 12% in 2011, 21% in 2012, and 50% in 2013. (Id., p. 27, ll 631-634).

In addition to demonstrating several endpoints where varying sulfate in surface water affected wild rice, the Mesocosm Experiments showed how wild rice populations could be impaired over time, as seed viability and seed weights progressively decrease.

In the Mesocosm Experiments, increasing the independent variable of surface water sulfate resulted in increased porewater sulfide. (Id., p. 30, ll 665-667). Median porewater sulfide concentrations in the control mesocosms were 68 μg/L – below the 75 μg/L level at which wild rice prevalence decreased in the Field Surveys. As sulfate concentrations were increased to 50, 100, 150 and 300 mg/L, the mesocosms yielded median sulfide porewater levels of 138, 190, 265 and 778 μg/L respectively. (Id., p. 37, ll 840-843), raising porewater sulfide above levels found in Field Surveys and hydroponic tests to impair wild rice.

Sediment Incubation Studies
WaterLegacy is troubled by MPCA’s failure to provide peer reviewers with the findings from the Sediment Incubation Studies. These studies were undertaken to evaluate whether there should be seasonal limitations on the application of the wild rice sulfate standard or whether the standard should be applied year-round. This question was directed by the Legislature and is particularly salient in light of a recent permit issued by the MPCA to allow unlimited discharge of sulfates upstream of wild rice waters from September through March.²

The MPCA Draft Analysis summarized findings that sulfate movement into sediments and reduction to sulfides occurred 49% faster in the warmer temperature of 23°C as compared to the colder temperature of 4.5°C. (MPCA Draft Analysis, p. 34, ll728-729). This summary is accurate and pertinent.

However, the MPCA omitted from its Draft Analysis the more significant Study results demonstrating that, under cold conditions, of 3,800 micrograms per centimeter squared (μg/cm2) total sulfate that fluxed into sediment during the 80-day loading phase, 3,000 μg/cm2 reacted to form sulfide. (Will DeRocher, Nathan W. Johnson, Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments, Report Dec. 31, 2013, pp. 30, 33). From this data, Mr. DeRocher and Dr. Johnson concluded, “regardless of adjustments of diffusion and reaction rates to field conditions, a great majority of the sulfate that diffuses into sediments during an “80 day loading phase is likely to be reduced to sulfide in either warm or cold conditions.” (Id., p. 35) Thus, “over an 80 day sulfate loading phase, a vast majority of the sulfate added to sediment reacts to form sulfide, even at 4°C when biological rates are slower.” (Id., p. 38)

Mr. DeRocher and Dr. Johnson further explained how their findings addressed the study question pertaining to seasonal sulfate loading and sulfide formation:

Elevated sulfate levels in the porewaters provide favorable conditions for sulfate reducing bacteria that, over time, could produce sulfide in excess of the iron availability in a system and

result in an accumulation of dissolved sulfide in pore fluids (Johnson 2014). Sufficient quantities of dissolved sulfide could have detrimental effects on aquatic vegetation and organisms. This study provided both a physical and mathematical model to describe the porewater sulfate response to seasonal sulfate loading into surface water under different temperatures. These results will help to answer the question of how much sulfate diffuses into, and reacts within sediment, as a function of temperature and inform management decisions regarding the timing of sulfate release to natural waterways. (I.d., p. 38)

WaterLegacy asks that peer review evaluate the Sediment Incubation Studies along with other study results to review whether there is a scientific basis for seasonal application of the wild rice standard.

Sulfate Standard and Sulfide Toxicity
WaterLegacy requests peer review of data from the Field Surveys to answer the primary wild rice sulfate study question: whether the existing 10 mg/L sulfate limit is appropriate and sufficient to protect natural beds of wild rice.

The MPCA’s presentation to the Wild Rice Advisory Committee on April 16, 2014, Wild Rice Sulfate Standard Study: Summary of Preliminary Analysis, contained the regression analysis attached as Exhibit A. WaterLegacy’s scientific advisors believe this slide, which was not included in the MPCA’s Draft Analysis, should be reviewed to evaluate the appropriateness of the existing wild rice sulfate standard.

WaterLegacy’s advisors explain that the regression analysis in Exhibit A indicates that sulfide levels in sediments may reach levels toxic to wild rice where surface water sulfate is below 10 mg/L. Even if the threshold of concern for porewater sulfide were as high as 300 μg/L, under a surface water sulfate of 10 mg/L, wild rice would be protected only approximately 80% of the time. Reviewing this data, our advisors have expressed the concern that the 10 mg/L standard may not be protective enough of wild rice and have suggested that a sulfate limit of 6 mg/L might be needed to protect wild rice in the natural environment.

WaterLegacy believes the Draft Analysis and peer review process should consider the evidence of a decline in wild rice prevalence with porewater sulfide exceeding 75 μg/L discussed previously and the relation between sulfate and sulfide levels in the Field Surveys reflected in Exhibit A to inform whether the existing 10 mg/L sulfate limit is appropriate or sufficient to protect wild rice.

Iron Mitigation Hypothesis
The MPCA’s discussion of the relationship between sulfate, iron and sulfide overstates the evidence that iron, in fact, mitigates the effects of sulfate and sulfide on the presence and growth of wild rice.

Although some text in the Draft Analysis (MPCA Draft Analysis, p. 45, ll 1076-1077) suggests that additional research is needed to account for the role of iron, the MPCA disregards this caution in concluding that iron in sediments removes sulfide from solution “and from potential harm to wild rice,” (I.d., p. 51, ll 1204-1207) and that “wild rice abundance is not diminished when iron concentrations are high in the porewater, but is diminished when sulfide concentrations are high and iron is low.” (I.d., p. 52, ll 1232-1233).

From the limited evidence in the Wild Rice Sulfate Standard Studies, the MPCA suggests that an equation derived from Field Survey can be employed, if the iron concentration of the sediment solid
phase is known, “to predict the potential employed to predict the potential maximum sulfide concentration that would be produced from a given concentration of sulfate in surface water” (Id., p. 52, ll 1258-1260). The MPCA further suggests that this “prediction” can be applied where a “site has the capacity to consume more sulfide” to assess whether increasing sulfate concentrations should or should not be viewed with caution. (Id., p. 52, ll 1260-1267).

WaterLegacy believes the MPCA Draft Analysis oversimplifies the relationships between sulfate and sulfide in the presence of iron. Attempting to predict sulfate concentrations in the natural environment from iron content of sediment is premature, and any assumption that such predictions can determine the ability of wild rice to thrive in high sulfate environments is scientifically unsubstantiated.

Although there are some interesting correlations pertaining to iron in the Wild Rice Sulfate Standard Studies, there are no experiments varying iron concentrations in sediments and measuring the levels of sulfide produced when a given concentration of sulfate is supplied in surface water. There are no experiments co-varying sulfate and iron concentrations and evaluating wild rice biological endpoints to determine whether iron mitigates effects of sulfide on wild rice and, if so, to what degree and under what conditions. Unrepresentative Field Survey correlations in stream and river environments are insufficient to demonstrate causal or protective relationships. There has been no analysis of biological effects of plaque formation on wild rice roots in the presence of iron and sulfide, and no analysis of rooting zone chemistry to determine to what degree sulfides remain sequestered in the presence of iron or cycle in and out of porewater in a natural environment.

No Hydroponic Experiments tested any aspect of the iron hypothesis.

The Mesocosm Experiments showed diminished iron in porewater with increased sulfate concentrations in surface water, (Id., p. 31, Figure 12). However, there was no evidence in the Mesocosm Experiments that iron mitigated the formation of porewater sulfide or mitigated adverse impacts of the sulfate treatments on the growth of wild rice.

Field Survey correlations are insufficient to support the assertion that iron mitigates adverse effects of sulfate and sulfide on wild rice abundance in a natural environment. As discussed previously, any conclusions regarding wild rice survival in the natural environment should be tempered by the knowledge that sampling was intentionally biased to find sites where wild rice was present despite high surface water sulfate. Caution is also required because lakes, streams and rivers in the Field Survey have dissimilar findings.

First, although the MPCA Draft Analysis characterizes the data sets as “lakes” and “streams,” the category of “streams” actually includes rivers as well as streams, including several sites along the St. Louis River and the Mississippi River. Only a small number of streams and rivers were actually sampled – 6 or 8 water bodies in total.³

The Draft Analysis acknowledges that data from streams, rivers and lakes is different, noting that the median sulfate concentrations at sites in which wild rice occurs in streams and rivers in the 2012-2013 Field Survey (14.2 mg/L) is more than five times higher than in Field Survey lakes (2.5 mg/L). (MPCA Draft

³ Personal communication with Ms. Myrbo, July 2, 2014. See also MPCA, Draft Analysis, p. 25, Table 6.
Analysis, p. 22, l 544 to p. 23, l 546). In addition, this median sulfate level for the 23 stream and river sites sampled during the 2012-2013 Field Survey was nearly six times higher than the median value for the eight stream sites with wild rice sampled during the 2011 Pilot Survey (2.4 mg/L). As the MPCA noted, this discrepancy “calls into question the assumption that the data from the streams sites sampled during the 2012-2013 Field Survey truly represents the population of stream sites where wild rice grows in Minnesota.” (Id., p. 23, ll 546-550).

To postulate that porewater iron permits wild rice survival despite high sulfate waters, the MPCA has conflated dissimilar data from lakes, streams and rivers. The Draft Analysis shows wild rice presence in higher sulfate waters almost entirely in streams and rivers, not in lakes, whether or not high levels of iron are present. (Id., p. 35, Figure 15). In lakes, wild rice is absent (<1% coverage) in many high iron sites, (Id., p. 35, Figure 15 and p. 36, Figure 16). For streams and rivers, on the other hand, irrespective of iron levels, there are few data points where wild rice is absent. (Id.) If dissimilar data from lakes, rivers and streams were not combined, it is unlikely that any correlations could be drawn between iron levels and wild rice presence.

WaterLegacy is concerned that the MPCA’s hypothesis that iron mitigates impacts of sulfate and sulfide on natural wild rice is based on several layers of conjecture. The Draft Analysis suggests that iron in sediments serves as an available reservoir that removes sulfide from solution. (Id., p. 44, ll 1049-1055). However, the United States Geological Survey (USGS) measure of iron in soil and sediment “is not predictive of the iron metrics used in this study.” (Id., p. 48, ll 1141-1142).

The MPCA proposes that, where ferrous iron is present, “sulfide immediately reacts with iron and precipitates, removing the sulfide from solution and from potential harm to wild rice.” (Id., p. 51, ll 1206-1207). However, Mesocosm Experiments show high sulfide with sulfate treatments, even when sediments had high iron levels and control iron exceeded 10,000 μg/L. (Id., p. 31, Figure 12). Dr. Johnson’s data on Sandy Lake and Second Creek suggests a build up of sulfide as the growing season progressed, even approaching or exceeding the high levels of sulfide (~300 μg/L or 9.35 micromoles/L) found to be toxic in hydroponic experiments. (Johnson, Response of Rooting Zone Geochemistry to Experimental Manipulation of Sulfate Levels in Wild Rice Mesocosms, Dec. 31, 2013, see Figures B1-B3, pp. 38, 40, 42). Iron and sulfides may or may not reach a steady state in the natural environment, and sulfides may both precipitate from and re-enter solution, affecting porewater concentrations and wild rice.

Given the lack of experimental evidence, the mesocosm and field data suggesting sulfide may reach toxic levels despite the presence of iron, and the inconsistencies and unrepresentative nature of Field Survey findings of wild rice presence in high sulfate waters, there is insufficient scientific support to predict either sulfide or wild rice presence in natural systems as a function of iron in sediments.

MPCA’s selection of an hypothesis from the literature that iron coatings on the roots of wild rice are benign (p. 52, ll 1223-1232) also seems to be wishful thinking, not objective analysis. The cited literature pertains to iron hydroxide on white rice, rather than the iron sulfides believed to coat the roots of wild rice when sulfate concentrations are elevated and iron is present. (See Pastor, Effects of Enhanced Sulfate Concentrations on Wild Rice Populations: Results from a Mesocosm Experiment, Report December 31, 2013, pp. 11-12). In addition to the salutary hypothesis selected by the MPCA, some published literature suggests that, at high levels, iron may itself become a toxicant to plants in wetland sediments. (See van der Welle et al., Detoxifying Toxicants: Interactions between Sulfide and Iron
Toxicity in Freshwater Wetlands, Environ. Tox. and Chem., 2006, 25(6): 1592–1597.) Additional research would also be needed to determine whether the plaque coating wild rice roots in the presence of iron and sulfide is benign or whether it impedes nutrient uptake or results in other adverse effects.

Mercury Methylation

WaterLegacy would also caution that sulfate discharge has adverse impacts beyond the effects of sulfide on wild rice. The MPCA has long expressed concern that sulfate discharge to water increases mercury methylation and adverse impacts to health and the environment. (MPCA, Strategy to Address Indirect Effects of Elevated Sulfate on Methylmercury Production and Phosphorus Availability, Oct. 19, 2006), attached as Exhibit B). Despite this history, the MPCA rejected the opportunity to measure mercury or methylmercury as part of the Wild Rice Sulfate Standard Studies. As a result, no information is available on the degree to which mercury and methylmercury in surface water, porewater or the aquatic food chain vary in relationship to sulfate, sulfide or iron.


WaterLegacy and the health and scientific professionals with whom we collaborate are concerned about mercury methylation in waters downstream of sulfate discharge. We believe that, before iron or any other variable can be considered as a site-specific mitigative factor for sulfate discharge adverse impacts, much more analysis must be done – not only of the relationship of iron in sediments to wild rice but of the relationships between sulfate, sulfide and iron in sediments to mercury methylation risks.

Conclusion

Based on the foregoing discussion of the Wild Rice Sulfate Standard Studies, WaterLegacy believes that the data shows the existing 10 mg/L sulfate standard should be preserved, if not reduced to 6 mg/L in order to protect natural beds of wild rice. The threshold of concern for sulfide porewater is exceeded between 150 and 300 μg/L, and may be as low as 75 μg/L in the natural environment. There is no scientific basis to restrict the temporal application of the wild rice sulfate standard and insufficient scientific evidence to propose that iron may mitigate sulfate impacts in any site-specific application.

Respectfully submitted,

DATE: July 8, 2014

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5. Quantile regression of sulfide on sulfate is a useful way to translate sulfide levels to surface water sulfate.
MPCA Strategy to Address Indirect Effects of Elevated Sulfate on Methylmercury Production and Phosphorus Availability

Summary: Although there is evidence that elevated sulfate loading can increase methylmercury production and phosphorus mobilization, it is premature to develop specific sulfate concentration limits or other regulatory responses based on these effects. The deleterious effects of sulfate may be restricted to certain areas of the state, certain background sulfate concentrations, or other environmental controlling factors. These factors will be explored in a multi-year data collection effort combined with ongoing data analysis. It is anticipated that sensitive areas of the state will be identified and appropriate controls on sulfate discharges will be developed if necessary. The primary focus of the strategy is to pursue research to further understand impacts from sulfate on methylmercury production and phosphorus mobilization and to use the research to guide the future need for additional requirements or controls in environmental review and NPDES permits.

Problem Statement: Research indicates a correlation between sulfate loading and methylmercury (MeHg) production and phosphorus (P) mobilization under certain conditions. Many waters of the state are impaired as a result of MeHg in fish tissues and excess nutrients. MPCA staff need to better understand the relationship between sulfate concentration and MeHg production/P mobilization so that appropriate responses, if necessary, can be developed. Sulfate is a common constituent in domestic and industrial wastewaters. Additional information is needed so that the MPCA can develop a permitting strategy for existing, expanding and new domestic and industrial process wastewater discharges. The strategy must reflect varying MeHg production and P availability under differing environmental conditions.

MPCA Actions to Monitor & Evaluate Sulfate Impacts

MPCA staff will evaluate the following hypotheses over three to five years:

1) Elevated sulfate discharge into low-sulfate receiving waters significantly increases MeHg concentrations (as percent of total mercury) and P concentrations.
2) Elevated sulfate discharge into high-sulfate receiving waters has no significant effect on MeHg concentrations (as percent of total mercury) and P concentrations.
3) Elevated sulfate discharge into low-sulfate waters has greater effect on P concentrations when the iron to P ratio is low in the sediments of the receiving water.

Environmental Analysis and Outcomes Division will coordinate the following activities to evaluate the above hypotheses and support eventual changes in the environmental review and permitting practices:

1) Continued research at Wetland 6 in the Marcell Experimental Forest north of Grand Rapids;
2) Milestone Monitoring – permanently add sulfate, TOC, total mercury, and MeHg to the MPCA’s ambient water quality monitoring sites; (In FY07 Milestones did include THg, MeHg, sulfate, and TOC, through use of the Mercury Trends allotment).
3) Continue to track and participate in the research of national / international work groups;

4) Compile and map existing surface water sulfate concentration data in Minnesota;

5) Compile and map existing effluent sulfate concentration data in Minnesota;

6) Compile and map existing stormwater sulfate concentration data in Minnesota (if few data have been collected, consider obtaining representative data);

7) Fish Consumption Advisory Monitoring - Work with DNR and MDH to collect fish for mercury analysis of fish tissue at a subset of sites where environmental data is being collected on water or sediments;

8) Implement the Environmental Review and NPDES Permitting actions (below) Regional, Municipal and Industrial Divisions will lead as appropriate; and

9) Compile data from the above activities and complete an evaluation of the hypotheses.

Environmental Review and NPDES Permitting

While research shows a relationship between sulfate concentration and MeHg production/P mobilization, there is currently insufficient information to reach firm conclusions on whether specific point source (non-stormwater) discharges containing sulfate may impact water quality or cause/contribute to water quality impairments. The following information will guide the development of programmatic direction and procedures to address sulfate discharges. This approach includes 1) further characterization of the problem, 2) development of interim permitting and environmental review procedures, 3) research of sulfate impacts from point source dischargers, and 4) annual incorporation of new knowledge into the permitting and environmental review procedures. Prior to development of the interim procedures, NPDES permit writers and environmental review staff will need to manage projects on a case-by-case basis. They will use the current knowledge (as outlined below and in Appendix A) and work with the program supervisor and Ed Swain to assess and respond to the environmental risk from sulfate discharges.

Environmental Review

If a new or expanding domestic or industrial process wastewater discharge triggers environmental review for a wastewater-related threshold (not a non-wastewater related threshold) or if wet air controls that contribute sulfate to a wastewater stream are proposed the impact from sulfate must be evaluated in the environmental review document. The environmental review should include available data on projected effluent design flow rate, sulfate concentration, and sulfate load as well as best estimates of receiving water flow rate (7Q10 and other statistics) and concentrations of sulfate, mercury, MeHg, iron, ortho-P, total P, and, as a measure of organic matter in the water, TOC and/or DOC. If receiving water flow was measured concurrently with water sampling, flow data should also be included. The environmental review must also include available data on the organic matter, mercury, iron, and P content of the sediments of receiving waters and lakes or impoundments downstream. It is understood that available data may be limited. To the extent possible, qualitative discussion of downstream conditions and mitigative options should also be included.
NPDES Permitting

If a new, expanding or existing domestic or industrial wastewater discharge for “high risk” situations is encountered, 1) the need for effluent and/or receiving water monitoring for sulfate, mercury, MeHg, iron, ortho-P and/or total P should be considered; and 2) if research or other information supports a likely impact from sulfate in a specific situation an evaluation of the treatment technologies and pollution prevention opportunities should be included with the permit application. Existing discharges will be addressed at the time of reissuance. A guidance for project proposers and NPDES permit writers will be developed by June 2007 to explain the procedures for addressing sulfate discharges. In the interim, permit writers will work with the program supervisor and Ed Swain to assess and respond to the environmental risk from sulfate discharges.

Currently, high-risk situations may include:

- Discharge of elevated sulfate concentrations into high-organic aquatic environments (e.g., wetlands that drain to fisheries, lakes with organic sediment, rivers with slow-moving back waters, ponds where rising water might inundate vegetation).
- Discharge of elevated sulfate into low-sulfate waters (< 40 ppm or so) where sulfate may be a limiting factor in the activity of sulfate-reducing bacteria (SRB).
- Discharge of elevated sulfate into streams with fluctuating water levels and bordering wetlands. Rising water levels would introduce sulfate into the high-organic wetland matrix, followed by falling water levels that hydraulically deliver elevated MeHg and/or phosphate to the stream.
- Discharge of elevated sulfate to waters that flow to a lake or impoundment downstream that may thermally stratify even temporarily in the summer or be cut off from the atmosphere from ice cover in the winter. Either stratification or ice cover can produce anoxic water, in which sulfate can be converted to sulfide, potentially enhancing both mercury methylation and phosphate release.

Conditions that decrease the risk that elevated sulfate loading may enhance mercury methylation:

- Discharge of elevated sulfate to waters with high background sulfate (>100 ppm or so), including downstream waters.
- Discharge of elevated sulfate to highly oxygenated, turbulent waters with low-organic sediment and no adjacent riparian or lacustrine wetlands, and none downstream.

Research Impacts of Sulfate from Domestic and Industrial Process Wastewater Discharges

MPCA staff will pursue funding to study specific impacts from domestic and industrial process wastewater discharges of sulfate on MeHg production and P availability in receiving waters. The study (or series of smaller studies) will include site-specific evaluations at facilities representing the various high risk situations identified in “Environmental Review and NPDES Permitting” above. This work may include effluent and receiving water monitoring for sulfate, mercury, MeHg, iron, ortho-P, total P, and supporting parameters that may reveal biogeochemical mechanisms, such as DOC, pH, oxygen, nitrate, and potassium. The work will include an evaluation of the data to determine whether domestic and industrial process wastewater discharges are impacting receiving waters during any time of the year with a particular focus on the summer months. Some of the study work may need to be contracted out to a research entity.
(i.e. UMD, NRRI, U of M St. Anthony, U of Toronto). Funding sources may include Legislative Initiative, CW Legacy Act, GLNPO, salary savings, or other related project savings.

**Action Items / Resource Needs**

1) Risk Managers need to select an EAO Division representative to coordinate the overall Sulfate Strategy by **August 28, 2006**. Action Complete: Marvin Hora will be overall coordinator.

2) Sulfate Strategy Coordinator (Marvin Hora) will work with the appropriate managers to recommend staff team members to develop guidance documents described in the Environmental Review and NPDES Permitting action items below by **September 25, 2006**. Recommendation: Team should include Ed Swain, Jeff Stollenwerk, Deb Lindlief, Dana Vanderbosch, Bruce Wilson and a GIS specialist (see MPCA Actions 4 & 5 above).

3) Water Policy Team reviews and approves the Sulfate Strategy including staff assignments by **October 31, 2006**. Jeff Stollenwerk will coordinate.

4) EAO staff should develop funding requests, detailed plans and funding applications, RFPs and conduct study oversight necessary to complete research on impacts of sulfate from domestic and industrial process wastewater discharges. **Ed Swain - Ongoing.**

5) The Sulfate ER/NPDES Permitting staff team (from item 2 above) further defines and characterizes high-risk situations/criteria and develops interim procedures for environmental review and NPDES permitting activities. **This action should be completed by February 28, 2007.** Estimated time commitment – 40 to 80 hours for each team member.

6) The Sulfate ER/NPDES Permitting staff team (from item 2 above) develops brief guidance for project proposers and MPCA staff that provides background on the sulfate issue and factors that will need to be evaluated as part of the environmental review and/or permit process. Guidance should also address permitting projects that do not require environmental review. The team should develop procedure documents that will be included in the program manual for the environmental review and the NPDES Permit Writers’ Manual. This document will provide background on the sulfate issue and issues that will need to be evaluated as part of the environmental review and/or permit process. **These actions should be completed and presented to the WQ Policy Forum for review and approval by June 29, 2007.** Estimated time commitment – 30 to 40 hours for each team member.

7) If necessary, revise the Illuminated EAW document and NPDES permit application to include background on the sulfate issue and issues that will need to be evaluated as part of the environmental review and NPDES permitting. **These actions should be completed by July 31, 2007.** ER Staff, Permit Staff and EAO staff – 10 hours each.

8) Complete technical review of environmental review submittals and NPDES permit applications. Develop responses to comments on specific projects. **Timeline is project-specific.** Environmental Review, Municipal/Industrial engineers and permit writers lead, and EAO staff support – workload could vary greatly.

9) Review research findings and if necessary incorporate into permitting and environmental review procedures. **Sulfate ER/NPDES Permitting staff team (from item 2 above) 10 to 20 hours – Annually.**
10) Provide technical assistance to permit writers regarding high-risk case-specific monitoring requirements and information protocols for targeted facilities or facility types. – EAO staff as needed – 40 to 80 hours per year.

11) Update agency managers on policy development needs, including needs to revise the sulfate standard - Strategy Coordinator – Annually.
Attachment A

MPCA Strategy
to Address Indirect Effects of Elevated Sulfate on
Methylmercury Production and Phosphorus Availability

Technical Background

Sulfur naturally cycles in aquatic systems between sulfate and sulfide, depending on multiple factors, including oxygen availability, hydrologic fluctuations, and organic matter degradation. Sulfate is a relatively inert chemical species, but its conversion to sulfide has a number of undesirable indirect effects that this strategy ultimately seeks to minimize. Under certain as-yet undefined environmental conditions, additional sulfate may enhance MeHg production and the availability of P for algal growth. The mechanisms associated with enhanced MeHg production and P availability are different, but are both associated with the tendency during decay of organic matter for natural bacteria to convert sulfate to sulfide after oxygen is depleted. This group of bacteria is called sulfate-reducing bacteria (SRB).

The initial tasks of the strategy involve collecting and interpreting data so that defensible quantitative permitting limits on sulfate discharge can be established. For instance, aquatic systems that are naturally elevated in sulfate due to local geological sources may not be sensitive to moderate increases in sulfate concentration. Other environmental attributes may make some systems more or less sensitive to added sulfate, including existence of wetlands and background dissolved iron concentrations.

Elevated sulfate can enhance MeHg production because SRBs are known to convert inorganic mercury (which is widely available due to atmospheric pollution) to MeHg, the only form that accumulates in fish. When the availability of sulfate controls the activity of SRBs, then additional sulfate may cause additional fish contamination. Recent research (Jeremiason et al. 2006) has documented increased MeHg production through increased sulfate concentrations in a wetland environment. SRBs produce MeHg when certain environmental factors coincide: low oxygen and adequate levels of bioavailable inorganic mercury, sulfate, and decaying organic matter. High organic matter can, of course, cause low oxygen because other bacteria will consume available oxygen in the first phases of organic matter degradation. SRBs are most active in aquatic systems because water decreases atmospheric oxygen availability and maintains a moist environment in which bacteria can thrive. SRB production of MeHg can be constrained by low mercury, low sulfate, low organic matter, or high oxygen. There is also a hypothesis that continued production of sulfide by SRBs can produce negative feedback by reducing mercury availability through the formation of sulfide-mercury chemical bonds. However, it is not clear how to model such negative feedback, and the production of sulfide is not necessarily permanent, as sulfide can oxidize back to sulfate. So, at this point, trying to maintain high sulfide does not seem like a viable strategy. However, data collection will provide empirical information on this hypothesis.

Elevated sulfate can enhance P availability because of an indirect effect of sulfide production. When aquatic systems become anoxic (common in both hypolimnia and wetlands) there is a tendency for enhanced P release from sediment to the water. While anoxic, iron oxides become soluble, which causes the dissolution of phosphate that had co-precipitated with the iron during an oxygenated phase. The phosphate will largely re-precipitate with the iron when the water is
oxygenated, unless the iron to phosphate ratio is too low. During anoxia, sulfide may be produced, which has the unfortunate ability to form a precipitate with the dissolved iron—unfortunate because elevated levels of sulfide can decrease the amount of iron that is available to co-precipitate the P. If the P is not precipitated upon oxygenation (either turnover of a lake or hydraulic movement in a wetland), then the additional P will likely stimulate algal growth above the historical range for that waterbody (Caraco et al. 1993).

Both of these indirect effects of elevated sulfate are difficult to model in a quantitative manner. One impediment is that the conversion to sulfide may be downstream from the site of sulfate discharge because the required combination of low oxygen and elevated organic matter may not occur immediately below the discharge. Sulfate conversion may occur when water flows laterally into adjacent wetlands or when the water reaches an impoundment or lake deep enough to have a hypolimnion. Enhanced loading of P and MeHg would occur when the anoxic water mixes back into surface water. This mixing would occur in a lake when the hypolimnion mixes with the epilimnion, and in rivers with lateral wetlands during a falling hydrograph.

Sulfate comes from a variety of sources. Generally, natural background sources result from marine rock and glacial till containing some marine rock such as limestone or shale. Surface water and ground water in the granitic Canadian Shield area is expected to have relatively low sulfate concentrations while waters in other parts of the state are expected to have relatively higher sulfate concentrations. Anthropogenic sources include air deposition (typically less than 1 mg/l) and domestic and industrial wastewater discharges. Wastewater sulfate concentrations can be elevated above surface water concentrations simply because of use of high-sulfate groundwater. In addition, sulfate may be elevated in wastewater by concentration through evaporation, capture of sulfur compounds by air pollution control equipment, or various industrial processes (e.g. lime addition in taconite production).

It is important to minimize the effect of sulfate on MeHg and P because Minnesota’s water quality is threatened by these chemicals state-wide. Federal NPDES permitting regulations prohibit the authorization of wastewater discharges that may cause or contribute to water quality impairments. Numerous water bodies in the state are listed as impaired because the MeHg concentrations in fish tissues make the fish unsuitable for frequent human consumption. Similarly, numerous water bodies are impaired because of excess P concentrations.

**Treatment technologies for sulfate removal from wastewaters are limited. Reverse osmosis and evaporation are energy intensive and generally considered infeasible.** A new treatment technology, submerged packed bed, has shown potential but there is an unevaluated risk of MeHg production within the treatment system. Land application or rapid infiltration basins may be effective but must be evaluated on a case-by-case basis.

While research indicates a strong correlation between sulfate loading and MeHg production in a sulfate-poor wetland, the factors that control MeHg production and P release in other surface waters are not documented. The research results do not, however, tell us how aquatic systems higher in sulfate react to increased sulfate loading. We have not reached a sufficient level of confidence with our understanding of the controlling factors such that firm effluent limitations based on these phenomena can be established. **Therefore, a permitting strategy will need regulatory and study/monitoring components to reflect our varying levels of understanding of MeHg production under differing environmental scenarios.** MeHg study and control is further complicated by the lack of a standard EPA analytical method and limited commercial laboratories that are prepared to conduct MeHg analyses. EPA has developed Draft Method 1630 (January 2001) for MeHg analyses. The draft method can be found at:
MPCA staff have used Frontier Geosciences in Seattle, WA for recent analyses. It is anticipated that the MDH lab, and possibly other labs in Minnesota, would gear-up to run Draft Method 1630 if demand for this work increased.

Notes: [since this note does not seem to be referred to anywhere, perhaps it should be moved up into the text.—otherwise, it is not contributing to the appendix]

1) As a general rule, the order of depletion of electron acceptors during bacterial metabolism in aquatic systems is O$_2$, NO$_3$, Fe$_2$O$_3$, MnO$_2$, then SO$_4$. SRBs are known to produce MeHg and it is thought that iron-reducing bacteria may also methylate mercury under certain conditions. In any given environmental setting, it is not easy to determine which bacteria are dominating degradation of organic matter. To achieve an understanding of biogeochemical mechanisms of the effects of elevated sulfate, it may be desirable to measure a number of parameters, including sulfate, total mercury, MeHg, iron, ortho-P, total P, and supporting parameters such as DOC, pH, oxygen, nitrate, and potassium (for an example of the utility of measuring this suite of parameters, see Balogh et al. 2004). For instance, elevated nitrate or oxidized iron could negate the effect of elevated sulfate because the bacterial community likely finds it energetically advantageous to consume either of those two chemicals as electron acceptors before consuming sulfate. Without information on nitrate and iron, the effect of elevated sulfate may appear to be inexplicably unpredictable. Potassium data may be useful in a different way—elevated potassium can be an indicator of a hydraulic source area in decaying organic matter such as a wetland. When potassium is correlated over time with DOC, MeHg, and P, then the weight of evidence tends toward wetlands as the source area for all of the materials.

Literature Cited:


Comments and Proposed Charge Questions for Peer Review of the Wild Rice Sulfate Standard Studies

WaterLegacy Proposed Charge Questions
The Wild Rice Sulfate Standard studies were funded by the Minnesota Legislature (Minn. Laws 2011, 1 Sp. c.2, art. 4, § 32(a)) and designed to evaluate questions related to potential rulemaking amendments to Minn. R. 7050.0224. Study objectives were to provide answers to these substantive questions:

1) whether Minnesota’s existing water quality standard limiting sulfate to 10 milligrams per liter in waters used for the production of wild rice is appropriate to protect natural beds of wild rice; and
2) whether Minnesota’s water quality standard limiting sulfate to protect natural beds of wild rice should or should not be limited to specific times of the year.

Based on these Study objectives and the multiple lines of research undertaken by scientists under contract with the MPCA, WaterLegacy believes the following charge questions are required in order to allow the independent peer reviewers to contribute their expertise to this process.

WaterLegacy Charge Question 1: Do the field data, mesocosm data and hydroponic data taken together support the existing 10 milligrams per liter (mg/L) sulfate standard to protect wild rice or some other standard? What is the best estimate of the degree to which the existing standard would be protective of natural beds of wild rice?

WaterLegacy Charge Question 2: Does the field, mesocosm and hydroponic data support a conclusion that sulfide in sediments is toxic to wild rice? Does the data allow determination of the threshold at which sulfide becomes toxic to wild rice? If so, what is the sulfide concentration where toxicity to wild rice is observed?

WaterLegacy Charge Question 3: Does the mesocosm study data support a conclusion that elevated concentrations of sulfate and sulfide have intergenerational adverse effects on wild rice seed weight and viability?

WaterLegacy Charge Question 4: Do study data related to sulfide toxicity and the data pertaining to conversion of sulfate to sulfide at various temperatures support any limits on the time of year during which the sulfate standard would apply to protect natural beds of wild rice? If so, what specific temporal limits?

WaterLegacy Charge Question 5: Are the wild rice sulfate studies on which the MPCA Analysis is based, in which neither sulfate or sulfide were manipulated in conjunction with iron, sufficient or insufficient to support the MPCA’s conclusions about the ability to predict sulfide based on iron content in porewater?

WaterLegacy Charge Question 6: Are the wild rice sulfate studies on which the MPCA Analysis is based sufficient or insufficient to support MPCA’s conclusion that high concentrations of iron in porewater prevent sulfate from diminishing wild rice abundance in the natural environment?

WaterLegacy Proposed Revisions of MPCA Charge Questions
MPCA’s charge questions appear to be constrained by political factors, overstate the support for an
“iron mitigation” theory advanced by the mining industry, and do not provide the peer review committee with sufficient latitude to provide independent analysis of the issues before the MPCA for which the research was instituted and funded by the Minnesota Legislature, which are summarized above. The study objectives should have been clearly stated in the charge to the peer review committee and the charge questions should be modified as explained below to allow peer reviewers a more substantive and effective role in the process.

1) Various MPCA charge questions are inappropriately narrow and constrain the peer reviewers from contributing substantively to the scientific discussion:
   MPCA Charge Questions 2 and 3 should be understood as part of Question 1.
   MPCA Charge Question 5 is not meaningful and should be eliminated.
   MPCA Charge Question 6 should provide peer reviewers greater latitude to respond to the issue.

2) Several charge questions inappropriately elevate an untested hypothesis advanced by the mining industry that anthropogenic iron would “mitigate” effects of sulfate discharge on wild rice and blur the distinction between correlations and experimental results.
   MPCA Charge Question 4 should be substantially revised.
   MPCA Charge Questions 7-8 assume that a correlation found in a targeted field sample establishes a causal relationship between sulfate, iron and sulfide. These questions should be eliminated.
   MPCA Charge Questions 9 should be reframed to allow the peer review committee sufficient professional latitude to comment on the Agency’s “iron mitigation” hypothesis.
   MPCA Charge Question 10 is redundant with Charge Question 9 and is not needed.

3) Once the Study objectives are clearly stated for the Peer Review Committee, WaterLegacy has no objection to MPCA Charge Questions 11, 12 and 13.

Amendments to the MPCA Charge Questions in Redline Format

**MPCA Charge Question 1:** Discuss the appropriateness of the sulfide seedling hydroponic test method and performance in evaluating the hypothesis that elevated sulfide in the sediment porewater can be toxic to wild rice. Among other issues, please comment on the reasonableness of uses of the initial exposure solutions as the operative exposure concentrations for the test and the MPCA’s use of regression analysis to determine effect levels.

**Charge Question 2:** Is it reasonable to use the initial exposure solutions as the operative exposure concentration for the test? Why or why not? If not, what approach do you suggest?

**Charge Question 3:** Is regression analysis to derive EC20 and EC50 values an appropriate way to analyze the sulfide seedling hydroponic data to identify effect levels? Why or why not? Is there an alternative approach to evaluate the data for effect levels that you would suggest the MPCA pursue?

**MPCA Charge Question 4:** Discuss whether the Analysis demonstrates that the lake and stream field survey data and results from selective sampling focused on water bodies that were high in sulfates where wild rice was present, including the following potential correlations: 1) elevated surface water sulfate and porewater sulfide; 2) elevated sulfate and iron and porewater concentrations of sulfide and iron, are sufficiently representative of Minnesota lakes and streams with wild rice to 1) examine the chemical relationships between sulfate in surface water and acid extractable iron, acid volatile sulfide, and porewater concentrations of sulfide and iron, and 2) inform protection of wild rice from elevated sulfate.
Please note any specific questions or concerns.

**Charge Question 5:** Does the MPCA Analysis make appropriate use of the mesocosm experiment data? Please describe any suggestions you have about how the data could be further analyzed, or any cautions about the existing or potential use of these data.

**MPCA Charge Question 6:** Do you agree or disagree with the MPCA’s assertion that the field survey and mesocosm experiment data further support the hypothesis that elevated sulfide in the sediment porewater above 300 μg/L can be toxic to wild rice? Explain why or why not and comment on the level at which the field data suggests sulfide can become toxic to wild rice.

**Charge Question 7:** Is the use of multiple quantile regression an appropriate tool for predicting porewater sulfide concentrations? Why or why not? If not, what other options for predicting porewater sulfide would be suitable?

**Charge Question 8:** In the multiple quantile regression, MPCA relied on the acid-extractable iron rather than the porewater iron to predict porewater sulfide concentrations based on surface water sulfate concentrations. Do you agree or disagree with this approach? Why or why not?

**MPCA Charge Question 9:** The MPCA Analysis focuses on sulfide in the porewater as the sulfur parameter impacting wild rice, and the role of sulfate and iron as key variables controlling sulfide concentrations in porewater. Was this focus appropriate to inform understanding of the effects of sulfate on wild rice? Why or why not? If not, what other variables do you suggest the MPCA explore?

**Charge Question 10:** Please identify any concerns you have about the Synthesis, particularly any key omissions or assumptions in the logic that should be further evaluated.

**Charge Question 11:** Please state your overall assessment of the five Study components. Did MPCA choose appropriate Study components to meet Study objectives (summarized above) and to support the Analysis? Why or why not?

**Charge Question 12:** Please provide any other comments you may have on the Study data collection and interpretation, or on the Analysis.

**Charge Question 13:** Please identify any other issues or critical data gaps for further research that should be considered when evaluating the relationship between wild rice and sulfate.

Respectfully submitted,  

DATE: July 8, 2014

Paula Maccabee, Esq.  
JUST CHANGE LAW OFFICES  
1961 Selby Ave.  
St. Paul MN 55104  
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e-mail: pmaccabee@justchangelaw.com  
Advocacy Director/Counsel for WaterLegacy
Pat and Shannon,

I am including new comments on the sulfide hydroponics experiment. I am also including copies of the comments submitted by Paula Maccabee for Water Legacy. I support the Water Legacy comments in their entirety. I also support the Water Legacy request for changes in the proposed charge questions for peer review of the wild rice sulfate standard studies.

Please let me know, if I need to repeat comments and concerns I have expressed to you via email, during MPCA meetings held in Duluth.

I reiterate, here, that the repeated statement to the advisory committee be upheld: the standards will be science-based, requiring that any experiments used to support change in a standard be independently replicated several times. This is required despite any political pressure to change a standard. If results from independent replications differ, then additional independent replications are required.

Please confirm receipt of these comments.

Thank you.

Sara

Sara Barsel, Ph.D.
I am submitting these additional comments to the MPCA wild rice sulfate standard advisory committee. I am a retired Ph.D. scientist with graduate training and research experience in genetics, plant physiology, and plant pathology.

Hydroponics experiment critique – Sara Barsel, Ph.D.

I have concerns about the technical skills of the individuals advising and performing this experiment. I also wish to reiterate the comments recorded on 4/16/14 regarding the sulfide hydroponic design and whether or not it matches the hypothesis it proposes to test.

On 4/16/14, Ed Swain presented data and statistical analysis of the hydroponics experiment examining the impact of sulfide concentration on the growth of wild rice seedlings.

I want to reiterate and expand my concerns about this experiment. I think that the entire set of “hydroponics” experiments are flawed and should be discarded. I will present my concerns concentrating on the sulfide experiments.

I will present my concerns out-of-order, because the first concern is the most serious.

1. During his presentation on 4/16/14, Ed stated that there was difficulty maintaining anaerobic/anoxic conditions in the containers.

   Experimenters should be reminded of the formula for the photosynthetic light reaction: 
   \[ 6\text{CO}_2 + 12\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 6\text{H}_2\text{O} \]

   Any expectation that photosynthesizing plant materials in any closed container system will not impact the growth conditions by production of oxygen and water, is naïve, at best. Neither the anaerobic/anoxic conditions or the sulfide and buffer concentrations would be expected to remain stable and/or similar to initial experimental conditions.

   **Statistical manipulation of any data taken from this experiment is meaningless, in my opinion.**

   In the Draft Germination Growth Test Method for Anoxic Conditions; Revised 11/07/13 (p. 4), contained within the Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment. Presented to the Minnesota Pollution Control Agency by John Pastor, Professor, Dept. of Biology, University of Minnesota Duluth, December
31, 2013, the following method is described for introduction and mixing of the experimental sulfide solution in the individual containers:

“1.6.8 Exposure jars are spiked with a volume of concentrated sulfide solution appropriate for the corresponding nominal sulfide concentration of that jar. Control exposure jars are spiked with deoxygenated distilled water. Sulfide solutions are spiked into the jars through the chlorobutyl septa in the cap using Hamilton volumetric glass syringes. Spike volumes range between 0.2 – 3.0 mL of spike solution depending on target exposure concentrations and nominal concentrations of stock sulfide solution concentration.
1.6.9 The jars are inverted twice to mix the solutions and are placed on a tray.”

I have the following comments/concerns:

a. Introduction of different volumes of aqueous sulfide solution into fixed volumes of modified Hoagland’s solution in PIPES buffer will result in different dilutions of the salts contained in the modified Hoagland’s solution/PIPES buffer.

While this may have been a laboratory convenience, using only a single aqueous sulfide stock solution, it is poor experimental design.

b. Section 1.6.9 (above) describes two inversions of the bottle. Presumably, this will achieve a thorough mixing of the solutions resulting in equal distribution of the experimental sulfide solution throughout the container.

There is no information regarding the densities of the sulfide solutions and the modified Hoagland’s solution/PIPES buffer. The assumption that two inversions of the bottle will result in equal distribution of the test solution(s) is unsupported. Consequently, variability in seedling response to specific experimental concentrations of sulfide may actually be responses to differential concentrations of sulfide.

Again, while this may be a laboratory convenience, it is poor experimental design. There are no reports of visually testing this method using dye in the sulfide solutions and seeking equal distribution within the larger containers of modified Hoagland’s solution/PIPES, or anything more sophisticated.
2. During the presentation on this experiment at the 2/28/13 Wild Rice Standards Study – Mid Project Review, John Pastor spoke about problems with the experiments. He mentioned using Tris buffer. I immediately spoke with Ed Swain and presented him with a copy of *Buffers: A Guide for the Preparation and Use of Buffers in Biological Systems*. Gueffroy, Donald E., editor. © 1975; CALBIOCHEM; La Jolla, CA to copy and share with John Pastor.

Limitations of Tris buffer in biological systems are widely known. This information is readily available in booklets describing biological buffer systems. I was immediately concerned by the apparent lack of general knowledge or library research prior to designing this experiment.

Subsequent to this meeting, PIPES buffer was substituted for Tris buffer in the “hydroponic” experiments. While I am pleased to see that sesquisodium salt of PIPES was used for solution preparation, eliminating the difficulty of PIPES solubility, I am concerned that discussion of selection of PIPES, *in lieu* of other zwitterionic Good buffers, does not occur in any report I have seen. Optimization of buffer conditions, particularly since the modified Hoagland’s salts are dissolved in this buffer, would be expected in any responsibly conducted plant physiology experiment.

The initial choice of Tris buffer and the lack of discussion justifying selection of PIPES as the replacement buffer, leave me uncomfortable with assuming that buffer was correctly prepared regarding temperature and pH adjustment, etc.

There is no mention of filter sterilization of the solutions used in the experiments. This would have been easy to accomplish. It would have reduced the chance of introducing contaminants into the experimental systems. Although there are methods for seed sterilization, it was apparent, from discussion, that these were never considered in these experiments.

3. Additional concerns include
   a. These experiments are described as “hydroponic” experiments. This is a misuse of the term, as generally understood in the literature and practice. Even Wikipedia has a more accurate description of hydroponics [http://en.wikipedia.org/wiki/Hydroponics](http://en.wikipedia.org/wiki/Hydroponics)
Materials in these sulfide experiments are completely submerged, and maintained in partially anaerobic/anoxic conditions.

Plants grown under hydroponic conditions have submerged roots, but vegetative portions are exposed to air.

b. **There is no relationship between the growth conditions for any of these experiments** (sulfate or sulfide exposure for seed germination or seedling growth) **with the normal, in vivo conditions under which Zizania palustris grows.**

These experiments are examining the growth of Zizania palustris seeds under artificial conditions, i.e. total submergence with artificial exposure to light and temperature, and in the sulfide experiments, without exposure to air.

c. General knowledge appears lacking in the literature regarding *Zizania palustris* genetic variability, seed storage conditions impact on seed viability, influence of seed size on germination and size of the resulting seedling, etc. .
Allelopathic Potential of Aquatic Plants Associated with Wild Rice (Zizania palustris): I. Bioassay with Plant and Lake Sediment Samples

- H. A. Quayyum,
- A. U. Mallik,
- P. F. Lee

Abstract

The allelopathic potential of eight aquatic plants associated with wild rice was investigated using lettuce and wild rice seedling bioassays. Rhizome aqueous extracts of Scirpus acutus, Potamogeton natans, Nymphaea odorata, Nuphar variegatum; shoot extract of Eleocharis smallii; whole plant extract of Myriophyllum verticillatum; and leaf extract of P. natans significantly reduced the root length of lettuce and wild rice seedlings. The lettuce seedling bioassay was more sensitive than the wild rice bioassay. Shoot growth was less affected than the root growth. Water extract of sediments associated with the aquatic plants had little growth inhibitory effect on wild rice. Our study did not yield any conclusive evidence that the wild rice-associated aquatic plants have allelopathic effects on wild rice. We emphasize the use of target species as a bioassay material in allelopathic studies. Further investigation on allelopathic effects of lake sediments associated with the neighboring plants of wild rice is necessary to evaluate their ecological significance.
Laurie Waite

From: Leonard Anderson [bander@northlc.com]
Sent: Monday, July 14, 2014 3:24 PM
To: Engelking, Pat (MPCA)
Subject: Comments on Wild Rice Sulfate Standard Studies

Pat, would you please forward these comments on to the Peer Reviewers. Thank you. Len

ADVISORY COMMITTEE FEEDBACK TO PEER REVIEWERS

7-14-14

My name is Leonard Anderson. I serve on the Wild Rice Research Advisory Committee. I am a lifetime wild rice hand harvester and a professional biologist.

Back in 2011 when we were designing the wild rice research protocol, the MPCA offered suggestions which included dovetailing this research with the much more extensive on-going Hg methylation research. Many of us on the committee agreed. In my Comments on Wild Rice Research Protocol that I submitted, I said, "In order for the taxpayer to get the most bang for their bucks, it is important that sulfate biochemistry be studied in a way that can dovetail with existing data and expertise. We can learn about the pore water sulfur and iron chemistry and learn how to best protect wild rice, but we should also consider how that relates to mercury methylation and harbor corrosion.” The Minnesota Chamber of Commerce and other industrial members on the committee objected strenuously to any consideration of methylation. However, now they are supporting a site specific standard for wild rice based on the availability of iron to mitigate pore water sulfide.

Manipulating sediment iron to scavenge pore water sulfide is an interesting idea. However, it has not been done. What needs to be done, and there is research going on that could address that dynamic, is to measure the impact of sulfate and iron concentrations on the activity of both sulfate reducing and iron reducing bacteria. In that way we can get at the mercury methylation by both kinds of bacteria and the sulfide that becomes available. We must not protect wild rice and then harm our children with mercury in the food web.

In Minnesota wild rice waters, down in the anoxic sediments, these biochemical processes are inextricably bound together. The dynamics of mercury, iron and sulfide and the impacts on wild rice and fish tissue mercury must be understood before we can safely use any site specific standard.

Respectfully submitted, Leonard Anderson

130 Twin Lakes Dr

Cloquet, Mn. 55720

218-879-6521
Hello Ms. Engelking,

On behalf of Kathryn Hoffman from the Minnesota Center for Environmental Advocacy, please find the attached Wild Rice Sulfate Peer Review comments. Please do not hesitate to contact me or Kathryn directly should you have any questions or concerns.

Thanks,
Leah Harms
Legal Secretary
Minnesota Center for Environmental Advocacy
26 E Exchange Street, Suite 206
St. Paul, MN 55101
(651) 287-4868

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July 14, 2014

Patricia Engelking
Environmental Analysis & Groundwater Services
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Re: Wild Rice Sulfate Standard Study Peer Review

Dear Ms. Engelking,

Minnesota Center for Environmental Advocacy (“MCEA”) appreciates this opportunity to provide input to peer reviewers for the Wild Rice Sulfate Standard Study.


At the outset, MCEA is concerned at the prospect of peer reviewers receiving input from advocacy and business groups who have a stake in the outcome of this process as part of an independent scientific review. The bulk of the comments will likely reveal far more about the tumultuous political environment surrounding Minnesota’s existing water quality standard than about the science of sulfate, sulfide and wild rice in Minnesota’s waters. This process is not consistent with a rigorous and objective peer review process in place at most academic and scientific institutions.

An objective peer-review process is for scientific communication and not opinion, regulations or politics. The ultimate goal of the process is finding out to what level the reviewed work meets certain standards of scientific quality, not determining a proper or enforceable water quality standard. The science that is being reviewed may ultimately be relied upon by other scientists and regulators as they research, develop and enforce their work. For that reason, it is essential that the peer review process is trustworthy so that those that rely on these studies in the future can have some certainty that the research met the standards of science. It is the future work of scientists and researchers to determine if the work completed in the sulfate study is correct, to build on it further, determine its shortcomings, or to replicate its results.

MCEA strongly encourages the Peer Reviewers to consider that the viewpoints of most, if not all, advisory group members are subjective, and to only consider appropriate scientific questions raised by these commentators.

The main purpose of the Clean Water Act is to “restore and maintain the chemical, physical, and biological integrity of the Nation’s waters” by reducing, and eventually eliminating the discharge of pollutants into these waters. Primary responsibility for establishing water quality standards is left to the states. However, the Environmental Protection Agency (“EPA”) sits in an oversight capacity over the state-implemented standards, with the power to approve or reject the proposed standards.

When establishing water quality standards, states must first classify the “beneficial uses” for which the particular water body is to be protected, such as fishing, swimming or drinking. Then, the states must determine the level of water quality necessary to protect those uses. The description of the level of water quality necessary to protect the beneficial uses may take the form of a narrative statement, or numeric pollutant concentration levels.

When determining whether to approve or disapprove a particular water quality standard, the EPA must determine, among other things, whether the state has “adopted criteria that protect the designated water uses.” “Such criteria must be based on sound scientific rationale and must contain sufficient parameters or constituents to protect the designated use. For waters with multiple use designations, the criteria shall support the most sensitive use.”

The following principles may be drawn from the above-cited statutory and regulatory provisions:

(1) Any water quality standard must be scientifically sound and protective of the designated use. Thus, water quality standards must be designed to protect all sites, and should not be designed to focus on exceptional circumstances.

(2) The sulfate standard for wild rice waters has already been adopted by the state and approved by the EPA. Thus, the EPA has already determined that 10 mg/l sulfate is a scientifically sound criteria designed to protect the designated use of “waters used for the production of wild rice.”

(3) Because an approved standard is already in place, the burden lies with the MPCA to show that any proposed change in the standard has a strong scientific basis, and is protective of wild rice waters.

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1 33 U.S.C. § 1251(a).
2 33 U.S.C. § 1251(b).
3 33 U.S.C. § 1313(c).
5 Id.
6 Id.
7 40 C.F.R. § 131.5.
8 40 C.F.R. § 131.11(a) (emphasis added).
9 Although the wild rice standard was put under the “agricultural” beneficial use category, as MPCA points out, the legislative history demonstrates that it was intended to protect both natural and cultivated stands of wild rice.
In other words, water quality standards are designed to be conservative in relation to the designated use. The goal of a water quality standard is not to protect the designated use at just some waterbodies. It is designed to protect the most sensitive use at the most sensitive sites. And it must do so based on a sound scientific rationale.

The conservative nature of the standard is evidence from the MPCA’s discussion of the conclusions of John Moyle, the Department of Natural Resources researcher upon whose field research the sulfate standard is based. Moyle noted that “no large stands of rice occur in water having sulfate content greater than 10 [mg/l], and the rice generally is absent from water with more than 50 [mg/l].” The state did not use this research to set the wild rice standard at 50 mg/l, reasoning that this would protect some rice at some sites. Rather, it chose the lower number to protect all sites, or at least all sites that Moyle observed, based on the science available at the time.

Any new proposed standard faces an even greater burden because the EPA is operating on the assumption that the current standard is scientifically sound. It is not enough that the new research suggests that a different number might make sense to protect wild rice. Perhaps if MPCA were to set this standard today, they would set it at 5 mg/l, or 20 mg/l. But that is not the point of the exercise. Instead, there must be sufficient evidence to show that 10 mg/l is not correct, and some other number is correct. And any effort to raise the number, potentially resulting in a standard that is not protective of the beneficial use at some locations, will, and should, face particular skepticism.

As the MPCA points out in its Charge for Peer Review, the purpose of the peer review process is not to review the existing wild rice standard. However, it is important to understand the regulatory context of these studies, which were designed to determine whether the existing wild rice standard is scientifically sound. Some groups will submit comments suggesting that the appropriate standard for sulfate should be something other than 10 mg/l. For instance, the Chamber of Commerce has proposed a sulfate limit of 1600 mg/l, extraordinarily, a level that not only exposes wild rice to harm, but humans and animals as well.10

Ultimately, the inquiry for the MPCA isn’t, “what numeric criteria for sulfate should be selected to be protective of wild rice waters?” That question has already been answered. The inquiry in this case is, “Is there a scientific basis, based on the studies we have, to support changing the sulfate standard for wild rice waters, while still protecting wild rice?” Unless the scientific evidence shows that the standard is clearly incorrect, the only reasonable course for the MPCA is to leave the standard in place, unchanged. Thus the limitations of the studies — that the laboratory hydroponic experiments are for only a very short duration and do not measure long-term effects; that the hydroponic experiments do not test indirect effects of sulfate conversion to sulfide; that field study sites were not randomly chosen; etcetera - become very significant for MPCA, and should not be ignored by the Peer Reviewers in their analysis.

10 See, e.g., Sulfate in Well Water, Minnesota Department of Health, available at http://www.health.state.mn.us/divs/eh/wells/waterquality/sulfate.html, noting that sulfate levels of 400 mg/l or higher are unsafe for infants, and higher sulfate levels can also cause diarrhea and dehydration in animals.
3. The Selection Of Waters Surveyed In The Field Survey Was Not Random.

As discussed by MPCA in the Analysis of the Wild Rice Sulfate Standard Study, the selection of waters for the field work was intentionally not random. However, there is another factor that MPCA did not mention. Some of the sites were made at the suggestion of the Wild Rice Advisory Group, the same pool of individuals from whom the Peer Reviewers are receiving these comments. Industry members had a strong incentive to suggest sites where both high sulfate levels and wild rice were present because they wanted those sites investigated further. Thus, the few sites with both high sulfate and wild rice (however sparse) may represent a disproportionate number of the selected field sites.11

While this approach may have been most efficient, as MPCA states, the impact of this bias should be discussed more fully, and must be considered by the peer reviewers.

4. The Focus On Iron Availability May Not Be Justified.

The “basic scientific questions to be addressed in this study” are stated on page 51 of the MPCA’s Analysis of the Wild Rice Sulfate Standard Study (“MPCA Analysis”):

a) What concentrations of sulfide are deleterious to wild rice growth;
b) To what degree is sulfate converted into sulfide;
c) What controls the accumulation of the sulfide in the porewater; and
d) Under what conditions does sulfide accumulate to concentrations that are deleterious to wild rice growth?

MCEA does not disagree with these questions. However, MCEA is concerned that the conclusion of the research, stated on page 51-52 of MPCA’s Analysis and revolves largely around the presence of dissolved ferrous iron, states too strongly a conclusion that the research was never designed to support.

First, only one of the three studies – the Field Study – is used to support this hypothesis. The Hydroponics Study never tested any hypotheses about the presence of dissolved ferrous iron. The Mesocosm Study is never mentioned from page 41 onward in MPCA’s report, where all data used to support hypotheses related to the role of iron in controlling sulfide come from the Field Studies. But field studies, of course, are natural rather than controlled experiments and exhibit wide variability. The Field Studies also demonstrated that lakes, rivers and streams exhibit different characteristics when it comes to sulfate in surface water. (MPCA Analysis, p. 22-23).

Second, little is known about the nature of the iron in the sediment at these sites. Is it naturally occurring, or is it the result of years of disturbance in the watershed from mining or other industrial activity? Does it have other deleterious effects on the wild rice over time? For instance, the MPCA notes a “coating of brown crust” that accompanies the reaction of ferrous iron with oxygen, but offers no hypothesis as to whether this “crust” has long-term implications for wild rice growth. (MPCA Analysis, p. 52). Until we know the answers to these questions, the conclusion that “unless the reservoir of available iron in the sediment was low, or the rate that

\[11\] This issue is hinted at in the MPCA Analysis, p. 49.
sulfide is produced has outpaced the rate that new iron is supplied (from groundwater or surface water), newly produced sulfide immediately reacts with iron and precipitates, removing the sulfide from solution and from potential harm to wild rice” is premature.

Again, MCEA appreciates the opportunity participate in this process.

Sincerely,

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KH/lh
Pat, Shannon: Please find attached the comments from the Fond du Lac Band and the 1854 Treaty Authority regarding the MPCA’s charge questions to the peer review team for the Wild Rice Standard Study and analyses. We look forward to seeing this scientific review process carried out in a manner that both strengthens protection for wild rice and also supports the agency’s science-based regulatory enforcement capacity.

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Environmental Analysis and Outcomes  
Minnesota Pollution Control Agency

Sent by email only

July 14, 2014

Re: Comments on Wild Rice Sulfate Studies and Peer Review Questions

Dear Ms. Engelking and Ms. Lotthammer:  

The Fond du Lac Band of Chippewa and 1854 Treaty Authority hereby submit these comments in connection with the Minnesota Pollution Control Agency (“MPCA”) 2014 Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review to summarize wild rice sulfate research, and; Peer Review Charge Questions.

Fond du Lac and the Bands that the 1854 Treaty Authority represents (Bois Forte and Grand Portage) are federally recognized Indian tribes. All the Bands retain hunting, fishing, and other usufructuary rights that extend throughout the entire northeast portion of the state of Minnesota under the 1854 Treaty of LaPointe¹ (the “Ceded Territory”). In the Ceded Territory, all the Bands have a legal interest in protecting natural resources. Of these resources, wild rice is of particular importance and concern since it has declined substantially from its widespread historic distribution across the upper Midwest. Wild rice is of deep cultural significance to the Bands. It is an integral part of their way of life and is used for subsistence. The importance of

wild rice to the Bands was left out of the Background and Current Standard section of the Charge for Peer Review of “Analysis of the Wild Rice Sulfate Standard Study” (MPCA Draft Analysis) and should be included (wild rice is not just important to Minnesotans).

The context for the specific questions to be posed to the peer review committee is obvious; there should be clear and direct correspondence to the Study Objectives derived by the MPCA in response to the directive from the state legislature to answer the following questions:

1. whether Minnesota’s existing water quality standard limiting sulfate to 10 mg/l in waters used for the production of wild rice is appropriate to protect natural beds of wild rice; and
2. whether Minnesota’s water quality standard limiting sulfate to protect natural beds of wild rice should or should not be limited to specific times of the year.

Each of the Wild Rice Standard Study’s components had a specific purpose, strengths and limitations, yet none of the Study components’ specific purpose was to determine or define an iron component to a potential revised sulfate criterion. The MPCA’s Charge Questions do not appear to be designed to allow the members of the peer review committee to independently determine whether the Study has sufficiently and appropriately addressed those critical questions. Rather, they appear to be designed to elicit responses to the MPCA’s preliminary conclusions from the Study, which in some cases go well beyond the Study’s objectives and experimental components.

Lab Hydroponic Study Component

**MPCA Charge Question 1:** Discuss the appropriateness of the sulfide seedling hydroponic test method and performance in evaluating the hypothesis that elevated sulfide in the sediment porewater can be toxic to wild rice.

**MPCA Charge Question 2:** Is it reasonable to use the initial exposure solutions as the operative exposure concentration for the test? Why or why not? If not, what approach do you suggest?

**MPCA Charge Question 3:** Is regression analysis to derive EC20 and EC50 values an appropriate way to analyze the sulfide seedling hydroponic data to identify effect levels? Why or why not? Is there an alternative approach to evaluate the data for effect levels that you would suggest the MPCA pursue?

The lab hydroponics data revealed unequivocally that sulfide is toxic to wild rice. MPCA Charge Question 3 asks whether regression analysis to determine sulfide seedling EC20 and EC50 values is an appropriate way to identify effect levels. Tribes have expressed concerns regarding the lab hydroponics testing from the very beginning because they are very short-term and limited in scope, lasting only 11 days. Wild rice has been growing within the Ceded Territory for centuries. Even when germination occurs and seedlings begin to grow, the hydroponics study cannot determine whether or not the seedlings will continue to grow for a full season in the initial exposure concentrations and produce viable seeds for the next season. Therefore, we recommend eliminating MPCA Charge Question 3.
Utility of Field Survey Data

MPCA Charge Question 4: Discuss whether the Analysis demonstrates that the lake and stream field survey data and results are sufficiently representative of Minnesota lakes and streams with wild rice to 1) examine the chemical relationships between sulfate in surface water and acid-extractable iron, acid-volatile sulfide, and porewater concentrations of sulfide and iron, and 2) inform protection of wild rice from elevated sulfate. Please note any specific questions or concerns.

In 2011, the Minnesota Legislature provided funding to the MPCA to implement studies to evaluate whether the existing wild rice sulfate standard and seasonality of the standard (April 1st through August 31st) was appropriate to protect this important resource. Therefore, question 2) for Charge Question 4 should be rephrased as: 1) “Does the 10 milligram per liter sulfate standard adequately protect at least 80% of natural wild rice stands?” “Is protecting 80% of natural wild rice stands (a diminishing resource) sufficient from a population ecology perspective?” “If not, what percentile of natural wild rice stands should be protected and what should the standard for sulfate concentration be to provide that protection?” We also suggest adding a new secondary question that relates to the evaluation of the seasonality of the sulfate standard: 2) “Is there a scientific basis for the seasonality of the wild rice sulfate standard? Does the seasonal approach adequately protect wild rice?” “Why, or why not?”

This study was not specifically designed to evaluate the relationship between sulfate, sulfide and iron. Although an extensive discussion of this relationship was included in the MPCA Draft Analysis Rooting Zone Depth Profiles study component, it was previously stated in that document and the “Charge for Peer Review of “Analysis of the Wild Rice Sulfate Standard Study” that further analysis and possibly additional study is needed before general findings can be drawn. Therefore, we suggest removing the original question 1) and any other questions regarding the relationship between sulfate, sulfide and iron and any role iron concentration would play in revising the sulfate standard.

Mesocosm Study Component

MPCA Charge Question 5: Does the MPCA Analysis make appropriate use of the mesocosm experiment data? Please describe any suggestions you have about how the data could be further analyzed, or any cautions about the existing or potential use of these data.

The mesocosm study was the only component of the Study that provided controlled experimental evidence for the effects of sulfate loading over time, and that can be replicated. We recommend adding two Charge Questions regarding the mesocosm study: 1) “Is there evidence from the mesocosm study component to determine whether there is a cumulative intergenerational adverse effect on wild rice seed weight and viability?”, and 2) “Did wild rice in any of the experimental sulfate treatments exhibit differential recovery over the growing season(s)?”
Wild Rice in Relation to Sulfate, Sulfide, and Iron

MPCA Charge Question 6: Do you agree or disagree with the MPCA’s assertion that the field survey and mesocosm experiment data further support the hypothesis that elevated sulfide in the sediment porewater above 300 µg/L can be toxic to wild rice? Why or why not?

We believe this question should be rephrased because wild rice presence in the field studies was diminished when sulfide concentrations in the porewater were above 78 micrograms per liter, a sulfide porewater concentration similar to the control mesocosms (10 milligrams per liter sulfate) with porewater concentrations of 68 micrograms per liter. Our recommendation is to ask: “When mesocosm and field data are considered together, is there a concentration of sulfide in porewater that does not cause an adverse effect or diminish wild rice viability?”

We also suggest an additional question regarding what percentile of protection is necessary to ensure no net loss of a diminishing resource: “When evaluating sulfide concentrations in porewater, should a concentration allowing wild rice presence in 69-80% of waters be considered protective given that it is a diminishing resource?” “If not, what percentile of waters having wild rice present should be considered protective?”

Control of Porewater Sulfide by the Availability of Sulfate and Iron

MPCA Charge Question 7: Is the use of multiple quantile regression an appropriate tool for predicting porewater sulfide concentrations? Why or why not? If not, what other options for predicting porewater sulfide would be suitable?

MPCA Charge Question 8: In the multiple quantile regression, MPCA relied on the acid-extractable iron rather than the porewater iron to predict porewater sulfide concentrations based on surface water sulfate concentrations. Do you agree or disagree with this approach? Why or why not?

Synthesis: How Sulfate, Sulfide, and Iron Interact to Affect Wild Rice

MPCA Charge Question 9: The MPCA Analysis focuses on sulfide in the porewater as the sulfur parameter impacting wild rice, and the role of sulfate and iron as key variables controlling sulfide concentrations in porewater. Was this focus appropriate to inform understanding of the effects of sulfate on wild rice? Why or why not? If not, what other variables do you suggest the MPCA explore?

We suggest eliminating or revising Charge Questions 7, 8 and 9 to reflect the fact that none of the study components were specifically designed to investigate the concentrations or speciation of iron that may possibly provide some mitigation for sulfide toxicity to wild rice in natural, variable ecosystems. MPCA is overstating their understanding of these relationships and their controlling factors based upon the completed Study components. At most, the peer review process may be used to inform further lines of study; it should not be used to attempt to validate a major regulatory revision for which there was no discrete research program of experimental treatments or field investigations.
MPCA Charge Question 10: Please identify any concerns you have about the synthesis, particularly any key omissions or assumptions in the logic that should be further evaluated.

General Questions

MPCA Charge Question 11: Please state your overall assessment of the five Study components. Did MPCA choose appropriate Study components to meet Study objectives (summarized above) and to support the Analysis? Why or why not?

MPCA Charge Question 12: Please provide any other comments you may have on the Study data collection and interpretation, or on the Analysis.

MPCA Charge Question 13: Please identify any other issues or critical data gaps for further research that should be considered when evaluating the relationship between wild rice and sulfate.

We do not object to MPCA Charge Questions 10 through 13, however we think the order in which the questions are asked should be reversed and the primary questions relating to the specific reasons the study was funded should be asked first.

Summary of Comments

In summary, we recommend the Charge Questions clearly reflect the purpose of the Study and each of its components, revising or deleting any questions that are not related to the purpose of the studies, particularly the questions regarding the possibility that some form of iron may mitigate the adverse effects of sulfide on wild rice. Additional questions and information about the sediment incubation component must be provided to the Peer Review Panel in order to answer the questions regarding the protectiveness of a seasonal application of the wild rice sulfate criteria. Mesocosms are the only study component that can be replicated and the only component designed to determine if there is a long-term cumulative adverse effect to wild rice grown in various concentrations of sulfate. There were no Charge Questions provided by MPCA to elicit information specifically about long-term effects of various sulfate concentrations on wild rice, and the appropriate percentile of protection necessary to ensure no net loss of a significant but diminishing cultural and subsistence resource.

Sincerely,

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Fond du Lac

Darren Vogt
Environmental Director
1854 Treaty Authority
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From: Mike Hansel [MHansel@barr.com]
Sent: Monday, July 14, 2014 5:25 PM
To: Engelking, Pat (MPCA)
Cc: Lloyd Grooms; Joan Harmon
Subject: RE: Update of Scientific Peer Review Process and Dates for Review Meeting

Pat,

On behalf of the Minnesota Chamber of Commerce, please find attached the Chamber’s comments on the MPCA’s June 2014 revised analysis of the Wild Rice Sulfate Standard Study. Thank you for the opportunity to comment on the MPCA’s revised analysis. We look forward to working with you and the Peer Review Team.

Mike Hansel, PE

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From: Engelking, Pat (MPCA) [mailto:pat.engelking@state.mn.us]
Sent: Wednesday, June 18, 2014 1:24 PM
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Subject: Update of Scientific Peer Review Process and Dates for Review Meeting

Greetings,
July 14, 2014

Pat Engelking
Environmental Analysis and Outcomes
Minnesota Pollution Control Agency
520 Lafayette Road N.
St. Paul, MN 55155


Dear Ms. Engelking:

Attached please find the Minnesota Chamber of Commerce (Chamber) analysis of the MPCA’s June 9, 2014 revised analysis of the Wild Rice Sulfate Standard Study for the Scientific Peer Review. Please forward this, as you indicated in your email of June 18, to the Peer Review Panel for their consideration.

Thank you for the opportunity to provide analysis on the MPCA’s revised analysis. We look forward to continuing our work with you and the agency on the wild rice matter, and to participating with the Peer Review Panel during the August 13-14, 2014 meeting.

Sincerely,

Lloyd Grooms
MN Chamber of Commerce

Cc: MN Chamber Wild Rice Sulfate Task Force
MN Chamber
Technical Analysis of MPCA’s “Analysis of Wild Rice Sulfate Standard Study:
Draft for Scientific Peer Review”

Prepared by
Barr Engineering

June 9, 2014
MN Chamber
Technical Analysis of MPCA’s “Analysis of Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review”

Prepared by
Barr Engineering

June 9, 2014
# MN Chamber

**Technical Analysis of MPCA’s “Analysis of Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review”**

**June 2014**

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1.0 Introduction and Overview

The Minnesota Chamber of Commerce (Chamber) previously submitted extensive comments\(^1\) on the research conducted by the Minnesota Pollution Control Agency (MPCA) and its contract researchers at the University of Minnesota (Twin Cities and Duluth campuses). The purpose of this analysis is to respond to the MPCA’s “Analysis of Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review” June 9, 2014. While the Chamber takes issue with many of the analyses and conclusions which the MPCA puts forth in that document, the Chamber has chosen to limit its comments to the following main areas of concern:

- Toxicity of sulfide to wild rice seedling leaves
- Sources and Sufficiency of Porewater Iron
- Use of quantile regression analysis
- Use of results from the mesocosm experiments
- MPCA’s Synthesis: How Sulfate, Sulfide and Iron Interact to Affect Wild Rice

The Chamber makes the following observations:

- Wild rice seedling leaves showed impact from sulfide only because they were exposed in the sulfide hydroponic experiments to conditions which do not represent normal wild rice growing conditions;
- Wild rice plant portions which would potentially be exposed to the toxic form of sulfide (e.g. those in direct contact with the sediment – germinating seeds, mesocotyl growth and roots) were unaffected by sulfide at concentrations as high as 2,400 µg/L
- The MPCA has failed to account for rhizospheric influences in its interpretation of the hydroponic results. As noted in the MPCA’s own referenced material\(^2\) (emphasis added):

  "Traditional toxicity testing, including hydroponic approaches, generally neglect rhizospheric effects, which makes it difficult to extrapolate results to real ecosystem processes."

  The deprivation of oxygen to the wild rice leaves and shoots in the MPCA’s hydroponic test exposures, in addition to not representing “a real ecosystem process”, also eliminates the potential for the seedlings to oxidize sulfide, subsequently rendering it non-toxic.

- There is and will continue to be sufficient iron to precipitate sulfide in porewater to prevent it from accumulating to levels which may be toxic to wild rice plants throughout most of Minnesota (outside of the areas of the state underlain by the Des Moines lobe of the most recent glaciation); this is true even at very low iron concentrations and at low Fe/AVS ratios
- There are significant of sources of iron available to precipitate sulfide, including convection/advection and diffusion of iron from groundwater, convection/advection and

\(^1\) Technical Analysis of Wild Rice Research, Minnesota Chamber of Commerce, February 2014

\(^2\) Sulfide as a soil phytotoxic – a review. Lamers et al, Frontiers in Plant Science
diffusion of iron from overlying surface waters, in addition to the reduction of sediment-bound iron

• The quantile regression analysis is based upon the “wedge” (see Figure 22, page 45)\(^3\), purporting to show the relationship between porewater sulfide and surface water sulfate. The “wedge” is only present because of an extreme outlier of one observation; without that observation, there is no “wedge” and there is no correlation between porewater sulfide and surface water sulfate.

• The 2013 mesocosm experiments are fatally flawed. The mesocosms also do not represent actual ecological systems, for a number of reasons, not least of which is the fact that water was not replenished, allowing iron and other nutrients to be depleted and allowing for potential accumulation of waste products. The small size of the mesocosm containers, coupled with the lack of renewal water, also creates a strong potential for thermal stratification in the containers. Thermal stratification can have serious impacts on plant health, creating zones of depleted oxygen that can lead to the creation of sulfide, ammonia, and other potentially deleterious substances. The Chamber strongly cautions against relying upon the results of those studies.

• The conclusions reached in the MPCA’s synthesis are not supported by the research or by proper technical and statistical analyses.

---

\(^3\) Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, Minnesota Pollution Control Agency, June 9, 2014
2.0 Toxicity of Sulfide to wild rice seedling leaves

2.1 Hydroponic Tests

The MPCA, relying solely on the sulfide hydroponic study, has determined that sulfide is “likely harmful” to rice at concentrations greater than 300 µg/L. The Chamber’s point of view is that sulfide is not “likely harmful” to wild rice at concentrations less than 2,400 µg/L, based on the same studies.

The MPCA conducted several statistical analyses of the sulfide hydroponic studies data, and concluded.

Therefore, both the average EC20 and EC50 estimates are compatible with the conclusion that a sulfide concentration greater than 300 µg/L is likely to be harmful to wild rice.\(^4\)

However, the sulfide hydroponic study found only impacts on the leaves and stems of the wild rice seedlings were impacted by sulfides.

\[\text{Enhanced sulfide under anaerobic conditions did not affect germination of seeds (p > 0.10, Table 9), mesocotyl weights (p > 0.10, Table 10), or mesocotyl lengths (p > 0.10, Table 11) in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 µM sulfide (see Appendix 7 for raw data and statistics). The rangefinder test was repeated, and because the same results were observed, so we did not proceed with any further tests.}\(^5\)

And

\[\text{Root lengths were not significantly affected by sulfide concentration (Table 13).}\(^6\)

Wild rice seeds do not sink very far, if at all, into the sediment. The Chamber noted in its original technical analysis of the MPCA research:

\[\text{Wild Rice seeds are quite thin and light. Seed used in the hydroponic experiments is approximately 2 cm in length and } < \frac{1}{2} \text{ cm in width}\(^7\). When this seed falls to the sediment it will likely remain in the top 1 – 2 centimeters. Cultivated wild rice is generally planted at depths of 1-3 inches (2.5 to 8 cm).}\(^8\)

\(^4\) Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, Minnesota Pollution Control Agency, June 9, 2014
\(^5\) Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013
\(^6\) Id.
Seedlings will not emerge when planted deeper than 3 inches\textsuperscript{9}. During the sulfate seed germination and mesocotyl growth experiments, the mesocotyls grew to lengths of 11.8 to 13.5 cm, while during the sulfide seed germination and mesocotyl growth experiments, the mesocotyls grew to lengths of 7.5 to 9.3 cm\textsuperscript{10}. In both cases, the tops of the mesocotyls would not be in sediment.

In addition, the top portion of the sediment is likely not anoxic, due to diffusion of oxygen, sulfate and other oxygenated compounds in the upper few cm of the sediment\textsuperscript{11}. Dr. Johnson noted very low levels of sulfide in the upper 3 inches (8 cm) of the sediment in the mesocosms (outdoor container) studies\textsuperscript{12}, and very low to non-detectable sulfide concentrations in the root zone geochemistry of the two field sites in May and June\textsuperscript{13}.

The juvenile seedlings used in the second portion of the sulfate hydroponics experiments began as 1-2 cm mesocotyl seedlings, and grew to 11 to 12 cm seedlings by the end of the test (10 days)\textsuperscript{14}. Plant development of the shoot portion (following mesocotyl growth) would require oxygen for photosynthesis; shoot parts of the plants would not be exposed to anoxic conditions, either in the upper sediment or in the overlying water. Therefore, exposure of the leaves of juvenile seedlings to anoxic conditions is inappropriate.\textsuperscript{15}(Emphasis Added)

2.2 Do the sulfide hydroponic test methods “mimic an exposure that never occurs in nature?”

Perhaps in response to the Chamber’s comments, the MPCA explored whether or not the hydroponic method “mimicked an exposure that never occurs”.

Recall that the hydroponic sulfide test method immersed photosynthesizing seedlings, with at least one leaf, in an anoxic solution containing variable amounts of sulfide. Although the effects of hydroponic exposure of the juvenile (3-day old) seedlings to elevated sulfide appear to be compatible with results from the Field Survey and the outdoor Mesocosm Experiment, it is uncertain if the leaves of seedlings would ever be exposed to sulfide in a natural setting. If, in natural settings, wild rice seeds germinate in sediment prior to sending a leaf upward out of a well-defined

\textsuperscript{9} Id.
\textsuperscript{10} Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013. Tables 2 and 11
\textsuperscript{11} Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan Johnson, University of Minnesota Duluth, December 2013 Appendix B.
\textsuperscript{12} Id.
\textsuperscript{13} Id.
\textsuperscript{14} Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth December 2013
\textsuperscript{15} Technical Analysis of Wild Rice Research, Minnesota Chamber of Commerce, February 2014
sediment-water interface into oxygenated water, then it might be argued that the hydroponic method mimicked an exposure that never occurs.\textsuperscript{16}

The MPCA then examines the concentration of oxygen in water above the sediment in field sites where "peepers" were inserted into the sediment, but measured the lowest 2 cm of overlying water. The MPCA concludes:

*Given that oxygen just above the sediment was below 0.35 mg/L at 25\% of the lake sites sampled in 2013, it is conceivable that the leaves of wild rice seedlings could be exposed to sulfide diffusing out of the sediment. Furthermore, the sediment-water interface may be poorly defined, potentially resulting in additional exposure of seedling leaves to sulfide (and which further complicates this question). Together this evidence suggests that it cannot be asserted with confidence that the experimental method used for the hydroponic sulfide seedling test mimicked an exposure that never occurs.*\textsuperscript{17}

However, the MPCA ignores three glaring facts that demonstrate that the hydroponic studies in fact mimic an exposure that never occurs in nature:

- Oxygen concentration was not zero in the overlying water in the lake sites; there was always some amount of oxygen present. Stream sites, in comparison, had a median dissolved oxygen level of 1.9 mg/L
- The sediment is the only area where zero oxygen conditions are documented. Parts of the plant exposed to these conditions include germinating seeds, roots and mesocotyls. All three parts of the plant that grow in the sediment were completely unaffected by sulfide
- Seedling lengths exceeded the 2 cm depth of water with low oxygen levels, effectively elevating the leaves out of areas that could potentially contain sulfide

### 2.2.1 Oxygen concentration was not zero, but there was oxygen present

The MPCA makes much of the fact that organisms in and near the sediment will use oxygenated compounds in a certain order, with oxygen being the preferable substrate:

*The scientific literature generally predicts that the bacterial community in an organic-rich saturated soil will proceed in a more-or-less orderly fashion through a sequence of electron acceptors, in the order predicted by energetics of: oxygen, nitrate, oxidized manganese, oxidized iron, sulfate, and carbon dioxide. Investigators have found that the different bacterial functional groups overlap with each other, but the general order holds fairly well.*\textsuperscript{18}

\textsuperscript{16} Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, Minnesota Pollution Control Agency, June 9, 2014

\textsuperscript{17} Id.

\textsuperscript{18} Id.
Since there is in fact oxygen in the 2 cm of water overlying the sediment in the 2013 field studies (see Table 9\textsuperscript{19} and Appendices A and B of the Johnson root zone study\textsuperscript{20}), and if microorganisms preferentially convert oxygen to water prior to converting sulfate to sulfide, it is likely that the organisms present will convert oxygen to water, rather than convert sulfate to sulfide. Indeed no or very little sulfide was observed in the top 4.5 cm of either the mesocosm studies\textsuperscript{21} or the two field surveys conducted by Dr. Johnson. Thus, based on the MPCA’s own studies, it is unlikely that there is sulfide present in the water above the sediment layer, (whether that layer is poorly or tightly defined) and it is quite likely that the hydroponic sulfide seedling test indeed mimicked an exposure that never occurs.

2.2.2 Germinating seeds and mesocotyl growth were unaffected by sulfide

In the sulfide hydroponic studies, there were actually two parts:

- Effects of sulfide on germination of wild rice seeds under anaerobic conditions; and
- Effects of sulfide on growth of juvenile seedlings under anaerobic conditions

In the first part, germinating seeds were exposed to a range of sulfide conditions (0 to 90 µM) for 10 days. Dr. Pastor concluded that:

Enhanced sulfide under anaerobic conditions did not affect germination of seeds ($p > 0.10$, Table 9), mesocotyl weights ($p > 0.10$, Table 10), or mesocotyl lengths ($p > 0.10$, Table 11) in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 µM sulfide (see Appendix 7 for raw data and statistics). The rangefinder test was repeated, and because the same results were observed, so we did not proceed with any further tests.\textsuperscript{22}

Table 11 shows that the mesocotyl lengths after 10 days ranged from 7.5 to 9.3 cm. Thus, even if there were sulfides in the sediment and in the 2 cm of water overlying the sediment, the juvenile plants would have grown through the sediment and the 2 cm of overlying water, and not be exposed to sulfide concentrations. Even if they were, it is clear that germinating seeds and mesocotyl growths are unaffected by sulfide at concentrations as high as 90 µM (~ 2,400 µg/L)

2.2.3 Seedling lengths exceeded the 2 cm depth of water with low oxygen levels

In the second part of the sulfide hydroponic test, 3 day old seedlings were exposed to a range of sulfide conditions (0 to 90 µM) for 10 days. Although the initial length of the seedlings was not measured, the experimental method notes that:

\begin{itemize}
  \item Enhanced sulfide under anaerobic conditions did not affect germination of seeds ($p > 0.10$, Table 9), mesocotyl weights ($p > 0.10$, Table 10), or mesocotyl lengths ($p > 0.10$, Table 11) in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 µM sulfide (see Appendix 7 for raw data and statistics). The rangefinder test was repeated, and because the same results were observed, so we did not proceed with any further tests.\textsuperscript{22}
\end{itemize}

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\textsuperscript{19} Id.
\textsuperscript{20} Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson, University of Minnesota Duluth, December 2013
\textsuperscript{21} Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth December 2013
\textsuperscript{22} Id.
Germinated seeds (referred to as a sprout) are selected with at least 1-2 cm of mesocotyl growth. A germinated seed (sprout) is described as growth of the mesocotyl that is longer than the seed coat.\textsuperscript{23}

An unintentionally ineffective randomization of the plants being assigned to the treatments at the beginning of the exposure could easily have occurred and then drastically affected the end results. Table 15\textsuperscript{24} shows that stem plus leaf length varied from 5.6 to 14.5 cm. (Note that one (1) cm of initial variation in the length of the sprouts is nearly 18 % of a final length of 5.6 and 7 % of a final length of 14.5 cm. This is why every plant’s response should have been recorded as change in length: final – initial.)

Again, this length is longer than the 2 cm of overlying water which the MPCA posits can be lacking in oxygen and containing sulfides (evidence notwithstanding). Thus, it is quite likely that the experiment wherein the entire stem and leaf is exposed to anaerobic conditions with varying sulfide concentrations mimicked an exposure that never occurs.

As demonstrated in standard sediment geochemistry and fluid dynamics textbooks and throughout the literature, the concentration of a chemical diffusing out of the sediment rapidly decreases above the sediment water interface (e.g., for O\textsubscript{2} see Jørgensen and Revsbech\textsuperscript{25}). The concentration of sulfides even a few millimeters above the sediment water interface is significantly lower than that observed in the pore water. The concentration in the water column, even just above the sediment water interface, is not equivalent to that in the porewater. Thus, the sulfide hydroponics experiments clearly do not represent a realistic exposure scenario for rice stems and leaves, and in fact provides an exaggerated estimate of toxicity.

Therefore, the portions of the sulfide hydroponic experiments which exposed juvenile seedlings to anoxic conditions and varying concentrations of sulfide do not represent real ecosystem processes, and are in fact an exposure that would never occur. Therefore, the alleged sulfide concentration that is harmful to wild rice – 300 µg/L – is not a true reflection of the toxicity of sulfide to wild rice plants.

In contrast, those portions of the plant which might be exposed to anoxic conditions with elevated sulfide concentrations – germinating seeds, mesocotyls and roots – are all unaffected by sulfide at concentrations as high as 90 µM (~2,400 µg/L). This portion of the experiment does mimic natural conditions.

\section*{2.3 EC20 and EC50 regression analyses}

The regression analyses used to determine the EC20 and EC50 are lacking significance testing or goodness of fit testing. In the absence of these types of diagnostics it is difficult to judge the quality of the fit and the robustness of the resultant EC20 and EC50 estimates. In addition, if the three experiments are truly representative of the dose-response one should be able to combine the three experiments and

\textsuperscript{23} Wild Rice Juvenile Seedling Growth Test: Anoxic Conditions, Dr. John Pastor, University of Minnesota Duluth, November 2013.
\textsuperscript{24} Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth December 2013
\textsuperscript{25} Diffusive boundary layers and the oxygen uptake of sediments and detritus, Jørgensen and Revsbech, Journal of Limnology and Oceanography, 1985 \url{http://www.aslo.org/lo/toc/vol_30/issue_1/0111.pdf}
recalculate the EC20 and EC50 for the combined dataset. However, the results show extreme variability in the control/low dose treatments. MPCA could normalize the results to control and then combine them. However, an explanation for the variability in the controls is required to judge the quality of the analysis.
3.0 Sources and sufficiency of porewater iron

3.1 Source of iron

The MPCA apparently concludes that the largest source of iron to replenish reduced pore water iron (the iron that precipitates sulfides) is the sediment itself.

*The total potential reservoir of iron is estimated as the concentration of acid-extractable iron (Fe) in the sediment*, whereas the portion that has been precipitated as an iron-sulfide can be estimated by the concentration of acid-volatile sulfide, AVS (since sulfide almost exclusively precipitates as an iron compound in Minnesota systems). Therefore, the progressive depletion of iron reserves by elevated sulfate in Minnesota waters is documented by a positive correlation between sulfate and AVS (e.g. Figure 20), and a progressive decline in the Fe/AVS ratio as sulfate increases (Figure 21). High values of Fe/AVS are indicative of systems that have a large reservoir of iron to keep sulfide from accumulating in porewater, and low values of Fe/AVS are indicative of systems where the production of sulfide is outpacing the rate that iron is supplied.26 (Emphasis added)

This argument ignores well established mass transfer principles and other obvious sources of iron:

- Iron can easily diffuse from overlying waters into the sediment and sediment porewaters
- Iron in groundwater is constantly convected into “gaining” streams (typically headwater streams where groundwater flows into the river from the surrounding watershed) and into the littoral zone of lakes (the shallow area near the shore where wild rice grows) and can easily diffuse into sediment porewaters.

See Figure 1.

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While there may be dissolved iron which is originating from the sediment, it is likely that the kinetics of diffusion (from groundwater into porewater and from overlying surface water into porewater) and convection/advection (groundwater upwelling into the sediment and surface water flowing past or, in the case of lakes, wave action bringing water into intimate contact with the sediment) are much faster than the biogeochemical liberation of iron from sediments.

Dr. Johnson studied the diffusion of sulfate into sediment, using one riverine and one lake sediment at two different temperatures. He modeled the flux of sulfate into the sediment and determined that it was significant, on the order of 42.5 µg cm\(^{-2}\) day\(^{-1}\) under cold (4°C) to 100.8 µg cm\(^{-2}\) day\(^{-1}\) under warm (23 °C) (Table 7).\(^{27}\) This resulted in equilibrium between porewater and surface water.

Dr. Johnson also measured and modeled the diffusion of chloride, fluoride and bromide as:

\textit{chloride, fluoride, and bromide are inert and stable under both oxidizing and reducing conditions and interact minimally with the solid phase.}\(^{28}\)

Clearly, if chloride, fluoride, bromide and sulfate can diffuse into sediment from overlying water, so too can iron. A review of the data from the field surveys\(^{29}\) shows that iron in the overlying water in the surveyed sites range from 5 µg/L to over 7,200 µg/L (although it is strange that all other surface water measurements have units of mg/L – only iron has units of µg/L).

Dr. Johnson showed that sulfate diffuses readily into porewater from the overlying surface water:

\(^{27}\) Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments. Will DeRocher, Nathan W. Johnson, University of Minnesota Duluth, December 2013

\(^{28}\) Id.

Table 7  Simulated steady state sulfate flux under cold and warm loading and recovery conditions

<table>
<thead>
<tr>
<th>Loading Phase (~300 mg/L overlying water)</th>
<th>Cold steady state flux [ug cm-2 day-1]</th>
<th>Warm steady state flux [ug cm-2 day-1]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>42.5</td>
<td>100.8</td>
</tr>
<tr>
<td>Recovery Phase (~40 mg/L overlying water)</td>
<td>4.6</td>
<td>16.2</td>
</tr>
</tbody>
</table>

One would expect iron to show similar flux rates, both from surface waters as well as from groundwater.

3.2 Sufficiency of Iron

The MPCA worries that:

> If sulfate becomes elevated and enough sulfide is produced to deplete iron faster than it is supplied, it likely takes multiple years for the available iron to become depleted to the point that little addition iron is available to go into solution.\(^{31}\)

Although the MPCA acknowledges it will likely take “multiple years”, the agency is concerned that iron will ultimately be depleted. This is not the case, except for the mesocosm experiments where essentially closed experimental systems were utilized.

Dr. Johnson notes:

> Phase II provided a means of quantifying dissolved sulfate within the porewaters at a given time based on the surface water concentration. This is of interest due to the predilection of bacteria to reduce sulfate to sulfide under anoxic conditions (Van Der Well, Smolders, OP Den Camp, Roelofs & Lamers, 2007). Though porewater sulfide was measured initially and at the end of each phase, a quantifiable rise in dissolved sulfide was not observed over the course of this nine-month study. A similar study conducted by Van der Well et al. 2007, observed a strong negative relationship between iron and sulfide concentrations within sediment porewaters. Their research was conducted over the course of 21 months and utilized in situ testing within a peat meadow.

> Based on the geochemistry of the sediments used for the present study where iron concentrations of 200 – 1000 uM were observed, low sulfide concentrations could be

\(^{30}\) Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments. Will DeRocher, Nathan W. Johnson, University of Minnesota Duluth, December 2013

\(^{31}\) If sulfate becomes elevated and enough sulfide is produced to deplete iron faster than it is supplied, it likely takes multiple years for the available iron to become depleted to the point that little addition iron is available to go into solution.
expected (Van Der Well et al. 2007), based on the formation of insoluble iron sulfide compounds. The initial hypothesis for the present study was that a decrease in porewater iron would be observed during the loading phase of the study as iron sulfide was formed; however, the sulfate exposure portion of this study was not long enough to allow a measurable titration of the high iron content (including solid phase) of the sediment and the appreciable accumulation sulfide in the porewaters. A longer-term sulfate loading study was conducted in field mesocosms as a part of the MPCA Wild Rice Sulfate Standard Study and is described in separate reports (Johnson 2014; Pastor and Dewey 2013). Though the field mesocosm studies contained a longer time course of sulfate loading, they occurred in hydrologically isolated tanks and therefore cannot capture the effects of groundwater or other sulfur and iron sources to natural field sites.\footnote{Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments. Will DeRocher, Nathan W. Johnson, University of Minnesota Duluth, December 2013} (Emphasis added)

Thus, it is unlikely that iron will become depleted, except in the mesocosms, which were “hydrologically isolated” and do not “capture the effects of groundwater or other sulfur and iron sources to natural field sites”.

The MPCA notes in the text on page 44 referring to Figure 21 that:

As sulfide is produced, and iron-sulfide precipitates are formed, the reservoir of iron that is potentially available to react with sulfide is diminished. The reservoir can potentially be replenished if the sediment dries and is exposed to oxygen, or if there is a source of iron, such as groundwater might supply.\footnote{Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, Minnesota Pollution Control Agency, June 9, 2014}

MPCA’s analysis of the Fe/AVS ratio vs SO\(_4\) is misleading and inaccurate. These results can easily be used to determine if the Fe reservoir has been depleted due to the conversion of sulfate to sulfide in the MN surface water data set. The molar ratio of Fe/AVS can be used to determine if all of the free iron has been bound to form iron sulfides. As an example, \(10^{0.5}\) (3.16) is a reasonable lower bound ratio of Fe/AVS. On a molar basis, this corresponds to 177 moles of iron and 64 moles of S. Thus, the molar ratio is just below 3. This indicates that even for the lower bound ratios reported in MN surface water, there is more than enough iron to bind with the AVS, leaving an abundance of iron available. Thus, the data indicates that in MN surface waters there is no evidence of the complete depletion of iron by the formation of iron sulfides; furthermore there is ample residual capacity to bind with additional sulfide. The fact that iron depletion was not observed in field studies\footnote{WILD RICE SULFATE STANDARD FIELD SURVEYS 2011, 2012, 2013: FINAL REPORT, Dr. Amy Myrbo, University of Minnesota, December 2013} or in the sediment incubation experiments\footnote{Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments. Will DeRocher, Nathan W. Johnson, University of Minnesota Duluth, December 2013} clearly supports the hypothesis that under current sulfate loadings, or even if loadings are modestly increased, sufficient iron is available to prevent the accumulation of sulfides in the rooting zone.
Further, the data collected to date does not indicate that the available iron has been consumed in the sediments associated with any of the surveyed Minnesota surface waters.

Finally, it is important to note that there are multiple possible mechanisms to explain the results presented in Figure 19, panel B on page 21.

**Figure 2** The relationship between porewater sulfide and porewater iron at the 119 sites sampled in the 2012-2013 Field Survey. Log-log plot, with lakes, streams, and cultivated paddies identified by symbol. (Spearman’s rho = -0.56, p < 0.001)

MPCA hypothesizes that the figure demonstrates depletion of iron. An alternative explanation is that iron is not depleted from the sediment pool but rather the concentration is controlled by kinetics. If one assumes an infinite pool of iron in the sediments, the concentration of dissolved iron will be controlled by the rate at which iron reacts with sulfide. As the rate of iron sulfide formation increases due to an increase in sulfate, the residence time of iron in the dissolved pool will decrease, resulting in a decrease in the steady state concentration of dissolved iron and an increase in the steady state concentration of sulfide. Thus, depletion of the total iron pool is not required to explain the observed iron and sulfide relationships.

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As mentioned previously, there are multiple sources of iron to replenish porewater iron and to precipitate sulfide to maintain sulfide concentrations below toxic levels (e.g. < 2,400 µg/L), including diffusion and convection/advection from ground water and surface water, as well as reduction of sediment bound iron. Except for the mesocosm studies, which do not mimic natural conditions (and which had “significant seedling mortality in all tanks after thinning”\(^{37}\) in the 2013 tests), there is and will continue to be in most parts of Minnesota more than sufficient iron to bind up sulfide as it is formed, and prevent any toxicity to wild rice. This appears to be true universally for streams. For lakes, the abundance of iron holds true except for those lakes located in the Des Moines glaciation lobe, where low iron material is the foundation for the lakes and surrounding groundwater. As described previously, the available Fe and AVS results can be used to show that there is little evidence that the available iron, which is the source of the porewater iron, has been exhausted. MPCA is misinterpreting the Fe/AVS ratios. A simple examination of the molar ratios can be used to demonstrate that the available Fe has not been depleted, even in the water bodies with a high sulfate concentration and the lowest Fe/AVS ratio.

\(^{37}\) Effects of enhanced sulfate concentrations on wild rice populations: results from a mesocosm experiment, Dr. John Pastor, University of Minnesota Duluth, December 2013
4.0 Use of Quantile Regression Analysis

The MPCA makes much of Figure 22 in their analysis, which purports to show the relationship between Porewater sulfide and surface water sulfate, as well as the relationship to porewater iron concentrations.

Figure 3  Relationship between sulfide in porewater and sulfate in surface water in the 119 sites sampled in the 2012-2013 Field Survey. The red dashed line indicates an empirically-fit maximum potential concentration of sulfide for a given concentration of sulfate.

Because, in the MPCA’s eyes, the data form a wedge, the MPCA assumes that quantile analysis is the appropriate statistical analysis. The Chamber respectfully disagrees. The “wedge” would not be a wedge, but for a single data point – Bean Lake in Becker County.

Bean Lake is a clear outlier, as demonstrated in the Chamber’s statistical analysis. Bean Lake had a porewater sulfide concentration of 499 µM or 16,000 µg/L. The next highest observation (one of 3 samples taken at Lady Slipper Lake) was a porewater sulfide concentration of 14,800 µg/L. All other

38 Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, Minnesota Pollution Control Agency, June 9, 2014
observations were less than 3,200 µg/L. Bean Lake has porewater sulfide higher 5 times higher than the highest, non-outlier observation and is 177 times the mean observed sulfide porewater.

If Bean Lake is properly eliminated as an outlier, the MPCA’s statistical analysis crumbles, as the purported quantile regression analysis would not be appropriate. The Chamber holds that the correct relationship between porewater sulfide and porewater iron was given in the Chamber’s original analysis:

*Put simply, if there is sufficient iron available in the sediment; it will tie up any sulfide generated in the sediment, making it biologically unavailable to wild rice. There is such a small exponent on the sulfate concentration (two orders of magnitude lower than the exponent on the iron concentration) that the surface water concentration is nearly inconsequential. In fact, nearly the same amount of variability can be explained by a power formula using only iron and the field survey data:*

**Equation 4-2**

\[
\text{Sulfide} = 6.51 \times Fe^{-0.446}
\]

This relationship had an \( r^2 \) between observed and predicted = 0.595 and was significant (prob. \(<0.001)\).

Based on the MPCA dose response curves (e.g. see Figure 6, page 16), essentially no rice should be present when sulfide is > 1000 µg/L. However, the field surveys found that almost 20% of these sites do in fact have rice. This suggests a bias in extrapolating the individual dose response results to population level effects. It also suggests that there are multiple, non-chemical variables which can influence the presence or growth of wild rice, including water depth, water flow, water clarity, water level fluctuations, sediment type and condition, presence of competitive plant species, invasive plant and animal species. The MPCA needs to take care that it is not overextending its analyses, because these other factors often outweigh any impact from low levels of sulfate or sulfide.

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39 Technical Analysis of Wild Rice Research, Minnesota Chamber of Commerce, February 2014
40 Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, Minnesota Pollution Control Agency, June 9, 2014
5.0 Use of Mesocosm experiments

A primary goal with any toxicity exposure is to ensure that observed effects to the test organism (wild rice) are caused by the stressor (sulfate), and not the test design. The mesocosm exposure system incorporated for this research deviates substantially from the larger, more natural mesocosm designs that have been historically used to assess the effects of stressors on aquatic plants. Upon review of the test design and data, it is quite likely that the test design contributed, at least in part, to the observed effects in the mesocosm.

As an example, consider a more typical mesocosm experimental design employed by Hill et al. in their exposure entitled *Lambda-Cyhalothrin: A Mesocosm Study of Its Effects on Aquatic Organisms*. In this exposure, the mesocosm was a pond 15 meters wide, 30 meters long, and 2 meters deep or about 240,000 gallons. The study authors also point out that the effects noted in this large mesocosm represent a “severe test of exposure, and may potentially overestimate typical field risk”.

In fact, the use of the term mesocosm to describe the MPCA’s research is likely inappropriate. In his paper on mesocosm design, John Gamble notes the minimum size for a simple mesocosm to be ~5 m$^3$, or 1320 gallons. The containers used in Dr. Pastor’s Exposure, in contrast, were 100 gallons, less than a tenth of the size of the minimum volume recommended for a mesocosm exposure.

The MPCA’s analysis of the small stock tanks they refer to as mesocosms does not discuss in detail the severe limitations the small container size and lack of water renewal could have on the observed results. As discussed below, results from the mesocosm study strongly suggest test design was insufficient to accurately measure the effects of the stressor and likely contributed to the observed deleterious effects.

The high plant mortality across all treatments in 2013 greatly complicates the interpretation of the mesocosm results, as does the fact that it appears that individual plants for a given sulfate treatment were considered as replicates in 2011 and 2012, while tanks were considered as replicates in 2013 (Table 1). This represents unequal treatment of the data across years. One additional area of complexity is that per-seed weight and seed viability data were recorded on a different basis for 2011-2012 compared to 2013.

Although MPCA hypothesizes that a late and cold spring are responsible for the high mortality, additional information would be useful to interpret this event and MPCA’s hypothesis. For example, was a similar mortality event observed in the natural rice stands? 2013 was, by most accounts, an average to good year for wild rice production in Minnesota. Also, in general, were the growth rates and other metrics observed in the mesocosms suppressed as compared to natural rice, indicating that the rice in the mesocosms were stressed and therefore not representative?

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42 Aquatic Mesocosm Studies in Ecological Risk Assessments, Graney et al.
43 MESOCOSMS: STATISTICAL AND EXPERIMENTAL DESIGN CONSIDERATIONS, John C. Gamble
The most troubling aspect of the mesocosm experiments is the fact that they do not realistically mimic field conditions. Deviations include

- Very small size in comparison to standard mesocosm exposures.
- An initially disturbed sediment column
- An underlying layer of clean sand
- Little to no water exchange. Additions to the tanks included only sulfate additions to maintain the target sulfate concentration and well water to replace evaporative loss.

Presumably one of the main goals of the experiment was to examine the relationship between sulfate and sulfide. The linkage between sulfide and iron is discussed throughout the draft document and by design the mesocosm experiments eliminate the possibility of an exchange of iron. In addition, the lack of water exchange (both surface water and groundwater) very likely led to the depletion of other nutrients and the accumulation of waste material. The creation of an artificially deoxygenated zone in the water column in these small containers during warm summer months is also quite possible given the test design. This potential for depletion and accumulation in a closed system is exactly why toxicity tests, including standard mesocosm exposures, are generally performed in larger, flow through (or regular exchange) systems such that the media is regenerated constantly as the experimental exposure progresses.

With respect to the statistical analyses conducted by Dr. Pastor and by the MPCA, it is clear that although three of the six parameters were consistent with the assumptions of two-way ANOVA (and were analyzed exclusively on that basis), three additional parameters exhibited data distributions with significant differences from normality that could not be fully remedied by data transformation, and substantial differences between the nonparametric and parametric results for sulfate were observed in all three cases.

Based on all of these considerations, the Chamber continues to hold that the mesocosm experiments are fatally flawed and cannot be relied upon to assess the toxicity of sulfate to wild rice.
6.0 Synthesis: How Sulfate, Sulfide and Iron Interact to Affect Wild Rice

The MPCA concludes in its analysis the following main points:

- sulfide was found to be toxic to wild rice at or above 10 micromoles per liter (320 µg/L);
- Sulfate in surface water penetrates into the anoxic, saturated, soils where wild rice grows and bacteria convert sulfate to sulfide;
- if dissolved ferrous iron is not available in the porewater, as bacteria produce sulfide the concentration of sulfide can build up in the porewater in which the wild rice is rooted;
- As sulfide is produced, and iron-sulfide precipitates are formed, the reservoir of iron that is potentially available to react with sulfide is diminished; and
- multiple quantile regression, by relating both sulfate and iron to sulfide in porewater, offers a method to predict the potential production of sulfide at a site without over-estimating sulfide concentrations at high iron sites, or under-estimating sulfide concentrations at sites low in iron.  

The Chamber’s analysis shows, point by point, that the MPCA’s analysis is flawed, and its conclusions are not supported by the facts:

- Sulfide is not toxic to those portions of the plant which are exposed in nature to anoxic or low oxygen conditions and sulfides at concentrations as high as 90 µM or 2,400 µg/L;
- It is true that sulfate diffuses into the anoxic saturated soils where wild rice grows, and true that bacteria convert sulfate to sulfide, but as the MPCA observes, other oxygen sources (e.g. O2, nitrates, oxidized manganese and oxidized iron) would be converted first;
- It is not true that the “reservoir of iron” will be depleted by reactions with sulfide, as there are multiple sources of iron, such as convection/advection and diffusion of groundwater into porewater, convection/advection and diffusion of surface water into porewater, as well as reduction of sediment bound iron
- More than sufficient porewater iron remains, even at the lowest field observed iron concentrations or at the lowest Fe/AVS ratios to prevent sulfide concentrations from accumulating to toxic levels
- The quantile regression analysis is fatally flawed, as the foundation for it is the alleged “wedge” (see Figure 22, page 45), which exists because of a single outlier observation. By rightly excluding that observation, there is no “wedge” and the quantile regression analysis loses credibility and utility.

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44 Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, Minnesota Pollution Control Agency, June 9, 2014
45 Id.
• The hypothesis that surface water sulfate is a key determinant in the formation of sediment porewater sulfide is not supported.

The Chamber analysis shows that there is a much simpler explanation, as provided in our original analysis of the MPCA research:46

• The field studies suggest that sulfide in sediment water would only affect wild rice at very high levels, and only in lakes in the western and southern portions of the state where iron concentrations in sediment water are very low;

The Chamber and the MPCA agree that sulfate in surface water is not toxic. The Chamber disagrees that sulfide is toxic to those portions of the plant in the root zone throughout the life stages of wild rice, at concentrations below 2,400 µg/L. The MPCA seemingly ignores the fact that there is sufficient iron moving into the sediments from groundwater and overlying surface waters (in addition to reduction of sediment bound iron) to prevent sulfide concentrations from reaching 2,400 µg/L, except perhaps in the southern and western portions of the state, where naturally occurring low iron concentrations occur. Only 4 lakes have been found to have sulfide concentrations as high as 2,400 µg/L. In contrast, nearly 20% of the sites surveyed had porewater sulfide greater than 1,000 µg/L support wild rice growth.

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46 Technical Analysis of Wild Rice Research, Minnesota Chamber of Commerce, February 2014
February 21, 2014

Shannon Lotthammer, Director
Environmental Analysis and Outcomes Division
Minnesota Pollution Control Agency
520 Lafayette Road N.
St. Paul, MN 55155-4194

Re: Comments on Wild Rice Research

Dear Ms. Lotthammer:

Attached is a technical analysis by the Minnesota Chamber of Commerce (Chamber) of Minnesota Pollution Control Agency (MPCA) wild rice research conducted under the 2011 Wild Rice Rulemaking and Research law. As you know, the Chamber has participated with MPCA and the advisory group established under the legislation in the wild rice research and rulemaking process set forth by the Legislature. The Chamber committed to that engagement because of the importance of the standard as a statewide issue with major ramifications for industry, municipalities and other dischargers.

The Chamber’s analysis of the MPCA research is in three parts:

- A critical analysis of each MPCA study (Section 2);
- An integration of the data from each study (and other credible, public information) to address MPCA’s goals and primary hypotheses (Section 3); and
- Responses to the regulatory questions posed by MPCA (Section 4).

The Chamber’s analysis was prepared by a team of scientists and policy experts holding post-graduate degrees and possessing decades of applied experience in aquatic toxicity assessment, water resources, soil science, rice nutrient dynamics, forest resources and genetics, chemical engineering, statistics, salinity effects on plants, and federal and state environmental permitting and rulemaking.

Based on its analysis, the Chamber makes the following recommendations regarding the three regulatory questions posed by MPCA (shown in italics below).  

- Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?

Given the high concentration of sulfate necessary to adversely affect the growth of wild rice, as well as the lack of a relationship between wild rice growth and surface water sulfate concentrations demonstrated in MPCA-sponsored field surveys, a sulfate water quality standard is unnecessary.

1 2011 Minn. Laws, 1st Special Session, Ch. 2, Art. 4, Sec. 31.
2 Wild Rice Sulfate Study Summary and next steps, MPCA December 2013
However, if MPCA determines there is a need for a sulfate water quality standard, the standard should be increased to 1,600 mg/L in surface water at the location where wild rice is present. Two sulfate hydroponics studies support such an increase. Moreover, MPCA-directed field surveys and private surveys document observations in waters with sulfate concentrations above 800 mg/L and 1,000 mg/L at the location where wild rice is present.

The studies show that sulfide in sediment water\(^3\) only affects wild rice at very high levels, only in lakes in the western and southern portions of the state, and only where iron concentrations in sediment water are very low. See Section 4 of the report.

- **Should there be a different standard for lakes/wetlands, or streams, or paddy rice?**

No. At an appropriately set standard of 1,600 mg/L, there is no need for a separate stream or lake standard. MPCA has conducted no research on wild rice in wetlands.

- **What more can be said about the period when the rice may be susceptible to high sulfate?**

Wild rice is not susceptible to sulfate concentrations in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). As a result, any sulfate water quality standard should not apply between early September and mid-April. If MPCA determines to impose a 1,600 mg/L sulfate water quality standard at locations where wild rice is present, that standard should apply only during the wild rice growing season (mid-April through early September).

We respectfully request that the Chamber’s analysis be submitted to the agency’s peer review panel, along with the agency studies. Thank you for the opportunity to participate in the advisory group process and to provide these comments on this important matter.

Sincerely,

Lloyd Grooms
Minnesota Chamber of Commerce

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\(^3\) Technically referred to as “porewater”
Technical Analysis of Wild Rice Research

Prepared by
Minnesota Chamber of Commerce

February 2014
Technical Analysis of Wild Rice Research

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Minnesota Chamber of Commerce

February 2014
# Technical Analysis of Wild Rice Research
February 2014

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1.0 Executive Summary

In 2011, the Minnesota Legislature enacted the Wild Rice Rulemaking and Research law, which provided the Minnesota Pollution Control Agency (MPCA) with funding to assess the effects of sulfates and other substances on the growth of wild rice. The legislation also created a Wild Rice Standards Advisory Committee, on which representatives from Minnesota Chamber of Commerce (Chamber) members and staff serve and which is responsible for reviewing MPCA’s research results. In January 2014, researchers submitted wild rice data collected since 2011 to MPCA, which the agency is now reviewing. To assist the agency’s review, the Chamber prepared a detailed technical analysis of MPCA’s research. The Chamber committed to that engagement because of the importance of the standard as a statewide issue, with major ramifications for industry, municipalities and other dischargers.

The Chamber's analysis of the MPCA research is in three parts:

- A critical analysis of each MPCA study (Section 2);
- An integration of the data from each study (and other credible, public information) to address MPCA’s goals and primary hypotheses (Section 3); and
- Responses to the regulatory questions posed by MPCA (Section 4).

The Chamber’s analysis was prepared by a team of scientists and policy experts holding postgraduate degrees and possessing decades of applied experience in aquatic toxicity assessment, water resources, soil science, rice nutrient dynamics, forest resources and genetics, chemical engineering, statistics, salinity effects on plants, and federal and state environmental permitting and rulemaking.

Based on its analysis, the Chamber makes the following recommendations regarding the three regulatory questions posed by MPCA (shown in italics below).

- Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?

Given the high concentration of sulfate necessary to adversely affect the growth of wild rice, as well as the lack of a relationship between wild rice growth and surface water sulfate concentrations demonstrated in MPCA-sponsored field surveys, a sulfate water quality standard is unnecessary.

However, if MPCA determines there is a need for a sulfate water quality standard, the standard should be increased to 1,600 mg/L in surface water at the location where wild rice is present. Two sulfate hydroponics studies support such an increase. Moreover, MPCA-directed field surveys and private surveys document observations in waters with sulfate concentrations above 800 mg/L and 1,000 mg/L at the location where wild rice is present.

The studies show that sulfide in sediment water only affects wild rice at very high levels, only in lakes in the western and southern portions of the state, and only where iron concentrations in sediment water are very low. See Section 4 of the report.

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1 2011 Minn. Laws, 1st Special Session, Ch. 2, Art. 4, Sec. 31
2 Wild Rice Sulfate Study Summary and Next Steps, MPCA December 2013
3 Technically referred to as “porewater”
Should there be a different standard for lakes/wetlands, or streams, or paddy rice?

No. At an appropriately set standard of 1,600 mg/L, there is no need for a separate stream or lake standard. MPCA has conducted no research on wild rice in wetlands.

What more may be said about the period when the rice may be susceptible to high sulfate?

Wild rice is not susceptible to sulfate concentrations in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). As a result, any sulfate water quality standard should not apply between early September and mid-April. If MPCA determines to impose a 1,600 mg/L sulfate water quality standard at locations where wild rice is present, that standard should apply only during the wild rice growing season (mid-April through early September).
2.0 Introduction

2.1 Overview of Minnesota Chamber of Commerce Analysis

The Chamber’s analysis of MPCA Wild Rice Research is divided into three parts:

- A critical review of each study;
- An integration of the data from each study (and other credible, public information) to address the agency’s goals and primary hypotheses; and
- Recommendations to answer the regulatory questions posed by the agency:
  - Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?
  - Should there be a different standard for lakes/wetlands, or streams, or paddy rice?
  - What more can be said about the “period when the rice may be susceptible to high sulfate”?  

These comments were prepared by the Chamber with significant contributions by a team of individuals with significant training and experience in a variety of fields related to wild rice, plant toxicology and other disciplines. See Section 5.0 for more detail.

2.1.1 Analysis of MPCA Wild Rice Research

The Chamber provides an analysis review of each of the studies, to answer the two most pertinent questions posed by the MPCA at the February 3, 2014 Advisory Committee Meeting:

- Are there concerns about the methods?
- Are there concerns about data quality?

In addition, the Chamber poses and provides its opinion to a third question:

- What conclusions can be drawn from the study?

In general, there were few concerns about the methods used in the following studies:

- Field survey;
- Sulfate hydroponics studies;
- Seed germination and mesocotyl growth portion of the sulfide hydroponic studies;
- Root zone geochemistry studies; and

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4 Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013
5 Input on Study Reports, MPCA, February 2014
• Temperature dependent diffusion rate studies.

Data and conclusions from these studies should be used to draw conclusions and to address the regulatory questions posed by the MPCA.

There were serious concerns with the methods used in the following studies:

• Juvenile seedling portion of the sulfide hydroponic studies; and
• Mesocosm (outdoor container) studies

Data from these studies should not be used to draw conclusions because of serious concerns about the methodology, and should not be used to address the regulatory questions posed by the MPCA.

In general, insufficient statistical analyses were provided in the MPCA’s reports; additional analyses are provided by the Chamber here.

Conclusions from all the studies are discussed in Section 2.

2.1.2 MPCA’s Goals and Primary Hypotheses – Chamber’s response

The MPCA set the following goals for all the studies:

The goal of the Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted.6

The MPCA also notes that the studies need to describe:

• The effect of sulfate on wild rice; and
• The effect of sulfide on wild rice

Based on the data in the MPCA studies, other credible studies and the Chamber’s analysis, the Chamber concludes that there is no correlation between sulfate in surface water and wild rice growth, and sulfate does not affect wild rice growth except at very high concentrations (e.g., in excess of 1,600 mg/L). Effects from very high concentrations of sulfate are likely caused by osmotic stresses resulting from high concentrations of any salt, including sulfate.

Based on the data in the studies, other credible studies and the Chamber’s analysis, the Chamber concludes that there is a correlation between sediment porewater sulfide and wild rice growth, but that porewater sulfide does not affect wild rice seed germination or mesocotyl growth except at very high levels (e.g., above 2.4 mg/L (above ~90 μM), only in lakes in the western and southern portions of the state, and only where iron concentrations in porewater are very low (e.g., less than 1 mg/L (~ 2 μM).

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6 Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013
The MPCA also proposed the following hypotheses:

- _wild rice can be impacted by sulfate via the conversion of sulfate to sulfide in the rooting zone of the plants and_

- _iron may mitigate the effects of sulfide production in the rooting zone of the sediment?_

The MPCA has acknowledged that no single study can elucidate these hypotheses; rather, an integration of the data from all of the studies (and perhaps other sources as well) is necessary to provide sufficient data to support these hypotheses.

Based on the data in the studies, other credible studies and the Chamber’s analysis, the Chamber concludes that the first hypothesis may be partially correct. In the absence of sufficient iron to precipitate dissolved sulfide, sulfate diffuses into the rooting zone of aquatic plants and is converted to sulfide. However, in all streams and most lakes in Minnesota, there is ample naturally occurring iron to precipitate dissolved sulfide. Only very high levels of porewater sulfide (e.g. greater than 90 μM) have been shown to impact wild rice plant growth.

Based on the data in the studies, other credible public studies and the Chamber’s analysis the Chamber concludes that the second hypothesis is strongly supported by the useable research and other credible evidence. In all streams and in the majority of the lakes surveyed, there is more than sufficient porewater iron to prevent the accumulation of soluble sulfide in porewater to any significant concentration. The few lakes where high sulfide was observed are lakes that reflect the parent material and groundwater in which they are located, and tend to be lower in porewater iron.

These conclusions are further discussed in Section 3.

2.1.3 Chamber’s Recommendations for Revisions of the Water Quality Standard for Protection of Waters Used for the Production of Wild Rice

The data developed in the studies and the statistical analysis herein demonstrate that the water quality standard for the protection of waters used for the production of wild rice should be revised, based upon the integration of the data provided by the MPCA’s research, and other credible, public information. The Chamber recommends the following with respect to the key questions posed:

- _Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?_

The scientific evidence indicates the standard should go up. Multiple observations of wild rice have been made in waters with concentrations well above the current 10 mg/L water quality standard, both in the University of Minnesota Field Survey and in private surveys.

7 Id.
Given the high concentration of sulfate needed to have an effect on the growth of wild rice (see below), and the fact the field survey showed no relationship between wild rice coverage and surface water sulfate concentrations, a sulfate water quality standard is unnecessary.

However, if the MPCA decides that there is a need for a sulfate water quality standard, the standard should be increased to 1,600 mg/L sulfate at the location where wild rice is present. In accordance with EPA guidelines and state procedures for establishing water quality regulations, two sulfate hydroponics experiments were conducted producing results which support this conclusion. Moreover, MPCA-directed field surveys and private surveys document observations in waters with sulfate concentrations above 800 mg/L and 1,000 mg/L at the location where wild rice is present.

The studies show that sulfide in sediment water only affects wild rice at very high levels, only in lakes in the western and southern portions of the state, and only where iron concentrations in sediment water are very low. See Section 4 of the report.

- **Should there be a different standard for lakes/wetlands, or streams, or paddy rice?**

No. At an appropriately set standard of 1,600 mg/L, there is no need for a separate stream or lake standard. MPCA has conducted no research on wild rice in wetlands.

- **What more can be said about the period “when the rice may be susceptible to high sulfate”?**

For clarification, the exact language of the current rule is:

... during periods when the rice may be susceptible to damage by high sulfate levels

The Chamber assumes the MPCA’s question is a short-hand restatement of the rule. In the Chamber’s analysis, the Chamber will use the rule precisely as currently drafted. Wild rice is not susceptible to any concentration of sulfate in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). There should be no sulfate water quality standards applicable during those times. The 1,600 mg sulfate/L water quality standard (at locations where wild rice is present) should apply only during the growing season of wild rice (mid-April through early September).

These recommendations are further discussed in Section 4.

### 2.2 Overview of MPCA Research

In order to avoid constant cross referencing of each of the studies, a succinct synopsis is provided for each study. These synopses are taken from each study and listed as direct quotes:

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8 Technically referred to as “porewater”
9 Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013
10 Minn. Rules 7050.0224, Subpart 2
when appropriate, Indented italics are used to denote quotations. Foot notes are provided either before the quotations (when citing a study for example) or at the end of the quotations. Additional text is provided by the Chamber to provide additional background and context for each study.

2.2.1 Field Surveys

Under contract from the Minnesota Pollution Control Agency (MPCA), the University of Minnesota conducted a survey of water bodies across Minnesota in the summers of 2011, 2012, and 2013, to assist the evaluation of the State’s sulfate water quality standard to protect wild rice waters (also known as the “wild rice sulfate standard”). This activity is referred to as the “field survey” (or “2011 survey,” “2012 survey,” and “2013 survey”) is intended to provide:

- information to the MPCA about correlations between wild rice presence and environmental parameters; and
- data collected in a comparable way at field sites and in mesocosms.

The 2011 survey was a preliminary effort to collect initial data on wild rice stands and to develop the methods for larger field surveys in 2012 and 2013.

The 2013 survey differed from the 2011 and 2012 surveys in three important ways. First, in 2013, selected sites were visited multiple times during the growing season, while in 2011 and 2012 each site was sampled only once. Second, in 2013, the wild rice experimental growth mesocosms (operated by Principle Investigator John Pastor) were sampled by field crews in addition to their field surveys of natural water bodies and cultivated wild rice paddies. Third, in 2013, field crews coordinated with project co-investigator Nathan Johnson to sample at the same time as his team retrieved porewater sampling devices (“peepers”) installed in field sites and mesocosms. The 2011 and 2012 field surveys were described in previous reports to the MPCA (Myrbo, 2012 and Myrbo, 2013, respectively). Except where noted, methods remained the same for the 2013 survey.

Site selection was conducted in close collaboration with MPCA personnel. Seventeen sites were selected as “multiple visit” sites that were visited three to five times between May and September 2013, while 19 sites were sampled one time. Sites included lakes, rivers, wetlands, and cultivated wild rice paddies, and were selected based on information provided by stakeholders, and on data on the chemistry and distribution of wild rice waters and other shallow water bodies.

For statistical purposes of investigating the hypothesized relationship between sulfate and wild rice growth, the team sought sites with a range of values in both parameters (i.e., low sulfate/low wild rice, low sulfate/high wild rice, high sulfate/low wild rice, and high sulfate/high wild rice). The team also attempted to sample widely across the state, and to sample sites that had a history of past or present drainage of high-sulfate waters into wild rice waters, as well as sites with no known history of high sulfate waters.
2.2.2 Hydroponics experiments

This report focuses on the hydroponics experiments to determine the effect of elevated sulfate and sulfide on wild rice growth and development.\(^\text{11}\)

In order to determine whether sulfate or sulfide have toxic effects on wild rice growth, it is instructive to perform hydroponics experiments where the chemistry of the growth solution and the presence of sulfate or sulfide can be controlled precisely. Accordingly, we began a series of hydroponics experiments to test the effects of sulfate and sulfide on wild rice seed germination and juvenile growth of seedlings under highly controlled conditions in growth chambers.\(^\text{12}\)

2.2.2.1 Sulfate hydroponic experiments

The selected seeds were placed into each of six numbered plastic cups to total fifty seeds each, then randomly assigned and transferred to each of six 1-pint Mason jars containing six sulfate treatment levels of 0, 10, 50, 100, 400, or 1600 mg SO\(_4\) \cdot L\(^{-1}\)

The experiment proceeded in a growth chamber at 20 °C in the dark. The solutions were exchanged with fresh solution of the appropriate treatment concentration every three days. Solution pH was measured both on the initial and exchanged solutions. The germinated seedlings were harvested after 11 days.\(^\text{13}\)

Hydroponic techniques were used to test growth of juvenile wild rice seedlings under aerobic conditions subject to various concentrations of sulfate. Seedlings from germinated seeds from Little Round Lake were used.

Each tube was considered a replicate for the corresponding test concentration. One seedling chosen as described above was placed with forceps into each Kimax tube, which was then filled with modified 1/5 strength Hoagland’s solution and an appropriate amount of sulfate.

The tubes were placed in an environmental growth chamber with lamps of maximum light intensity of 800 or greater μmol m\(^{-2}\) sec\(^{-1}\) (measured 6 inches below the lamps) produced by either fluorescent lamps or an LED light system. Tests were performed under a 16h: 8h light: dark photoperiod. Temperature was maintained at 21°C during lighted periods and 19°C during dark periods, and relative humidity was maintained at 85%. Plants were harvested after 10 days.

2.2.2.2 Sulfide hydroponic experiments

The techniques used here were the same as for the germination trials under various sulfate concentrations, except that extra care was necessary to ensure anaerobic conditions. Fifty conditioned seeds were placed in 700 mL borosilicate glass jars capped using phenolic screw caps with chlorobutyl septa 5 mm thick. The 1/5 Hoagland's

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\(^\text{11}\) Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013

\(^\text{12}\) Id.

\(^\text{13}\) Id.
nutrient solution was deoxygenated with purified nitrogen before being added to the bottles.

The target concentrations were 0, 3, 10, 30, and 90 μM sulfide. The bottles were placed in a growth chamber in continuous darkness 20° C ± 1°C. Solutions were exchanged every two days. After 11 days, the germinated seeds were harvested.\textsuperscript{14}

It is critical to note that the fully-enclosed test system would have severely limited gas exchange during wild rice life stages that would not be exposed to anaerobic conditions.

\subsection{2.2.3 Outdoor Container Experiments}

Sulfate amendments to tank mesocosms similar to those used by Walker et al. 2010 began in Summer 2011 and continued through 2012 and 2013 at five different amendment levels: control (~10 mg sulfate / L), low (50 mg sulfate / L), medium-low (100 mg sulfate / L), medium (150 mg sulfate / L), and high (300 mg sulfate / L). Water and sulfate levels in mesocosms were maintained by weekly sampling and appropriate additions of well water (~10 mg/L sulfate) and sodium sulfate stock solution to account for rain water dilution, evaporation, and flux of sulfate into sediment. During the fall of 2012, overlying water sulfate levels were not maintained actively after plant senescence in September, and sulfate amendments did not begin again until June 2013.

The outdoor containers with sediment from lake bottoms over sand were used to determine the response of wild rice to a range of sulfate concentrations in the surface water, and associated sulfide in the rooting zone, across the growing season.\textsuperscript{15} The experiments were established in 2011 and run through 2012. However, only data from 2013 are available, and no initial conditions were measured. Also, in 2013, there was significant seedling mortality in all tanks after thinning but before the floating leaf stage\textsuperscript{16}, which not only precluded sampling of individual plants, but also casts doubt about the viability of the plants being tested and their response to the sulfate treatments.

Additional sulfate was added to tanks to reach nominal sulfate concentrations in the overlying water of 0, 50, 100, 150 and 300 mg/L sulfate. A variety of biological endpoints, including seed weight, number of viable seeds, and plant biomass were measured at the end of the growing season and the results of each treatment compared to one another.

It is critical to note that the mesocosm systems used contained sediment with a past history of wild rice growth in sulfate exposures in overlying waters. Key sediment components such as iron and various trace nutrients were not replaced nor were fresh sediments added to the test systems prior to initiation of the experiments. Thus, the mesocosm test system was likely limited in its ability to provide plant nutrients and would have been deficient in the iron necessary to complex any dissolved sulfide naturally generated in sediment porewater.

\textsuperscript{14} Id.

\textsuperscript{15} Effects of enhanced sulfate concentrations on wild rice populations: results from a mesocosm experiment John Pastor, University of Minnesota Duluth, December, 2013

\textsuperscript{16} Id.
2.2.4 Root Zone Geochemistry

2.2.4.1 Outdoor Containers

During the summer of 2012, passive porewater equilibrators (peepers) were deployed to collect depth-profiles of samples approximately monthly in duplicate control, low (50 mg/L), medium (150 mg/L), and high (300 mg/L) sulfate mesocosms.

During the summer of 2013, peepers were again deployed monthly at the four sulfate treatment levels. Once a month from May to September, duplicate peepers were deployed within an individual tank at each treatment level. One peeper was inserted in the plant-free zone and the other peeper was located near the center of the tank where wild rice was allowed to grow. Sediment cores were extracted monthly during peeper retrieval in locations coincident with peepers, and sectioned into intervals consistent with peeper well spacing.\(^{17}\)

2.2.4.2 Field sites

In an attempt to characterize seasonal variability of porewater geochemistry in a field setting, peepers were also deployed at two field sites: one river site, Second Creek (47.52042 -92.1925), and one lake site, Sandy Lake (47.61872 -92.59314) during the summer of 2013. Both sites have sulfate concentrations in the overlying water elevated above regional background levels (150 to 700) mg/L in the overlying water [and as high as 838 mg/L sulfate] in contrast to regional background concentrations of less than 10 mg/L. Both sites have potential for groundwater flow due to coarse sediment and regional hydraulic gradients imposed by human alterations of the landscape. At each site, duplicate peepers were deployed and collected monthly; one in an area of coarser sediment and one an area of finer, organic sediment. The solid phase was also characterized monthly at these field sites, but was limited spatially to an analysis of the homogenized top 10 cm of sediment.\(^{18}\)

2.2.5 Sediment Incubation Experiments

Experimental sediments were retrieved from two locations within the St. Louis River watershed. The Partridge River (PR) sampling location is near the headwaters of the St. Louis River on the East-central portion of the Mesabi Iron Range in Northern Minnesota (Figure 2-1). The Partridge River site provided high organic sediment from a slow-moving part of a sulfate-impacted river where wild rice had been observed in recent years. The second sampling location, North Bay (NB), is near the tail waters of the St. Louis River, approximately 15 km upstream from the entrance into Lake Superior in the St. Louis River Estuary. The North Bay site, a protected bay away from the main channel, provided a lower organic sediment from a location where rice had also been observed in recent years. In January 2013, approximately 50 L of sediment was recovered from the top 10 cm.

\(^{17}\) Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan Johnson, University of Minnesota Duluth, December 2013

\(^{18}\) Id.
cm of the river beds, transported back to the University of Minnesota – Duluth, and homogenized.19

![Map showing sediment sample locations near Duluth and Lake Superior.](image)

**Figure 2-1  Sediment Sample Locations**

Microcosms consisted of polycarbonate plastic tubing with a sealed bottom and Rhizon® soil moisture samplers fixed and sealed at varying depths along microcosm’s profile to extract water from the pore space of the sediment, (Seeberg-Elverfeldt et al., 2005). The Rhizon® samplers were used to take 3 mL samples of porewater at specific time points throughout the experiment for the monitoring of anion transport within the sediment. Homogenized sediment was transferred to microcosm tubes, and gently consolidated by the use of a vibration table to minimize settling during the experiment. Fresh site water was placed over the sediment and the microcosms and allowed to equilibrate to lab conditions for three weeks prior to the beginning of experiments.

An aeration system was also included to provide oxygen and mixing to the overlying water. The microcosms were incubated in dark, temperature-controlled conditions throughout the experiment to minimize disturbances and to eliminate variables such as photosynthesis. Triplicate microcosms were constructed for each temperature. Three microcosms filled with North Bay Sediment were incubated at 4.5 °C for the duration of the experiment and three identical microcosms were incubated at 23 °C. An analogous set of microcosms were constructed and incubated using sediment from the Partridge River.

The experimental portion of the study occurred in three phases to analyze the flux of sulfate and several inert, tracer anions across the sediment-water interface. Water overlaying the sediment in each microcosm was continuously mixed and aerated to eliminate chemical gradients near the sediment-water interface in an effort to mimic conditions in a shallow natural stream that might receive sulfate-enriched discharges.

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19 Id.
20 Id.
After an initial three-week equilibration period, Phase I began with a chloride spike (30 mg/L) into the surface water of the all of the microcosms. During Phase I, the sulfate concentration in the overlying water was monitored weekly, and was replaced when necessary to maintain concentrations similar to those experienced in the field.\textsuperscript{21}

Phase II, the sulfate loading phase intended to mimic the onset of a sulfate discharge, was initiated by replacing the overlying water with fresh site water spiked with sodium sulfate (approximately 300 mg/L as sulfate) and sodium fluoride (16 mg/L as fluoride) and no chloride (Table 2 of Outdoor Containers report). Sulfate was spiked at the outset of Phase II and re-spiked three times throughout the 11 week loading phase.

Phase III, the recovery phase, was initiated by replacing the overlying water with fresh site water spiked with sodium bromide (20 mg/L as bromide; no additional chloride, sulfate, or fluoride) which was maintained for 9 weeks. The sulfate recovery phase was designed to simulate the end of sulfate-elevated discharge to surface waters, with the goal of determining how long sediment in a river system may be exposed to residual sulfate after sulfate concentrations in the overlying water are reduced. In between each phase was a three-day interlude in which only clean, unamended site water was present before starting the next phase.

To maintain target experimental conditions, surface water samples were collected and analyzed weekly from all replicate microcosms. The overlying water was changed as often as necessary to maintain the ion concentration gradient between the sediment and water or as otherwise deemed necessary. The amended overlying water used during incubations was retrieved from the same locations where the sediments were obtained.\textsuperscript{22}

\textsuperscript{21} Id.
\textsuperscript{22} Id.
3.0 Technical Analysis of MPCA Wild Rice Research

3.1 Overview
For each study, the Chamber considered two of the questions posed by the MPCA at the February 3, 2014 Advisory Committee meeting:

Are there concerns about the methods?

Are there concerns about data quality?

The Chamber asks one additional question for each study:

What conclusions can be drawn from the study?

3.2 MPCA’s Field Surveys
3.2.1 Are there concerns about the methods?
Yes. Water bodies were not selected randomly, but were selected based upon the presence of wild rice or the "expected" presence of wild rice. This limits the robustness of any statistical analyses. Given redox potential was not measured, it is difficult to make detailed geochemical predictions on the data.

3.2.2 Are there concerns about data quality?
Yes. The limited scatter plot analyses did not elucidate statistical relationships between surface water sulfate and sediment porewater sulfide and the presence or absence of wild rice. A thorough statistical analysis of the data is undertaken in Section 3.3, where data are integrated from all studies.

3.2.3 What conclusions can be drawn from the study?
Because statistical analyses of the field surveys by the MPCA were lacking, the Chamber undertook its own statistical analyses. Our analyses concluded that there is no statistically significant correlation between surface water sulfate concentrations and wild rice cover. There is, however, a weak correlation between porewater sulfide concentrations and wild rice cover.

Recognizing that a large number of chemical and physical parameters were measured during the field survey, an attempt was made to determine whether other variables may impact the presence or absence of wild rice. Since the updated data set was not supplied by the MPCA until January 29, 2014 the Chamber could not analyze all parameters for significance.

A binary logistic regression was conducted on field data from 2012-2013 to determine whether other redox-related water quality parameters, organic matter, or macronutrients may influence the presence vs. absence of wild rice across the sampling sites. The resulting statistical model
identified porewater total sulfide, sediment total sulfur, and sediment percent total organic carbon as having statistically significant predictive value for the presence vs. absence of wild rice.

Overall, the results of binary logistical regression models of different components indicate that these three parameters are likely to be predictors of wild rice presence vs. absence, and that sediment porewater iron’s contribution to predicting wild rice presence vs. absence occurs indirectly, via porewater total sulfide. In contrast, surface water sulfate concentration does not have any significant predictive value for wild rice presence vs. absence in this field study.

See Section 3.3 for a statistical analysis of this data, as part of the data integration discussion.

3.3 MPCA’s Hydroponics experiments

3.3.1 MPCA’s Sulfate Hydroponic Experiments

3.3.1.1 Are there concerns about the methods?

Yes, there are a few. Transfer of seedlings in these experiments likely increased their sensitivity to osmotic stress, i.e. sulfate or any other elevated ion that would have been present. Evidence of transfer stress was present in the method development testing conducted prior to these experiments. While the composition and concentration of the base nutrient solution differed, results of the University of Minnesota Duluth experiments are in general agreement with the results obtained in a separate study by Fort Environmental Labs, Inc.23

3.3.1.2 Are there concerns about data quality?

Data quality appears to be good, and sufficient replicates were taken to allow meaningful statistical analyses.

3.3.1.3 What conclusions can be drawn from the study?

Dr. Pastor concludes:

*Sulfate did not affect either seed germination or seedling growth other than a slight depression of root lengths at extremely high concentrations (1,600 mg SO₄⁻·L⁻¹). These high concentrations, while possible in nature, are not likely to be common.*24

The data and statistical analysis support this conclusion. The slight depression of root lengths at the highest sulfate concentration was statistically significant only when compared against 50 mg SO₄/L (Analysis of Variation (ANOVA) followed by Tukey’s post hoc separation of means).

23 Definitive Hydroponics-Based Wild Rice (Zizania palustris) Sulfate Toxicity Testing, Fort Environmental Laboratories, December 2013
24 Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013
The Fort Environmental Labs\textsuperscript{25} study found a No Observed Effect Concentration (NOEC) value of 5,000 mg/L sulfate for the majority of plant endpoints evaluated. The Fort Labs study followed Good Laboratory Practices.\textsuperscript{26} Fort Labs also followed the US EPA Guidance for ecological effects testing.\textsuperscript{27} The conclusions reached in that study include:

Results from the present 00325 study indicated that exposure of developing wild rice to sulfate generally did not induce an adverse response at concentrations $\leq 2,500$ mg sulfate/L at study day (SD) 10 (Table 1) and $\leq 5,000$ mg/L at SD 21. For example, the no observed effects concentration (NOEC) for three of the ten SD 10 NOEC values were 2,500 mg/L sulfate or lower, and seven of ten SD 10 NOEC values were 5,000 mg/L sulfate. For SD 21, eight of ten concentration endpoints exhibited NOEC values of 5,000 mg/L sulfate, indicating that sulfate was generally not toxic at the highest concentration that could be tested within the limits of solubility of the salts.\textsuperscript{28}

While seedlings were not adversely affected in the Fort Environmental Lab study up to and including exposures of 2,500 mg/L sulfate, the Chamber was concerned that exposures exceeding 1,600 mg/L may, like any ion, pose a salinity stress (osmotic stress) potentially adversely affecting wild rice during its life cycle (at least the portion of life cycle tested). This conservative assessment assumes that wild rice is salt sensitive and that exposure exceeding 1,600 mg/L could potentially impose an abiotic stress.

### 3.3.2 MPCA’s Sulfide Hydroponic Experiments

The sulfide hydroponic experiments consisted of two parts:

- Range finding experiments on seed germination and mesocotyl growth and
- Range finding and “definitive” or additional testing on juvenile seedlings.

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\textsuperscript{25} Dr. Fort has over 27 years of experience working with the Federal Insecticide Fungicide and Rodenticide Act (FIFRA), Toxic Substances Control Act (TSCA), and most recently adaptations of FIFRA to the FQA (1996). Graduating with a B.S. in Biology - Chemistry from Southwestern College and a M.S. and Ph.D. in Zoology from Oklahoma State University, Dr. Fort has served as Study Director on 25 GLP studies involving a variety of plant and animals exposures. He assists private clientele and governmental agencies with interpretation of toxic substances (TSCA) and pesticide regulations (FIFRA), and most recently the Registration, Evaluation, Authorisation and restriction of Chemical substances program (REACH) program in Europe. Dr. Fort has worked on various pesticide risk assessments on behalf of both government and manufacturers, and has an extensive knowledge of the ecotoxicology and general health risks of pesticides in the workplace and environment. Dr. Fort directed several large studies involving the development of a multi-generational study of mycids and reproductive studies of freshwater amphipods and marine copepods. Dr. Fort has directed 11 large vascular plant phytotoxicity studies involving both traditional soil and hydroponic exposures.

\textsuperscript{26} USEPA, Toxic Substance Control Act (TSCA), Good Laboratory Practice Standards, 40 CFR Part 792, Chapter 1, Subchapter R, 1989

\textsuperscript{27} Ecological Effects Test Guidelines OCSPP 850.4100: Seedling Emergence and Seedling Growth US EPA 712-C-012 January 2012

\textsuperscript{28} Definitive Hydroponics-Based Wild Rice (Zizania palustris) Sulfate Toxicity Testing, Fort Environmental Laboratories, December 2013
The experiments on seed germination and mesocotyl growth were range-finding experiments, and not definitive toxicity experiments. However, because there was no impact observed on those life stages, MPCA researchers determined that definitive toxicity experiments on these plant life stages were unnecessary. The experiments on juvenile seedlings consisted of both range-finding and “definitive” studies designed to better define the toxic threshold of sulfide to wild rice seedlings.

3.3.2.1 Are there concerns about the method?

For the range finding experiments on seed germination and mesocotyl growth, there were very few concerns. The placement of seeds, the light regime and temperature controls seem reasonable. While exposure of seeds and mesocotyls to anaerobic conditions may be appropriate, there is some concern about whether the entire mesocotyl would be exposed to totally anoxic conditions. Given that wild rice reseeds itself by falling to the sediment, it is unlikely that the entire mesocotyl is always exposed to anoxic conditions. It is very likely that a larger juvenile seedling would not be exposed to anoxic conditions.

Wild Rice seeds are quite thin and light. Seed used in the hydroponic experiments is approximately 2 cm in length and < ½ cm in width.29 When this seed falls to the sediment it will likely remain in the top 1 – 2 centimeters. Cultivated wild rice is generally planted at depths of 1-3 inches (2.5 to 8 cm).30 Seedlings will not emerge when planted deeper than 3 inches.31 During the sulfate seed germination and mesocotyl growth experiments, the mesocotyls grew to lengths of 11.8 to 13.5 cm, while during the sulfide seed germination and mesocotyl growth experiments, the mesocotyls grew to lengths of 7.5 to 9.3 cm.32 In both cases, the tops of the mesocotyls would not be in sediment.

In addition, the top portion of the sediment is likely not anoxic, due to diffusion of oxygen, sulfate and other oxygenated compounds in the upper few cm of the sediment.33 Dr. Johnson noted very low levels of sulfide in the upper 3 inches (8 cm) of the sediment in the mesocosms (outdoor container) studies34, and very low to non-detectable sulfide concentrations in the root zone geochemistry of the two field sites in May and June.35

The juvenile seedlings used in the second portion of the sulfate hydroponics experiments began as 1-2 cm mesocotyl seedlings, and grew to 11 to 12 cm seedlings by the end of the test. (10

31 Id.
32 Id.
33 Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013. Tables 2 and 11
34 Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan Johnson, University of Minnesota Duluth, December 2013 Appendix B.
35 Id.
days). Plant development of the shoot portion (following mesocotyl growth) would require oxygen for photosynthesis; shoot parts of the plants would not be exposed to anoxic conditions, either in the upper sediment or in the overlying water. Therefore, exposure of juvenile seedlings to anoxic conditions is inappropriate.

There are a multitude of concerns with the design of this experiment. First, in this experiment, juvenile seedlings were germinated in an aerobic environment (open vessels) and then transplanted to an anaerobic environment (closed vessels). Seedling transplantation may have resulted in stress to all portions of the plant. Furthermore, if seedlings were photosynthesizing and producing oxygen, sulfide concentrations may have been influenced by this plant process. Dr. Pastor noted this in his report:

Although the solutions for each of these experiments were exchanged every two days, the initial sulfide concentrations declined during those two days, perhaps because of the production of oxygen by the photosynthesizing plants.

Finally, placing plant parts adapted to photosynthesis in an aerobic environment in an anaerobic environment may have resulted in additional stresses and increased susceptibility to effects from sulfide exposure.

3.3.2.2 Are there observations about data quality?

The data were presented clearly, especially for the germination and mesocotyl growth portions of the experiment. Statistical analysis of the data included tests for normality of distribution, a one-way ANOVA, and post hoc Tukey’s means separation tests.

3.3.2.3 What conclusions can be drawn from the study?

Dr. Pastor draws the following conclusions:

Enhanced sulfide under anaerobic conditions did not affect germination of seeds (p > 0.10, Table 9), mesocotyl weights (p > 0.10, Table 10), or mesocotyl lengths (p > 0.10, Table 11) in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 μM sulfide (see Appendix 7 for raw data and statistics). The rangefinder test was repeated, and because the same results were observed, we did not proceed with any further tests.

Exposures up to 90 μM sulfide in a hydroponic experiment did not affect wild rice seed germination or mesocotyl length. While exposures on seed germination and mesocotyl growth were conducted appropriately, experiments conducted on juvenile seedlings were not. Concerns were raised above that the entire seedlings (root and shoot) were exposed to sulfide treatments during the test. The plant parts above the root-shoot node (crown) would not grow in anaerobic conditions where sulfide would form in the field. Rather the shoot emerges into the aerobic water above the sediment; sulfide production does not occur under aerobic conditions. Since shoot parts were

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36 Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth December 2013
37 Id.
38 Id.
more sensitive to sulfide than root parts, developing an upper sulfide limit based on shoot sensitivity is not appropriate given the highly inappropriate exposure regime for the plant shoots. Therefore, the Chamber recommends that results from this test not be used to inform or develop water quality regulations. The highest observed porewater sulfide concentrations in the field surveys were observed in Bean Lake in Becker County (sulfide concentration 16 mg/L) and Lady Slipper Lake in Lyon County (one measurement at 14.8 mg/L, other measurements below 10 mg/L). These concentrations are the equivalent of approximately 500 μM sulfide, more than five times the NOEC from the sulfide hydroponic studies.

Three other lakes had maximum porewater sulfide concentrations near the 90 μM NOEC:

- South Geneva Lake in Freeborn County (99 μM)
- Sandy Lake in St. Louis County (96 μM) and
- Rice Lake in Stearns County (93 μM).

It must first be stressed that the 90 μM NOEC observed in the sulfide hydroponic studies reflects a no effects concentration, and the sulfide concentration above 90 μM where effects would occur is unknown. While it is not known whether slightly higher concentrations of porewater sulfide could result in effects on wild rice, these three lakes, sulfide concentrations are within 10% of the NOEC value. Given the variability associated with biological endpoints, it is likely that the concentrations observed are within the margin of error of the experiment.

Table 3-1 shows that several of these lakes had multiple samples, with multiple concentrations of sulfide. Both Lady Slipper and Sandy Lake ranged over an order of magnitude in sulfide concentration. Thus, the highest concentrations observed may be temporal in nature, varying widely over time.
Table 3-1  Lakes with Multiple Sample Dates

<table>
<thead>
<tr>
<th>LacCore field ID</th>
<th>Class</th>
<th>Site name</th>
<th>DNR/State ID</th>
<th>County</th>
<th>Site type (Lake/Stream/Paddy)</th>
<th>Wild Rice presence (yes/no)</th>
<th>Date</th>
<th>Porewater Total Sulfide (TS, mg S/L)</th>
<th>Porewater Fe (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS-85</td>
<td>4 Survey</td>
<td>Bean</td>
<td>03-0411-00-201</td>
<td>Becker</td>
<td>L</td>
<td>no</td>
<td>8/21/2012</td>
<td>16.00</td>
<td>50</td>
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<tr>
<td>FS-79</td>
<td>5 Survey Duplicate</td>
<td>Lady Slipper</td>
<td>42-0020-00-203</td>
<td>Lyon</td>
<td>L</td>
<td>no</td>
<td>7/27/2012</td>
<td>1.63</td>
<td>1,320</td>
</tr>
<tr>
<td>P-55</td>
<td>1 Pilot Survey</td>
<td>Lady Slipper</td>
<td>42-0020-00-204</td>
<td>Lyon</td>
<td>L</td>
<td>no</td>
<td>9/22/2011</td>
<td>14.84</td>
<td>638</td>
</tr>
<tr>
<td>FS-78</td>
<td>4 Survey</td>
<td>Lady Slipper</td>
<td>42-0020-00-202</td>
<td>Lyon</td>
<td>L</td>
<td>no</td>
<td>7/27/2012</td>
<td>1.68</td>
<td>98</td>
</tr>
<tr>
<td>FS-184</td>
<td>4 Survey</td>
<td>Rice</td>
<td>73-0196-00-216</td>
<td>Stearns</td>
<td>L</td>
<td>no</td>
<td>7/30/2012</td>
<td>2.97</td>
<td>25</td>
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<tr>
<td>FS-345</td>
<td>6 Survey 2nd Year</td>
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<td>73-0196-00-216</td>
<td>Stearns</td>
<td>L</td>
<td>yes</td>
<td>8/7/2013</td>
<td>2.08</td>
<td>&lt;10</td>
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<td>FS-251</td>
<td>4 Survey</td>
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<td>0.12</td>
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<td>69-0730-00-203</td>
<td>St. Louis</td>
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<td>6/11/2013</td>
<td>0.09</td>
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<td>69-0730-00-204</td>
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<td>6/11/2013</td>
<td>1.08</td>
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<td>69-0730-00-204</td>
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<td>7/9/2013</td>
<td>3.08</td>
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<td>St. Louis</td>
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<td>8/13/2013</td>
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</tr>
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<td>FS-349</td>
<td>6 Survey 2nd Year</td>
<td>Sandy-3</td>
<td>69-0730-00-205</td>
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<td>Sandy-1</td>
<td>69-0730-00-203</td>
<td>St. Louis</td>
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<td>no</td>
<td>9/17/2013</td>
<td>0.14</td>
<td>29,500</td>
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<td>FS-381</td>
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<td>Sandy-2</td>
<td>69-0730-00-204</td>
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<td>9/17/2013</td>
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<td>yes</td>
<td>9/17/2013</td>
<td>0.03</td>
<td>23,900</td>
</tr>
</tbody>
</table>

Based on the data in the above table, there is no relationship between sediment porewater sulfide concentrations and the presence of wild rice.

It is interesting to note that while no wild rice was observed on the following lakes:
• Bean (1 sample)
• Lady Slipper (3 samples)
• South Geneva (1 sample)

Wild Rice was found in two of the lakes:

• Sandy Lake (9 samples (+ 1 duplicate), wild rice found in 3 samples)
• Rice Lake (2 samples, wild rice found in 1 sample)

While wild rice was not found when the highest sulfide readings were observed, wild rice was found later in the season, sometimes only a month after the highest sulfide concentration was observed.

It is also interesting to note that the following lakes are listed in the DNR’s 2008 survey of wild rice waters:\(^39\):

• Bean
• Sandy
• Rice

(Note that a Geneva Lake in Freeborn County is listed in the DNR report, but it has a different Lake ID number than the one sampled in the field survey.)

Bean and Rice Lakes are listed without any estimated wild rice coverage. Sandy Lake is listed as having 100% wild rice cover. However, this is at odds with the field survey observations\(^40\) and recent observations by Bois Forte, which found wild rice with only scattered locations with wild rice plants.\(^41\)

Although Sandy Lake had one sulfide observation greater than 90 \(\mu\)M, and one at 41 \(\mu\)M, the other observations were less than 10 \(\mu\)M. The value at 90 \(\mu\)M is not statistically different than the rest of the observations when the data were analyzed on a log normal distribution\(^42\). The median concentration observed including the two high values is 4 \(\mu\)M. It is inaccurate to state that Sandy Lake routinely has sulfide porewater concentrations greater than 90 \(\mu\)M. It is more accurate to say that Sandy Lake occasionally has high sulfide concentrations. It should be noted that Sandy Lake was sampled in three different locations as shown in Figure 3-1. The two highest readings occurred at the Sandy-2 sampling location. Again, the lake sulfide

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\(^{39}\) Natural Wild Rice In Minnesota, Department of Natural Resources, February 2008


\(^{41}\) Sandy Lake and Little Sandy Lake Monitoring (2010-2013), Bois Forte Reservation Technical Report 13-06, December 2013

\(^{42}\) Environmental data frequently follow lognormal or right-skewed distributions (e.g., Gilbert 1987 – Statistical Methods for Environmental Pollution Monitoring)
concentrations are compared to the no effects concentration appropriately derived in the sulfide hydroponics test evaluating plant endpoints typically exposed to anaerobic environments. The toxic concentration of sulfide to these plant life stages may be well in excess of 90 μM.
Figure 3-1  Sandy Lake Sample Locations
The lack of wild rice presence during the high sulfide (96 μM on July 9, 2013) observation is not clearly due to the porewater sulfide concentration. A month later, the sulfide concentration had dropped by an order of magnitude and wild rice was present. A month after that (September 2013) the sulfide concentration had dropped by another order of magnitude and wild rice was also present.

Fluctuating water levels likely confound the chemical analysis results (and perhaps other variables not measured). Sandy Lake is a very shallow lake, with a maximum depth of three feet, and is subject to fairly wide fluctuations in water level. The Bois Forte study measured water levels bimonthly approximately every two weeks from May through early November each year in 2010-2013. Water levels in 2013 varied by nearly 10 inches between May and November. Between June and July there was an increase of nearly 6 inches in water depth, or an increase of approximately 16 percent. While the highest sulfide reading at Sandy 2 occurred during June 2013, the month prior to the highest sulfide reading it is not clear whether water level fluctuations and sulfide levels are related or are simply confounding results.

Based on this analysis, Sandy Lake should not be considered as a lake with porewater sulfide greater than 90 μM. Bean and South Geneva lakes were only sampled once. Porewater sulfide from those samplings was greater than 90 μM. Even though Lady Slipper and Rice lakes have other measurements that are less than 90 μM, the other concentrations are sufficiently high that they will also be considered as lake with porewater sulfide greater than 90 μM. Again, despite periods of time when porewater concentrations exceed 90 μM, both Rice and Sandy lakes were observed to have wild rice present.

The four lakes with porewater sulfide concentrations greater than 90 μM are located in the Prairie Parkland ecological province or on the border between that province and the Eastern Broadleaf Forest (Figure 3-2). The significance of this finding is discussed further in Section 3.

43 MN DNR topographic map at [http://files.dnr.state.mn.us/lakefind/data/lakemaps/c0826010.pdf](http://files.dnr.state.mn.us/lakefind/data/lakemaps/c0826010.pdf) and MPCA water quality database at [http://cf.pca.state.mn.us/water/watershedweb/datasearch/waterUnit.cfm?WID=69-0730-00](http://cf.pca.state.mn.us/water/watershedweb/datasearch/waterUnit.cfm?WID=69-0730-00)
44 Sandy Lake and Little Sandy Lake Monitoring (2010-2013), Bois Forte Reservation Technical Report 13-06, December 2013
Figure 3-2  Porewater Sulfide Concentration >90 μM no effects concentration
3.4 MPCA’s Outdoor Container Experiments

The MPCA’s outdoor container experiments were seriously flawed, and no conclusions should be drawn from them. First, only one year’s raw data were included in the appendices, making it impossible to analyze results from previous years’ experiments. As a result of omitting data from years 2011 and 2012, it is not possible to know initial container conditions, including baseline sediment, porewater, and surface water physical conditions and chemical concentrations. Second, the containers are hydrologically isolated, preventing infusion of groundwater carrying iron or other constituents (e.g., plant micronutrients) that would be present in the natural environment. Nutrient depletion may also have occurred over time (without replenishment). Third, the mesocosms had been used in other experiments prior to initiation of testing in 2013. As a result, their history of sulfate exposure is unknown. The systems appear to be aged and to be potentially depleted of micronutrient prior to their use in 2013. Finally, in 2013, Dr. Pastor reported significant seedling mortality following thinning. As discussed by Dr. Pastor, seedling mortality may have been influenced by removal of five plants per tank in years 2011 and 2012 (one sixth of the population) resulting in depletion of the seed bank for future population growth.\footnote{Effects of enhanced sulfate concentrations on wild rice populations: results from a mesocosm experiment, John Pastor, University of Minnesota Duluth, December 2013} In 2013, decreases in total plant biomass were not significantly correlated with increases in sulfate concentration.

3.4.1 Are there concerns about the methods?

Yes. Unlike the hydroponic experiments conducted by Dr. Pastor and Fort Labs, no test acceptability criteria were established to determine whether the test data were acceptable. In Dr. Pastor’s sulfate hydroponic experiments, the following test acceptability criteria are established:

Tests were deemed acceptable if: 1. At least 90% of control juvenile seedlings were living at test termination; 2. Mesocotyl length of juvenile seedlings from control exposures were at least 5.0 cm at the end of the 10 day duration of growth; and 3. Control juvenile seedlings did not indicate any visible phytotoxic or developmental symptoms at any time during the test and the controls grew. See Appendix 2 for more details.\footnote{Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013}

Dr. Pastor’s sulfide hydroponic experiments had similar test acceptability criteria:

Tests were deemed acceptable using the same criteria as described above for the tests of sulfate on germination. See Appendix 3 for more details.\footnote{Id.} (for seed germination and mesocotyl growth)

The Fort Environmental Labs study applied more rigorous test acceptability criteria (Table 3-2).\footnote{Id.}
Table 3-2  Fort Labs Hydroponic Studies Acceptability Criteria

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Acceptable Limits</th>
<th>Criterion Passed? (d21 value, if applicable)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control activation</td>
<td>95%</td>
<td>√ (100%)</td>
</tr>
<tr>
<td>Control mesocotyl emergence</td>
<td>≥30%</td>
<td>√ (38.3%)</td>
</tr>
<tr>
<td>Control survival</td>
<td>≥90%</td>
<td>√ (100%)</td>
</tr>
<tr>
<td>Positive control (BA) phytotoxicity</td>
<td>≥80%</td>
<td>√ (100%)</td>
</tr>
<tr>
<td>pH</td>
<td>6-7.5 in all replicates of control and treatments</td>
<td>√ (within range)</td>
</tr>
<tr>
<td>Water temperature</td>
<td>21° ± 2°C (day), and nightly, 12 ± 2°C (night) in all replicates of control and treatments</td>
<td>√ (within range)</td>
</tr>
<tr>
<td>Sulfate concentration</td>
<td>Inter-replicate CV ≤20% for control and treatments for individual measurement set (Study Day 0, 10, and 21)</td>
<td>√</td>
</tr>
</tbody>
</table>

No test acceptability criteria were established for the outdoor container studies. 49 Significant but undefined mortality occurred in 2013 across all concentrations, including controls. High mortality is indicative of a test system unable to support healthy plants absent the presence of the test variables (i.e., increased sulfate). In most laboratories, the results would subsequently be qualified or rejected as unreliable, especially given the poor rate of control survival (i.e., 15 percent in 2013). Although not directly applicable, an attempt was made to compare the results of the outdoor container study to the test acceptability criteria for the hydroponics study. That comparison is provided in Table 3-3.

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49 Definitive Hydroponics-Based Wild Rice (Zizania palustris) Sulfate Toxicity Testing, Fort Environmental Laboratories, December 2013
49 Effects of enhanced sulfate concentrations on wild rice populations: results from a mesocosm experiment, John Pastor, University of Minnesota Duluth, December 2013
Table 3-3  Outdoor Container Study Acceptability Criteria

<table>
<thead>
<tr>
<th>Hydroponic Experiment Acceptability Criteria</th>
<th>Outdoor Container Study – Criteria Passed?</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 90% of control juvenile seedlings were living at test termination</td>
<td>Fail – less than 15% of control seedlings survived</td>
</tr>
<tr>
<td>Length of juvenile seedlings from control exposures were at least 5.0 cm at the end of the 10 day duration of growth;</td>
<td>Passed. Initial seedling stem and leaf length was 6.1, 6.6 and 6.8 cm. Final control seedling stem and leaf length were 10.1, 11.4 and 12.9 cm</td>
</tr>
<tr>
<td>Control juvenile seedlings did not indicate any visible phytotoxic or developmental symptoms at any time during the test and the controls grew.</td>
<td>Passed in part, unknown in part. Control seedlings grew (see above). Phytotoxic or developmental symptoms of controls were not reported.</td>
</tr>
</tbody>
</table>

Based on Dr. Pastor's criteria for the hydroponic experiments, the outdoor container studies do not pass the acceptability test.

The test design did not include groundwater or surface water recharge. Instead, the water levels were maintained by intermittent additions of well water or precipitation, and water quality was infrequently monitored. Although well water is considered ground water, it does not have the same chemical composition as shallow groundwater that would be in contact with water bodies in nature. Without nutrient and iron infused recharge, this experimental design more closely resembles a seasonal pond or pothole, where wild rice may not grow or grow as well as in a natural setting. The test design likely stressed the entire wild rice population and made the results questionable. Conditions with no groundwater infusion, and no inflow or outflow carrying additional nutrients are important constraints that confounded results.

It appears that the tanks were nutrient deficient including iron and perhaps other limiting trace metal nutrients. As discussed by Dr. Johnson in Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, in hydrologically isolated mesocosms without the delivery of iron, it is likely that sulfide would build up. Without the benefit of measurements of initial conditions and data from previous years' experiments, no one can analyze the 2013 results. Similarly, without the benefits of measurements of initial conditions, no one can determine whether sulfide build up (unprecipitated by iron) that occurred or other substances (or lack of other substances) affected the test organisms. It may be that the third year of testing (2013) was a part of the normal life cycle of wild rice. Dr. Pastor notes:

Delays in the release of nitrogen from these litters in subsequent years may be responsible for the population oscillations of 3-5 year periods often seen in wild populations (Pastor and Walker 2006, Walker et al. 2010, Hildebrandt et al. 2012).50

The Great Lakes Indian Fish and Wildlife Commission also note;

50 Id.
Rice abundance can vary widely from year to year, especially on the most “lake-like” beds. The rule-of-thumb for lake beds: A typical four year period will include a bumper year, two fair years, and a bust year.\textsuperscript{51}

The results between 2011 and 2013 may have been simply part of the natural low-density cycle of wild rice, caused, perhaps, by delays in release of nutrients from the litter.

Measured sulfate concentrations in the overlying water fluctuated considerably; therefore, assigning precise sulfate concentrations to each treatment was difficult. From the study, and from an analysis of the data provided for 2013, each treatment only achieved the nominal treatment level once, sometime in June 2013. Concentrations were less than the nominal treatment goal at all other times during the experiment.

Based on all of these considerations, the effects of sulfate or sulfide on wild rice could not be evaluated. The study should not be relied upon to inform or develop water quality regulations.

\subsection*{3.4.2 Are there concerns about data quality?}

Only one year (2013) of data was made available, so verification of additional data cannot be conducted. Additional statistical analysis should be conducted, including ANOVA. Results from regression analysis do not allow for a comparison of means of multiple samples.

\subsection*{3.4.3 What conclusions can be drawn from the study?}

Given the serious concerns with the methodology, this study cannot be used to inform or develop water quality regulations. In particular, the MPCA cannot rely upon the presentation and analysis of data from 2011 and 2012 if that data is not publicly available.

\section*{3.5 MPCA’s Root Zone Geochemistry}

\subsection*{3.5.1 Are there concerns about the methods?}

This study consisted of two parts:

- Root zone geochemistry studies of the outdoor containers; and
- Root zone geochemistry studies of two field sites (one stream, one lake).

As discussed above, the outdoor container study did not produce acceptable results, based on the condition of the containers, so the data from the root zone geochemistry study of the outdoor containers cannot be used.

The results of the root zone geochemistry study of the two field sites (with two different substrate sites at each field site) is useful, and can help provide data on sulfate to sulfide transformations. The field sites for the root zone geochemistry studies were chosen as ones where wild rice was

\textsuperscript{51}Wild Rice Ecology-Harvest-Management, Great Lakes Indian Fish and Wildlife Commission, undated
observed in the field surveys; however it was not clear from the report that wild rice was growing
in the precise locations where the peepers were deployed.

3.5.2 Are there concerns about data quality?

Only limited chemistry data were collected (as opposed to that collected in the field study, for example), limiting the types of geochemical models that can be developed. Other substances (e.g. manganese, nitrate, phosphorus) could also precipitate or tie up sulfide, and sediment interactions are much more complex than those measured in this experiment. The geochemical model is not sufficiently robust to address sediment complexity or be used as a predictive model to revise the wild rice sulfate standard. The geochemical model is also of limited utility because it was derived from an experimental approach that was not mass-balanced with respect to sulfur loading.

3.5.3 What conclusions can be drawn from the study?

Dr. Johnson reaches the following conclusions:

_Sulfide concentrations in sediment pore fluids were almost always less than 10 μM (compared with 1000 – 7800 μg/cm² sulfate in the overlying water), and were often below the method reporting limit of 0.7 μM. The steep gradients of sulfate, sustained throughout the summer, and a lack of buildup in porewater sulfide indicate a consistent removal mechanism for sulfur in sediments. Iron concentrations at field sites were frequently in excess of 500 – 1,000 μM and precipitation of sulfide as iron sulfides provide a likely explanation for the low dissolved sulfide concentrations._

The Chamber agrees with these conclusions.

3.6 MPCA’s Temperature Dependent Diffusion Rate Studies

3.6.1 Are there concerns about the methods?

This study must be considered an exploratory study of the fate and transport of sulfate into and
out of sediments, and not a definitive test. Only two sediments were analyzed, limiting
conclusions about other sediment characteristics. Sediments could not be extracted whole, but
were homogenized prior to testing. The homogenization would have altered basic sediment
chemical characteristics by exposing anaerobic sediments to oxidizing conditions.

3.6.2 Are there concerns about data quality?

Yes, first one of the sediment samples had significant quantities of sulfate in the porewater.
Second, there were difficulties in achieving equilibrium. Third, there were difficulties in defining
the water/sediment interface.

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52 Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms,
Nathan W. Johnson, University of Minnesota Duluth, December 2013
3.6.3 What conclusions can be drawn from the study?

Dr. Johnson draws the following conclusions:

Negligible dissolved sulfide was generated in sediment pore waters despite the 300 to 400 mg/L sulfate in overlying waters for 11 weeks.\textsuperscript{53}

Sufficient sediment iron clearly eliminates the build-up of dissolved (toxic) sulfide in sediments. Increases in soluble sediment iron concentrations during the test indicate iron will not be depleted.\textsuperscript{54}

The Chamber agrees with Dr. Johnson’s conclusions that with sufficient naturally occurring porewater iron concentrations, there will negligible accumulation of porewater sulfide concentrations.

3.7 Summary

Given the high concentration of sulfate needed to have an effect on the growth of wild rice (1,600 to 2,500 mg/L in the hydroponic experiments), and the MPCA’s field survey showed no relationship between wild rice coverage and surface water sulfate concentrations, a sulfate water quality standard is unnecessary.

However, if the MPCA decides there needs to be a surface water standard, the standard should be 1,600 mg/L sulfate where wild rice is present. Two sulfate hydroponics experiments were conducted producing results support such an increase\textsuperscript{55}:

- 1,600 mg/L (Sulfate did not affect either seed germination or seedling growth other than a slight depression of root lengths at extremely high concentrations (1,600 mg SO\textsubscript{4}·L\textsuperscript{-1}).
  - not statistically significant)\textsuperscript{56}

- 2,500 mg/L (The no observed effects concentration (NOEC) for three of the ten Study Day 10 NOEC values were 2,500 mg/L sulfate or lower, and seven of ten SD 10 NOEC values were 5,000 mg/L sulfate. For Study Day 21, eight of ten concentration endpoints exhibited NOEC values of 5,000 mg/L sulfate, indicating that sulfate was generally not toxic at the highest concentration that could be tested within the limits of solubility of the salts.\textsuperscript{57})

\textsuperscript{53} Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments. Nathan W. Johnson, Will DeRocher, University of Minnesota Duluth, December 2013

\textsuperscript{54} Id.

\textsuperscript{55} In accordance with EPA guidelines and state rules for establishing water quality regulations, hydroponic testing is required. See Minn. Rules 7050.0218 and “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses,” USEPA, Office of Research and Development, Environmental Research Laboratories, Duluth MN; Narragansett, RI, Corvallis, OR, 1985

\textsuperscript{56} Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth, December 2013

\textsuperscript{57} Definitive Hydroponics-Based Wild Rice (Zizania palustris) Sulfate Toxicity Testing
While seedlings were not adversely affected in the Fort Environmental Labs study up to and including exposures of 2,500 mg/L sulfate, the Chamber is concerned that exposures exceeding 1,600 mg/L may, like any ion, pose a salinity stress (osmotic stress) potentially adversely affecting wild rice during its life cycle. This conservative assessment assumes that wild rice is salt sensitive and that exposure exceeding 1,600 mg/L could potentially impose an abiotic stress. Exposures up to 90 µM sulfide in a hydroponic experiment did not affect wild rice seed germination or mesocotyl length.

The juvenile seedling sulfide hydroponic studies and the outdoor container study raised serious concerns about the methodology. The entire seedlings (root and shoot) were exposed to sulfide treatments during the test; plant parts above the root-shoot node (crown) would not grow in anaerobic conditions where sulfide would form. Large seedling die-off occurred in 2013 for reasons that are not clear. Since MPCA did not provide the data from 2011 or 2012, it is not possible to evaluate initial tank conditions. Data from these studies cannot provide useable data for determining the relationship between surface water sulfate, porewater sulfide, porewater iron and wild rice growth.
4.0 MPCA's Goals, Primary Hypotheses - Chamber's analysis

4.1 Overview

In Section 3, the Chamber reviewed each of the MPCA's studies to determine whether the studies could be used to address the questions posed by the MPCA at the February 3, 2014 Advisory Committee Meeting:

- Are there concerns about the methods?
- Are there concerns about data quality?\(^{58}\)

In addition, the Chamber reviewed each MPCA study to determine whether the studies could provide scientifically valid conclusions.

In this section, the Chamber integrates all of the studies, along with other public, credible data to address the goals and hypotheses posed by the agency.

The MPCA sets the following goal of all of the studies:

*The goal of the Wild Rice Sulfate Standard Study is to enhance understanding of the effects of sulfate on wild rice and to inform a decision as to whether a revision of the wild rice sulfate standard is warranted.*\(^ {59}\)

The MPCA also notes that the studies need to determine:

- The effect of sulfate on wild rice and
- The effect of sulfide on wild rice

*Note: To inform the wild rice sulfate standard review, an important aspect of this analysis will be determining what concentrations of sulfate and sulfide are protective of wild rice, which may be different than the concentrations at which effects are observed in the study results.*

The first step is to determine how studies can inform the effects of sulfate and sulfide on wild rice. The Chamber notes that the United States Environmental Protection Agency (US EPA) specifies methods to set water quality standards for aquatic plants:

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\(^{58}\) Input on Study Reports, MPCA, February 2014

\(^{59}\) Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013
The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important\textsuperscript{60}.

Given the very low toxicity of sulfate to wild rice as confirmed in two independent studies, the MPCA also assumed that it was likely that sulfate by itself would not impact wild rice, and proposed the following hypotheses:

- **Wild rice is impacted by sulfate via the conversion of sulfate to sulfide in the rooting zone of the plants and**
- **Iron may mitigate the effects of sulfide production in the rooting zone of the sediment**\textsuperscript{61}

The MPCA has acknowledged that no single study can prove the hypotheses; rather, an integration of the data from all of the studies (and perhaps other sources as well) may demonstrate the veracity of the hypotheses.

Based upon the field surveys and hydroponic sulfate studies there is no correlation between sulfate in surface water and wild rice growth; sulfate does not affect wild rice growth except at very high levels (e.g., 1,600 mg/L) (which effects are likely caused by osmotic stresses brought on by high concentrations of any salt, including sulfates).

Based upon the field surveys and hydroponic sulfide studies and the data analysis presented here, there is a correlation between sulfide in porewater and wild rice growth, but that porewater sulfide does not affect wild rice seed germination or mesocotyl growth even at very high concentrations (e.g., above 2.4 mg/L or \(\sim 90 \mu M\)). Only lakes in the western and southern portions of the state have sulfide levels exceeding 90 \(\mu M\), and only where iron concentrations are very low (e.g., less than 1,000 \(\mu g/L \sim 2 \mu M\)).

The data analysis presented here indicates that the first hypothesis is only partially supported by the useable research provided by the MPCA and other credible, public evidence. There is evidence that sulfate in the overlying surface water diffuses into the sediment and is converted to sulfide in the rooting zone of the plants (porewater), but only in the absence of sufficient iron to precipitate the sulfide. However, in all streams and most lakes in Minnesota, there is ample iron to precipitate the sulfide. Where there is no discharge of sulfate to the water body, the sulfide in porewater is dependent on other factors such as parent soil material in which the lake is located, which is in turn affected by a number of climatic, geologic and biologic factors.

The second hypothesis is strongly supported by the useable research and other credible, public evidence. In all streams and in the majority of the lakes surveyed, there is more than sufficient


\textsuperscript{61} Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson, University of Minnesota Duluth, December 2013.
porewater iron to prevent the accumulation of sulfide in porewater to any significant concentration. The few lakes where high sulfide is observed are lakes that reflect the parent material and groundwater in which they are located, and tend to be lower in porewater iron.

### 4.1.1 Effects of Sulfate and Sulfide on Wild Rice

From the scatter plots presented in the field survey report, there is no statistically significant correlation between surface water sulfate and wild rice cover. Taking the data as presented (e.g. untransformed), the probability of fitting a linear \((p=0.375)\), quadratic \((p=0.110)\), and cubic \((p=0.179)\) relationship to the data indicate that there is no significant relationship between surface water sulfate and wild rice cover. Viewing the data in three dimensions (plant cover, surface water sulfate, and porewater iron) shows that there is no correlation between surface water sulfate and wild rice growth (Figure 4-1).

![Wild Rice Cover vs. Porewater Iron vs. Surface Water Sulfate](image)

**Figure 4-1  Wild Rice Cover vs. Porewater Iron vs. Surface Water Sulfate**

There is, however, a statistically significant correlation between porewater sulfide and wild rice cover. Taking the sulfide data as presented (e.g. untransformed), the probability of fitting a linear \((p=0.115)\), quadratic \((p=0.066)\), or cubic \((p=0.144)\) relationship to the data is not significant\(^6\). However, if the highest outlier (Bean Lake, porewater sulfide = 16.0 mg/L (500 μM) is removed, a quadratic \((p=0.088)\), and cubic \((p=0.166)\) relationship is not significant, but a linear relationship is \((p=0.028)\). The linear regression was \(y = -6.401x + 16.90\); adjusted \(R^2\) is 2.1% \(x-\)

\(^6\) In keeping with standard practice, \(p \leq 0.05\) is taken as statistically significant.
intercept is 2.64 mg S/L (Figure 4-2). Also, despite the statistically significant linear relationship between sediment porewater sulfide and wild rice cover, the very low $R^2$ value (correlation coefficient) indicates that very little data variability is accounted for by the regression. Thus, other factors play a more important role in the presence of wild rice.

![Fitted Line Plot](image)

**Figure 4-2  Fitted Line Plot**

The significance of the x-intercept is that one would expect that when the porewater sulfide is greater than 2.64 mg S/L (83 μM), one would expect that wild rice would not be present. Three lakes (no streams or paddies) had porewater sulfide concentrations greater than 2.2 mg S/L (83 μM), plus two outlier lakes (Bean Lake, as noted above and one of the samples at Lady Slipper Lake). None of these lakes had wild rice present in any of the surveys. Given the poor correlation coefficient for the relationship between wild rice cover and porewater sulfide, the resulting 83 μM sulfide intercept (the concentration at which wild rice would likely not grow) should only be considered an approximate sulfide concentration potentially limiting wild rice growth under some field conditions. A better value to use is the value derived from the sulfide hydroponic studies – 90 μM as the No Observed Effect Concentration (NOEC).

Wild rice cover was plotted (Figure 4-3) as a three-dimensional space with porewater iron and porewater sulfide.
Figure 4-3  Wild Rice Cover vs. Porewater Iron vs. Porewater Sulfide (no Outlier)

Because the updated field survey data were not available until January 29, 2014, only the relationships between wild rice growth, sulfide and iron could be explored fully in order to meet the MPCA’s timeline for a preliminary determination.

4.1.2 MPCA caveats regarding sediment interactions and limitations on research

Sediment interactions of sulfur are extremely complex, involving physical interactions (e.g. diffusion) between sediment and overlying water and between sediment and groundwater, chemical interactions (e.g. precipitation of dissolved sulfide) and biological interactions (e.g. conversion of sulfate to dissolved sulfides, precipitation of sulfides by iron (and perhaps other substances), the role of nutrients in these and other pathways). Other component interactions are occurring simultaneously. The MPCA attempted to illustrate these interactions in Figure 4-4.
Figure 4-4  Sulfur interactions in wetland sediments that might affect wild rice growth

The research conducted by the MPCA only began to explore the sulfur interactions, and failed to explore interactions of other components, nor were the relationships between sulfur and other components explored.

4.1.3 Integration of Data – Sulfate Water Quality Standard

First, the Chamber attempted to integrate the data from the studies and from other publicly available data to answer two of the regulatory questions posed by the MPCA:

- Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?
- Should there be a different standard for lakes/wetlands, or streams, or paddy rice?

4.1.4 Integration of Data – Period when "rice may be susceptible to high sulfate"

Secondly, the Chamber attempted to integrate the data from the studies and from other publicly available data to answer the third regulatory question posed by the MPCA:

What more can be said about the "period when the rice may be susceptible to high sulfate?"
4.2 Integration of Data – Sulfate Water Quality Standard

First, the data from the field survey were analyzed to determine whether they support the MPCA’s two primary hypotheses – that wild rice is impacted by sulfate via the conversion of sulfate to sulfide in the rooting zone of the plants and iron reduces sulfide production in the rooting zone of the sediment by precipitating it out of solution as iron sulfide.\textsuperscript{63}

It is also noted that several mining companies have been requested or required to undertake wild rice field surveys. These surveys have been conducted using methods similar to those used in the field survey conducted by the University of Minnesota. Surface water grab sampling near wild rice stands was carried out as part of the private surveys, but not for the extensive list of analytes conducted by the University. All areas of wild rice identified in these surveys had surface water sulfate concentrations greater than 10 mg/L; the highest observed (1,040 mg/L) was in an area also monitored by the University as having 838 mg/L sulfate.

A list of the surveys which have been submitted to the MPCA is included in Appendix A. The MPCA should incorporate the results of these surveys in their regulatory deliberations as further evidence of wild rice growth at surface water sulfate concentrations above 10 mg/L.

4.2.1 Confirmation of Hypotheses – Integration of Data

A relationship between sulfide, sulfate and iron was developed from the field survey data for lakes and streams data (paddies not included). That relationship is given in Equation 4-1:

\textbf{Equation 4-1}

\[ \text{Porewater Sulfide (mg/L)} = 6.42 \times \text{Surface Water Sulfate (mg/L)}^{0.00427} \times \text{Porewater Iron (\mu g/L)}^{-0.445} \]

Or

\[ \text{Sulfide} = 6.42 \times \text{SO}_4^{0.00427} \times \text{Fe}^{-0.445} \]

This relationship had an \( r^2 \) between observed and predicted sulfide of 0.595, and was significant (prob. <0.001). (Figure 4-5). The relationship was derived from 198 observations. Forty Six (46) observations with missing sulfide sulfate or iron data were eliminated from the original data set. Two (2) sulfide outlier values were eliminated, as were all true duplicates. Values which were reported as less than detection limits (e.g., < values) were included at the detection limit.

\textsuperscript{63} Id.
Figure 4-5  Relationship between observed sediment pore water sulfide in lakes and stream sediments and that predicted by a non-linear relationship with surface water sulfate and sediment pore water iron as predictor variables, n = 198 (1:1 line indicated).

The explanatory power of this equation is surprisingly high considering that the data represented 198 samples from uncontrolled natural systems (lakes and streams) and the samples were collected over a period of three years during varying times of the growing season.

Put simply, if there is sufficient iron available in the sediment; it will tie up any sulfide generated in the sediment, making it biologically unavailable to wild rice. There is such a small exponent on the sulfate concentration (two orders of magnitude lower than the exponent on the iron concentration) that the surface water concentration is nearly inconsequential. In fact, nearly the same amount of variability can be explained by a power formula using only iron and the field survey data:

**Equation 4-2**

\[
\text{Sulfide} = 6.51 \times \text{Fe}^{0.446}
\]

This relationship had an \( r^2 \) between observed and predicted = 0.595 and was significant (prob. <0.001) Figure 4-6.
Figure 4-6  Relationship between observed sediment pore water sulfide in lakes + stream sediments and that predicted by a non-linear relationship with only sediment pore water iron as a predictor variable, n = 198 (1:1 line shown)

The relationships in Equation 4-1 and Equation 4-2 do not explain all of the variability observed in the field, but they do account for a large portion of that variability, and ample demonstrates that the MPCA’s hypothesis was well founded with respect to the role of iron in mitigating the presence of dissolved sulfide. The hypothesis that surface water sulfate is a key determinant in the formation of sediment porewater sulfide is not supported.

In other words, Equation 4-2 confirms one of the primary hypothesis:

    .... if elevated sulfate has a negative effect on the growth of wild rice, it is mediated through the formation of hydrogen sulfide in the rooting zone of wild rice, and that elevated iron would mitigate the toxicity of the sulfide by forming insoluble iron sulfide compounds^{64}.

The derivation of these relationships (Equation 4-1 and Equation 4-2) is provided Appendix B.

4.2.2 Alignment of Hydroponic Experiments

Next, the Chamber reviewed the Hydroponic Experiments to see whether the data were consistent with the relationship developed from the field surveys.

^{64} Wild Rice Sulfate Study Summary and Next Steps, MPCA, December 2013
4.2.2.1 Sulfate Hydroponic Experiments

The sulfate hydroponic experiments demonstrated that sulfate was not toxic to wild rice plants at any life stage tested, even at extremely high sulfate concentrations. The University of Minnesota Duluth study found no impacts up to 1,600 mg/L, while the Fort Environmental Labs study found LOECs of 5,000 mg/L sulfate and > 5,000 mg/L sulfate for the full 21 d exposure period, depending upon the biological endpoint. None of the field surveys showed sulfate concentrations near these levels. The statistical analysis of the field data showed that there was little, if any, correlation between porewater sulfide and surface water sulfate.

These conclusions are consistent with the relationship developed from the field surveys. The amount of sulfate in the overlying surface water had little influence on the amount of porewater sulfide, and sulfate by itself does not impact the growth of wild rice.

4.2.2.2 Sulfide Hydroponic Experiments

The portion of the sulfide hydroponics experiments that addressed seed germination and mesocotyl growth demonstrated that sulfide was not toxic above 90 μM sulfide. That concentration can be considered a No Observed Effects Concentration or NOEC value.

Only a few of the lakes (no streams or paddies) in the field survey had sediment sulfide concentrations greater than 90 μM (Figure 4-7).
These include the two outlier lakes:

- Bean (Becker Co – 600 μM)
- Lady Slipper (Lyon Co – 543 μM) and

as well two others:

- South Geneva (Freeborn Co – 99 μM)
- Rice (Stearns Co – 93 μM)

Recall that one of 10 observations from Sandy Lake in St. Louis County had sulfide porewater concentrations greater than 90 μM. Because only one of ten observations was greater than 90 μM, the Chamber determined, based on the weight of evidence approach, that Sandy Lake did not consistently have sulfide porewater concentrations greater than 90 μM. See Section 2.3.2.3 above.
The location of these lakes is shown in Figure 3-2.
These lakes are all located in the Prairie Parkland Ecological Province or on the border of the Prairie Parkland and Eastern Broadleaf Forest Ecological Province.

Table 4-1 summarizes the sulfide, sulfate and iron data from the field survey. Note that none of these observations were associated with the presence of wild rice.

### Table 4-1 Lakes with Sulfide >90μM

<table>
<thead>
<tr>
<th>Lake Name</th>
<th>Inventory Number</th>
<th>County</th>
<th>SO4 (mg/L)</th>
<th>Sulfide (μM)</th>
<th>Fe (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td>03-0411-00</td>
<td>Becker</td>
<td>83</td>
<td>500</td>
<td>50</td>
</tr>
<tr>
<td>Lady Slipper</td>
<td>42-0020-00</td>
<td>Lyon</td>
<td>108</td>
<td>463</td>
<td>638</td>
</tr>
<tr>
<td>South Geneva</td>
<td>24-0015-00</td>
<td>Freeborn</td>
<td>14</td>
<td>99</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Rice</td>
<td>73-0196-00</td>
<td>Stearns</td>
<td>3</td>
<td>93</td>
<td>25</td>
</tr>
</tbody>
</table>

1 Multiple samples were taken at these lakes. Only the sample set with porewater sulfide concentrations >90μM are shown.

These four lakes have very low porewater iron concentrations, with three in the lowest five percentile of iron concentrations and one (Lady Slipper) in the lowest 10 percentile. The data from these four lakes are consistent with the results of the sulfide hydroponic studies.

The Minnesota Department of Natural Resources (MN DNR) lists two of the lakes as part of their Natural Wild Rice in Minnesota Inventory:65

- Bean (but no wild rice acreage indicated)
- Rice (but no wild rice acreage indicated)

It should be noted that the “Geneva” Lake listed in the DNR inventory is North Geneva Lake, a different lake, which has much lower porewater sulfide, but no wild rice was found during the most recent survey.

Next the size, depth, and clarity of these lakes are explored in Table 4-2.

### Table 4-2 Lakes with Sulfide >90μM Lake Properties

<table>
<thead>
<tr>
<th>Lake Name</th>
<th>Inventory Number</th>
<th>County</th>
<th>Acres</th>
<th>Littoral Area (acres)</th>
<th>Maximum Depth (ft)</th>
<th>Water Clarity (Secchi Disk - ft)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td>03-0411-00</td>
<td>Becker</td>
<td>14</td>
<td>14</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Lady Slipper</td>
<td>42-0020-00</td>
<td>Lyon</td>
<td>286</td>
<td>286</td>
<td>11</td>
<td>1.7</td>
</tr>
<tr>
<td>South Geneva</td>
<td>24-0015-00</td>
<td>Freeborn</td>
<td>2,214</td>
<td>2,214</td>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>Rice</td>
<td>73-0196-00</td>
<td>Stearns</td>
<td>1,509</td>
<td>958</td>
<td>41</td>
<td>3.6</td>
</tr>
</tbody>
</table>

1 Multiple samples were taken at these lakes. Only the sample set with porewater sulfide concentrations > 90 μM are shown.

65 Natural Wild Rice In Minnesota: A Wild Rice Study. Minnesota Department of Natural Resources, February 2008
All four are very shallow lakes, likely subject to winter fish kills (when the dissolved oxygen levels drop to levels too low to support fish in the lake). While not all four lakes had information on fish kills, the information from the MN DNR LakeFinder website for Lady Slipper Lake might be typical of such shallow, prairie pothole lakes:

*Both partial summer kills and winterkills have occurred in Lady Slipper, but these have not been documented since the late 1990’s. However, a winterkill assessment was conducted during late April of 2010 for Lady Slipper Lake.*

And

*Oxygen levels in Lady Slipper were near 13 ppm during late December of 2009, 3 ppm during mid-January of 2010, 1.7 ppm by mid-February of 2010, 1 ppm during late February of 2010, and 15 ppm by mid-March of 2010. The aeration system was started during mid-January of 2010 on Lady Slipper. One surface aerator quit during late February.*

Similarly the information for Rice Lake from the MN DNR LakeFinder website for Rice Lake may also be typical for such shallow prairie pothole lakes in agricultural areas:

*Nutrient runoff enters Rice from agricultural row crops, feedlots/pasture areas, city storm sewer, and lake residential sources. Water clarity was poor during mid-July of 2012 (secchi=3.0 feet). Dissolved oxygen levels were less 1 ppm below 18 feet deep during the survey. Low water levels due to drought conditions and high summer air temperatures were the norm during the 2012 summer. Nutrient levels (total phosphorus=0.049 ppm, chlorophyll a=36.3 ppm) were moderately high during June of 2007.*

Finally, carp are known to harm wild rice by uprooting the wild rice plants and consuming seeds from the sediment. Rice Lake was noted as having carp in the MN DNR FishFinder website.

### 4.2.3 Alignment of Root Zone Geochemistry Experiment

The Chamber reviewed the Root Zone Geochemistry Experiments to see if the data presented there aligned with the relationship developed from the Field Study. Dr. Johnson concludes that root zone geochemistry supports the theory of sulfide being mitigated by sediment iron, and this conclusion is consistent with Equation 4-1 and Equation 4-2:

*This oversaturation of porewaters with iron and sulfide highlights the critical role of ferrous iron in controlling dissolved sulfide in porewaters* and indicates that the precipitation of

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66 MN DNR LakeFinder interactive website: [http://www.dnr.state.mn.us/lakefind/index.html](http://www.dnr.state.mn.us/lakefind/index.html)
67 Id.
68 Effectiveness of Temporary Carp Barriers for Restoring Wild Rice Beds in Upper Clam Lake: 2010 to 2013, Freshwater Scientific Services, October 2013
69 MN DNR LakeFinder interactive website: [http://www.dnr.state.mn.us/lakefind/index.html](http://www.dnr.state.mn.us/lakefind/index.html)
solid phase iron sulfides in surficial sediments represents a sink for removing dissolved iron and sulfide from sediment porewaters.\textsuperscript{70} (emphasis added)

Thus, while sulfate can diffuse into sediment and can be converted to sulfide, accumulation occurs only where there is insufficient iron in the porewater. With only a few exceptions, ample naturally occurring iron is present to precipitate dissolved sulfide and prevent its accumulation in porewaters. The mean porewater sulfide concentration observed in the field surveys was 3.9 μM.

4.2.4 Alignment of Temperature Dependent Diffusion Rate Studies

Dr. Johnson concluded in his study of transport and reaction of sulfate between overlying waters and sediment:

Based on the geochemistry of the sediments used for the present study where iron concentrations of 200 – 1000 μM were observed, low sulfide concentrations could be expected (Van Der Well et al. 2007), based on the formation of insoluble iron sulfide compounds. The initial hypothesis for the present study was that a decrease in porewater iron would be observed during the loading phase of the study as iron sulfide was formed; however, the sulfate exposure portion of this study was not long enough to allow a measurable titration of the high iron content (including solid phase) of the sediment and the appreciable accumulation sulfide in the porewaters\textsuperscript{71} (emphasis added)

And:

Though porewater sulfide was measured initially and at the end of each phase, a quantifiable rise in dissolved sulfide was not observed over the course of this nine-month study. A similar study conducted by Van der Well et al. 2007, observed a strong negative relationship between iron and sulfide concentrations within sediment porewaters. Their research was conducted over the course of 21 months and utilized in situ testing within a peat meadow\textsuperscript{72} (emphasis added)

Dr. Johnson’s research supports the MPCA’s hypothesis that:

- iron may mitigate the effects of sulfide production in the rooting zone of the sediment\textsuperscript{73}

\textsuperscript{70} Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson University of Minnesota Duluth December 2013
\textsuperscript{71} Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments Nathan W. Johnson, Will DeRocher, University of Minnesota Duluth, December 2013
\textsuperscript{72} Id.
\textsuperscript{73} Id.
4.2.5 Alignment with geology and geochemistry

According to the Minnesota Geological Survey (MGS), groundwater in the Quaternary deposits (e.g., shallow groundwater in soil, as opposed to groundwater in sedimentary or metamorphic bedrock) is chiefly glacial in origin.\footnote{Major Constituent Chemistry of Selected Phanerozoic Aquifers in Minnesota, Roman Kanivetsky, Minnesota Geological Survey 1986}

Areal variations in the chemical characteristics of the groundwater are controlled by mineralogical composition of the Quaternary deposits, the length of time the water remains in contact with the glacial materials, climatic factors (especially precipitation and temperature which influence evapotranspiration), and physiography.\footnote{Id.}

Each of these factors which influence the Quaternary deposits and the water which runs through it are explored below.

4.2.5.1 Glacial origin of Quaternary Deposits

The most recent glacier to cross the state was the Des Moines lobe. About 14,000 years ago, this ice extended through the Red River lowland in northwestern Minnesota south to Des Moines, Iowa. Des Moines lobe till is gray to brown and is distinctive because it contains Cretaceous shale imported from North Dakota and Canada.\footnote{Minnesota at a Glance: Quaternary Glacial Geology. Minnesota Geological Survey, B.A. Lusardi, 1994; revised March 1997} A map showing the extent of the Des Moines lobe is shown in Figure 4-8. Note that all of the lakes with high (> 90 μM) sulfide are on or near deposits of the Des Moines lobe.

This is significant in terms of groundwater chemistry because the groundwater takes up the minerals in the glaciated deposits. From the MGS:

Toward the west, the groundwater quality gradually changes from the calcium-magnesium carbonate type to the calcium magnesium and sodium-potassium sulfate and chloride types. This occurs because the glacial deposits of western Minnesota contain a high fraction of pulverized Cretaceous shale, which contains gypsum and disseminated crystals and nodules of iron sulfide. The shale also retains absorbed sodium and potassium ions on its constituent clay minerals. Fragments of shale, incorporated in the glacial drift, have high exchange capacity and readily give up sodium and potassium ions for calcium and magnesium ions in the circulating water. High concentrations of sulfate are caused by direct leaching of sulfate minerals, such as gypsum, and by oxidation of sulfide. In areas of increasing sulfate concentration total dissolved solids increase to more than 1000 ppm. Similar changes occur with depth,
because Cretaceous shale makes up more of the glacial drift near the bottom of these materials than it does near the top.\textsuperscript{77} (emphasis added)

In contrast, the eastern part of the state was largely influenced by the Superior and Rainy lobes. "Till from the Superior lobe is distinctly red in color and contains rocks derived from the Superior basin—red sandstone, shale, and agates."\textsuperscript{78} The red color, of course, is due to iron. In addition, "sulfate concentration in waters of eastern Minnesota is low, because most of the readily soluble sulfate minerals have been leached."\textsuperscript{79}

Figure 4-8 shows the highest sulfide sediment lakes (those greater than 90 $\mu$M). As can be seen in Figure 4-8 all these lakes are in or near the deposits put down by the Des Moines Lobe of the Wisconsin Era glaciation.

\textsuperscript{77} Major Constituent Chemistry of Selected Phanerozoic Aquifers in Minnesota, Roman Kanivetsky, Minnesota Geological Survey 1986
\textsuperscript{78} Minnesota at a Glance: Quaternary Glacial Geology. Minnesota Geological Survey, B.A. Lusardi, 1994; revised March 1997
\textsuperscript{79} Major Constituent Chemistry of Selected Phanerozoic Aquifers in Minnesota, Roman Kanivetsky, 1986
4.2.5.2 Climatic factors

Minnesota can be thought of as lying astride the Precipitation – Evaporation (P-E) divide (Figure 4-9). To the east of this divide, precipitation exceeds evaporation, while west of the divide, the opposite is true. This is significant because it explains how, over thousands of years, minerals either leached out of soils or remain as a source for groundwater.

Water in contact with Rock that contains high soluble gypsum or anhydrite acquires calcium and sulfate ions from these minerals.

Once dissolved:

sulfate [is] reduced to sulfide in reducing conditions in MN groundwater.

South of the Minnesota River, direct leaching of sulfate minerals and oxidation of sulfide change the water from the bicarbonate to the sulfate type.⁸⁰

Figure 4-9 shows the highest sulfide sediment lakes (those greater than 90 µM). As shown in Figure 4-9 most are on the semi-arid side or in the transition of the climatic divide. The two highest sediment sulfide concentrations of 600 µM (Bean Lake) and 543 µM (Lady Slipper Lake) were observed in net-zero or negative precipitation minus evapotranspiration zones.

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⁸⁰ Id.
⁸¹ Minnesota’s Water Supply: Natural Conditions and Human Impacts, MN DNR, September 2000
4.2.5.3 Groundwater Provinces

Figure 4-10 shows the highest sulfide porewater lakes (those greater than 90 μM). As shown in Figure 4-10, while the lakes are in a number of groundwater provinces, those groundwater provinces are wholly or partially within the Des Moines glaciation lobe. The soils within these groundwater provinces tend to be high in sulfates and low in iron, similar to the porewater observed in these lakes.
Figure 4-10  Groundwater Provinces

GROUNDWATER PROVINCES
AND GLACIAL LOBES
Minnesota Chamber of Commerce
State of Minnesota
4.2.5.4 Ecological Provinces

Figure 3-2 shows the highest sulfide sediment lakes (those greater than 90 μM). As shown in Figure 3-2, all are in the Prairie Parkland province in the transition from the Eastern Broadleaf Forest and the Prairie Parkland province. The original surface vegetation reflects the combination of the glacial origins of soils, the groundwater interaction with those soils, the climate and other factors. Since the mid-1800's, wholesale conversion of the landscape to agriculture has resulted in increased application of nutrients, and increased runoff of sediments and nutrients to lakes and streams.
The "Moyle's isopleth"

Moyle's isopleth denotes a line that Dr. John Moyle drew based on his field observations that abundant wild rice and low sulfate levels were primarily found north and east of this line, whereas less abundant or no wild rice and high sulfate levels were primarily found south and west of this line. Figure 4-11 shows the highest sulfide sediment lakes (those greater than 90 μM) and all are near the Moyle's isopleth, which is the area where wild rice was not found.
Figure 4-11  Moyle's 1956 Isopleth

Dr. Moyle had limited tools at the time of his observations (mid- to late 1940s). He could not measure sulfide, could not measure iron at the low levels found in porewater, and probably could only measure bulk sediment properties. He did measure sulfate, and made observations about wild rice.
"Moyle's isopleth" reflects a line dividing:

- Semi-arid from semi-humid climates
- Soils with high sulfate and lower iron content from soils with low sulfates and high iron content
- Nutrient rich prairie topsoil (Prairie Parkland Ecological Province) from nutrient deficient forest topsoil (Forest Ecological Province)

Lakes which are to the west of the "Moyle's isopleth" may be waters which do not support the production of wild rice, not because of sulfate concentration, but because of the ecosystem, soil and climate in which they were formed and in which they exist today.

4.2.6 Summary

One of the MPCA's primary hypotheses is borne out by the data from all of the studies. A relationship between porewater sulfide, surface water sulfate, and porewater iron is described by Equation 4-1:

Equation 4-1

\[
\text{Sulfide} = 6.42^{*}\text{SO}_4^{0.00427}^{*} \text{Fe}^{-0.445}
\]

In fact, nearly the same amount of variability can be explained by Equation 4-2 using only iron and the field survey data:

Equation 4-2

\[
\text{Sulfide} = 6.51^{*} \text{Fe}^{-0.445}
\]

These relationships were used to predict the expected porewater sulfide concentrations under a number of permutations and combinations of porewater iron and surface water sulfate. We used Equation 4-1 to estimate sulfide concentrations at the 5th, 25th, 50th, 75th and 95th percentiles of iron distributions for the edited data set from the field survey (n = 198) and plotted these data against various surface water sulfate concentrations of interest.

We selected a range of sulfate values that could help illuminate the question of whether the current surface water standard should be modified. The values for sulfate were 1.2 mg sulfate/L representing the 25th percentile in the data set (Figure 4-12), 838 mg sulfate/L representing the maximum observed in the data set (Figure 4-13), and 1,600 mg sulfate/L representing the NOEC observed in the hydroponics study (Figure 4-14). In the case of iron, the concentrations shown in each figure are 90, 2.79, 6.65, 13.9, and 33.3 µg iron/L in sediment pore water respective to the percentiles listed above. Results are shown in Figure 4-12, Figure 4-13, and Figure 4-14.

Equation 4-1 is the best tool for predicting the concentrations of porewater sulfide in sediment, Equation 4-1 and the associated figures below integrate data from the field survey, the
porewater geochemistry data, the hydroponic study, experiments. Although Equation 4-1 does not provide a mechanistic explanation for sulfide levels, Equation 4-1 shows that sulfate contributes positively to sulfide in sediments (although only slightly) and that iron significantly reduces sulfide in sediments.

This analysis that the NOEC observed in the sulfide hydroponic experiments (90 μM sulfide) is extremely unlikely to occur in natural systems. As noted above, 90 μM was exceeded only four times in the edited data set. At the maximum observed sulfate concentration in the dataset (838 mg/L), and at low observed iron concentrations (5th percentile), the estimated sulfide concentration (27.56 μM) is substantially below the NOEC level.

| Predicted Pore Water Sulfide Concentrations based on Pore Water Iron and Surface Water Sulfate Concentrations |

**Sulfide Hydroponic NOEC**

![Bar chart showing predicted pore water sulfide concentrations based on pore water iron and surface water sulfate concentrations.](chart)

**Figure 4-12** Results of predicted sediment pore water sulfide using the observed 25th percentile surface water sulfate and the 5th, 25th, 50th, 75th and 95th percentiles of porewater iron distributions (for the edited data set from the field survey) as predictor variable.
Figure 4-13  Results of prediction of sediment pore water sulfide using the 838 mg/L surface water sulfate (maximum observed in field survey) and the 5th, 25th, 50th, 75th and 95th percentiles of porewater iron distributions (for the edited data set from the field survey)
Thus, even at the highest observed surface water sulfate concentration at the lowest (5th percentile) porewater iron concentration, the sulfide concentration is well below the NOEC observed in the sulfide hydroponic study. The few (4) lakes observed with the highest sulfide are located in or on the Prairie Ecological Province, underlain by Des Moines lobe soils derived from high gypsum, low iron Cretaceous shale, in the semi-arid portion of the state. These conditions, coupled with the fact that most of these lakes are extremely shallow, have low dissolved oxygen and high nutrient levels, leading to enhanced anoxic conditions help explain why these lakes have relatively high sediment sulfide concentrations and no wild rice.

4.3 Integration of Data – Period when “rice may be susceptible to high sulfate”

4.3.1 Overview

The current standard for protection of wild rice applies only “during periods when the rice may be susceptible to damage by high sulfate levels” (MN Rules 7050.0224, Subp. 2). However this
phrase is not defined in rule or statute. The MPCA has issued only two permits which specify this period: the permit for Minnesota Power Cohasset plant, originally issued in 1975 and the Mesabi Nugget permit issued in 2012. The 2011 Legislation requires the MPCA to conduct a rulemaking to "designate the specific times of year during which the standard applies" (2011 Special Sessions Laws, 1st Special Session, Ch. 2, Art. 4, Sec. 31).

The Chamber integrated the results of the studies, particularly the sulfide hydroponic study, the root zone geochemistry study, and the sediment incubation experiments to provide a basis for the MPCA to make the designations required by the legislation and define the "periods when the rice may be susceptible to damage by high sulfate levels."

4.3.2 Life cycle of wild rice plants

Dr. Pastor provides a succinct overview of the life cycle of wild rice and the nutrient uptake during that cycle in his hydroponics experiments:

Wild rice is an annual plant. It grows in both lakes and rivers in water between 0.3 and 0.67 m depth where there is some water flow. Native stands of wild rice grow in waters that are circum-neutral pH, of low conductivity and hardness, and generally low in nutrient concentrations. In lakes, the most common sediment is an organic-rich silt, but the sediment types range widely (Day and Lee 1990). Sediment in the riverine habitat also ranges widely and may be higher in mineral sediment in the main channels than in backwaters (Meeker 1996).

Seeds germinate in the spring and first develop a mesocotyl, or primordial shoot, and a radical, or primordial root. The mesocotyl then grows above the sediment surface, where it develops into a green shoot with a primordial leaf in late spring and early summer. The plant is now at the seedling stage. When the shoot of the seedling reaches the water surface, the plant generates a long narrow leaf which floats atop the water surface; this stage is therefore called the floating leaf stage. Photosynthesis by the floating leaf is used to expand the root system and the beginnings of an aerial shoot which emerges from the leaf axil of the floating leaf and the stem below the water surface.

Once the aerial stem and the first aerial leaf emerge, the floating leaf dies and the plant grows taller, putting out additional aerial leaves until late July or early August. Nutrient uptake is very rapid during this stage, and approximately 60-70% of the plant’s annual requirement for nitrogen, the most limiting nutrient to both vegetative growth and seed production in most environments, is taken up then (Grava and Raisanen 1978, Sims et al. 2012a,b). In late July or early August, vegetative growth slows and the plant begins to produce a flowering shoot containing male (pollen producing) flowers above female (seed producing) flowers below. Wild rice does not self-pollinate well; instead, as for most graminoids, pollination is largely by wind although bees and flies occasionally visit the male flowers to gather pollen (J. Pastor, personal observations).

During the seed production and ripening stage, there is another burst of nutrient uptake from the sediment and the lower vegetative leaves begin to senesce as the nutrients they contain are translocated to the ripening seeds (Grava and Raisanen 1978, Sims et
al. 2012a, b). Seeds ripen in late August and through September, although the first two weeks of September are commonly the period of peak ripening. The seeds contain a long awn, which helps stabilize them vertically when they are dispersed into the water and thereby allow them to drill into the sediment (Ferren and Good 1977, J. Pastor personal observations). After seed dispersal, the plant dies and its stem, leaf, and root litter are returned to the sediment. Delays in the release of nitrogen from these litters in subsequent years may be responsible for the population oscillations of 3-5 year periods often seen in wild populations (Pastor and Walker 2006, Walker et al. 2010, Hildebrandt et al. 2012).  

A graphical depiction of Pastor's description is shown in Figure 4-15.

![Cumulative Nutrient Uptake](image)

**Figure 4-15  Cumulative Nutrient Uptake by Wild Rice**

For much of the year, after seed maturation and harvest (or seed drop), there is no nutrient uptake by the wild rice plant, because it has senesced. During germination and early mesocotyl growth, the energy comes from within the seed itself, and there is little uptake or interaction with the environment. During the remainder of the plant's life cycle, nutrient uptake increases with plant growth, until seeds have been set and there is no further need for nutrients.

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82 Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013
Sulfur is one of the six macronutrients for plant growth, and low availability of sulfur may therefore limit primary production\(^83\). Therefore, concentrations of sulfate and sulfide in the environment would have only minimal impact on a plant that is not taking up nutrients (e.g. the senescing plant or the dormant seed).

### 4.3.3 Integration of Sulfide Hydroponics Study

Exposures up to 90 µM sulfide in a hydroponic experiment did not affect wild rice seed germination or mesocotyl length. Dr. Pastor concludes, and the data show that:

> Enhanced sulfide under anaerobic conditions did not affect germination of seeds, mesocotyl weights, or mesocotyl lengths in a rangefinder test at nominal exposure concentrations of 0, 3, 10, 30 and 90 µM sulfides. The rangefinder test was repeated, and because the same results were observed, we did not proceed with any further tests.\(^84\)

Based on these results, the presence of sulfate or sulfide in the porewater will not impact wild rice plants from roughly September through April. (Germination of course will be determined by the onset of spring, and will vary from year to year.)

### 4.3.4 Integration of Root Zone Geochemistry Experiments

With regard to the field locations, Dr. Johnson concluded:

> Sulfide concentrations in sediment pore fluids were almost always less than 10 µM (compared with 3,800 – 7,800 µg/cm\(^2\) sulfate in the overlying water), and were often below the method reporting limit of 0.7 µM. The steep gradients of sulfate, sustained throughout the summer, and a lack of buildup in porewater sulfide indicate a consistent removal mechanism for sulfur in sediments. Iron concentrations at field sites were frequently in excess of 500 – 1,000 µM and precipitation of sulfide as iron sulfides provides a likely explanation for the low dissolved sulfide concentrations.\(^85\)

Based upon this data and conclusions, as well as the data observed in the field surveys, concentrations of sulfide in porewater will not interfere with wild rice seed germination or mesocotyl growth.

### 4.3.5 Integration of Temperature Dependent Diffusion Rate Studies

The sediment incubation experiments “investigated the diffusion of sulfate (SO\(^4\)-) into and out of the anoxic regions of two contrasting freshwater aquatic sediments under warm and cold

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\(^{84}\) Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor University of Minnesota Duluth, December 2013

\(^{85}\) Response of rooting zone geochemistry to experimental manipulation of sulfate levels in Wild Rice mesocosms, Nathan W. Johnson, University of Minnesota Duluth December 2013
temperatures. This research addresses whether there are periods when high surface water sulfate levels may occur without damaging or interfering with the growth of wild rice.

Dr. Johnson loaded the two freshwater sediments with 270-280 mg/L sulfate at the beginning of the loading phase (following an equilibrium phase). However, sulfate concentrations in the surface water rose to 350-365 mg/L in one sediment and as high as 650 mg/L in the other, likely due to "bioturbation by naturally occurring organisms within the sediment, oxidizing the available iron sulfide." By normalizing the sulfate concentrations in the surface water, Dr. Johnson found that "changes in porewater concentration between the 6th week and 9th week were minimal in both North Bay and Partridge River sediments, indicating steady state concentrations had been reached."

In other words, by six (6) to nine (9) weeks following the start of loading of high levels of sulfate in the overlying water, concentrations of sulfate in pore water reached equilibrium within 1 ½ to 2 months. However, during this same phase, sulfide concentrations increased much less:

> Within twelve weeks of the sulfate spike to overlying water, sulfide concentrations had slightly increased in the porewaters of North Bay cold microcosms (2 µM at 4-6 cm below the sediment water interface) and Partridge River warm microcosms (1.3 µM at 3 cm below the surface, however this is near the 0.7 µM reporting limit of the Hach method used for analysis).88

In the next phase – the recovery phase – lower concentrations of sulfate were introduced to the overlying water. Sulfate diffused back out of the porewaters and into the overlying water. This occurred quite rapidly.

> Sulfate flux out of the sediment occurred rapidly over the first week of the surface water being replaced with fresh site water during Phase III, the recovery phase. After two overlying water replacements within one week, sulfate levels remained steady at 30-40 mg/L in all of the microcosms except the warm Partridge River trials...89

Porewater sulfide concentrations at the end of Phase II and throughout Phase III were at or near detection limits in the Partridge River Sediment, and less than 2 µM in the North Bay sediment at the end of Phase II and throughout Phase III. Again, sulfide concentrations are much lower than were observed in the outdoor containers at all treatments.

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86 Temperature Dependent Diffusion Rates of Sulfate in Aquatic Sediments, Nathan W. Johnson, Will DeRocher, University of Minnesota Duluth, December 2013
87 Id.
88 Id.
89 Id.
4.4 Summary

Wild rice is not susceptible to any concentration of sulfate in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). There should be no sulfate water quality standards applicable during those times.

If the MPCA decides there needs to be a surface water standard, the standard should be 1,600 mg sulfate/L where wild rice is present. The surface water standard should apply only during the growing season of wild rice (mid-April through early September).
5.0 Recommendations for Revisions of the Water Quality Standard

5.1 Does the scientific evidence indicate that the standard should go up or down and, if so, generally by how much?

Given the high concentration of sulfate needed to have an effect on the growth of wild rice (1,600 to 2,500 mg/L in the hydroponic experiments), and the MPCA’s field survey showed no relationship between wild rice coverage and surface water sulfate concentrations, a surface water sulfate standard is unnecessary.

However, if the MPCA decides there needs to be a surface water standard, the standard should be 1,600 mg/L sulfate where wild rice is present. Two sulfate hydroponics experiments were conducted producing results support such an increase:

- 1,600 mg/L. (Sulfate did not affect either seed germination or seedling growth other than a slight depression of root lengths at extremely high concentrations (1,600 mg SO4 · L-1). -- not statistically significant).
- 2,500 mg/L. The no observed effects concentration (NOEC) for three of the ten Study Day 10 NOEC values were 2,500 mg/L sulfate or lower, and seven of ten SD 10 NOEC values were 5,000 mg/L sulfate. For Study Day 21, eight of ten concentration endpoints exhibited NOEC values of 5,000 mg/L sulfate, indicating that sulfate was generally not toxic at the highest concentration that could be tested within the limits of solubility of the salts.

Wild rice has been observed growing at concentrations near the 1,600 mg/L level. Concentrations above 2,500 mg/L may impact wild rice because of the overall salt content, not because of the specific toxicity of sulfate to wild rice. Multiple observations of wild rice have been made in waters with concentrations well above the current 10 mg/L water quality standard, both in the University of Minnesota Field Survey and in private surveys. Wild rice has

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90 In accordance with EPA guidelines and state rules for establishing water quality regulations, hydroponic testing is required. See Minn. Rules 7050.0218 and “Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses,” USEPA, Office of Research and Development, Environmental Research Laboratories, Duluth MN; Narragansett, RI, Corvallis, OR, 1985
91 Effects of enhanced sulfate and sulfide concentrations on wild rice germination and growth: results from a hydroponics experiment, John Pastor, University of Minnesota Duluth, December 2013
92 Definitive Hydroponics-Based Wild Rice (Zizania palustris) Sulfate Toxicity Testing
   Fort Environmental Laboratories, Inc. December 2013
been observed at concentrations of 838 mg/L (field survey FS303, Second Creek)\textsuperscript{93} and 1,040 mg/L (Mesabi Nugget Wild Rice Survey).\textsuperscript{94}

Based on laboratory hydroponic studies, porewater sulfide does not affect wild rice seed germination or mesocotyl growth. Field studies show that porewater sulfide only affects wild rice at very high concentrations, only in lakes in the western and southern portions of the state, and only where porewater iron concentrations are very low.

5.2 Should there be a different standard for lakes/wetlands, or streams, or paddy rice?

No. At an appropriately set standard of 1,600 mg/L, there is no need for a separate stream or lake water quality standard. MPCA has not conducted research on wetlands.

5.3 What more can be said about the “period when the rice may be susceptible to high sulfate”?

Wild rice is not susceptible to sulfate concentrations in overlying water between senescence (early September) through seed germination and early stages of seedling growth (mid-April). As a result, any sulfate water quality standard should not apply between early September and mid-April. If MPCA determines to impose a 1,600 mg/L sulfate water quality standard at locations where wild rice is present, that standard should apply only during the wild rice growing season (mid-April through early September).

5.4 Summary

A surface water sulfate standard is not necessary given the lack of any impact from surface water sulfate on the growth of wild rice. There is little, if any, correlation between surface water sulfate and wild rice growth or density. If the MPCA decides to set a surface water quality standard, it should be set at or near 1,600 mg/L sulfate. The standard should apply to lakes and streams. There should be no water quality standard for sulfate (where wild rice is present) during the time when wild rice has senesced and the time when juvenile seedling growth is established (early September through mid-April). The 1,600 mg/L sulfate water quality standard (where wild rice is present) should apply only during the growing season for wild rice (mid-April through early September).

\textsuperscript{93} Wild Rice Sulfate Standard Field Surveys 2011, 2012, 2013: FINAL REPORT, Amy Myrbo, University of Minnesota, December 2013
\textsuperscript{94} 2013 Wild Rice Survey and Water Quality Monitoring Partridge River and Second Creek Prepared for Mesabi Nugget Delaware, LLC, January 2014
6.0 Acknowledgements

The Chamber’s comments were prepared by a team of scientists and policy experts that consisted of several individuals holding post-graduate degrees (M.S. and Ph.D.) and decades of applied experience in aquatic toxicity assessment, water resources, soil science, rice nutrient dynamics, forest resources and genetics, chemical engineering, statistics, salinity effects on plants (laboratory and field, a professor emeritus in soil science and statistics, and federal and state environmental permitting and rulemaking.

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Mike Bok, PhD, Senior Manager — environmental statistician

**Barr Engineering Co.**

Rachel Walker, PhD, Senior Environmental Scientist — wild rice biology and nutrient dynamics, wild rice surveys, project management
Mike Hansel, Senior Chemical Engineer and Vice President — water quality rulemaking, environmental permitting
John Borovsky, Senior Environmental Scientist and Vice President — soils and groundwater interaction, environmental permitting
Lindsey Tuominen, Biostatistician — statistical analyses

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**University of California Davis**

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**Fort Environmental Labs**

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**ALLETE/Minnesota Power**

Kurt Andersen, Environmental Audit Manager, aquatic toxicity assessment
Appendix A

Private Wild Rice Surveys
<table>
<thead>
<tr>
<th>Company</th>
<th>Year</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcelor Mittal</td>
<td>2011</td>
<td>2011 Wild Rice Field Survey for ArcelorMittal</td>
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<tr>
<td></td>
<td>2010</td>
<td>NPDES Wild Rice and Water Quality Monitoring Report – SD030 - NPDES Wild Rice and Water Quality Monitoring Report – SD030 to SD012 (Wyman Creek)</td>
</tr>
<tr>
<td>Cliffs - Dunka</td>
<td>2011</td>
<td>Wild Rice Literature Review and 2011 Field Survey for the Dunka Mining Area Technical Memo</td>
</tr>
<tr>
<td>Cliffs - Northshore</td>
<td>2013</td>
<td>Wild Rice Literature Review and 2011 Field Survey for the Dunka Mining Area Technical Memo</td>
</tr>
<tr>
<td>Essar Steel</td>
<td>2010</td>
<td>2010 Water Quality and Wild Rice Monitoring Report</td>
</tr>
<tr>
<td>HibbTac</td>
<td>2011</td>
<td>2011 Wild Rice Survey for Hibbing Taconite Company Technical Memo</td>
</tr>
<tr>
<td>Keetac</td>
<td>2009</td>
<td>2009 Water Quality, Hydrology, and Wild Rice Monitoring - Swan Lake, Hay Lake, Moose Lake, Hay Creek, and Hart Creek</td>
</tr>
<tr>
<td></td>
<td>2011</td>
<td>2011 Water Quality, Hydrology, and Wild Rice Monitoring Year End Report - Swan Lake, Hay Lake, Moose Lake, Hay Creek, and Swan River</td>
</tr>
<tr>
<td>Mesabi Nugget</td>
<td>2009</td>
<td>2009 Wild Rice Survey and Sulfate Monitoring</td>
</tr>
<tr>
<td></td>
<td>2010</td>
<td>Lower Partridge River and St. Louis River, October 2009</td>
</tr>
<tr>
<td></td>
<td>2012</td>
<td>2010 Wild Rice Survey and Sulfate Monitoring</td>
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<td></td>
<td>2013</td>
<td>St. Louis River and Second Creek March 2011</td>
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<td>Minntac</td>
<td>2013</td>
<td>2013 Wild Rice and Water Quality Sampling Report Dark River and Dark Lake</td>
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<td>PolyMet</td>
<td>2009</td>
<td>2009 Wild Rice and Sulfate Monitoring - Spring Mine Creek, Embarrass River, Partridge River, Pike River, and Lower St. Louis River</td>
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<td></td>
<td>2011</td>
<td>2011 Wild Rice and Water Quality Monitoring - Second Creek, Spring Mine Creek, Trimble Creek, Unnamed Creek (PM 11), Wyman Creek, Embarrass River, Partridge River, and Pike River</td>
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<td></td>
<td>2012</td>
<td>2012 Wild Rice and Water Quality Monitoring Summary</td>
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<td>Utac</td>
<td>2011</td>
<td>Wild Rice Field Survey for United Taconite LLC Technical Memo</td>
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Appendix B

Development of Power Relationship
The relationship in Equation 4-1 and Equation 4-2 were developed as discussed below. Minitab Statistical Software was used for the statistical analyses presented here.

Step 1 – Eligible Data

The data from the field survey were explored with respect to sulfate, sulfide, and iron. A total of 267 samples were included in the field survey database. Because of the interest in natural systems, data from paddies (12 samples) were not used. Only the stream and lakes data were explored in this analysis (initial data set of 255 samples from multiple stream and lake locations, some locations having multiple samples).

Upon examination of the data, several data use issues were identified. First, several field samples (46 samples) were missing surface water sulfate, sediment pore water iron and/or sediment pore water sulfide observations. These samples were removed from the data set. Second, duplicate samples (class = “Survey Duplicate”) were also not included in the statistical analysis. Third, it was noted that for 26 samples the reported surface water sulfate was < 0.5 mg/L.

There are numerous possible methods for handling left-censored data (i.e., values reported as below the method detection limit), the simplest methods being: 1) analyze these data as 0.5 mg/L (i.e., set the values as equal to the detection limit), or 2) delete all samples with reported less than values.

Unilaterally deleting 26 samples (> 10% of the database) from a preliminary analysis is ill advised, so the Chamber used method 1) above to address left-censored data. While other more sophisticated methods of handling left censored data are available (e.g., using a random number generator to generate random numbers between 0 and the detection limit, using ½ the detection limit, or using regression imputation), time constraints for performing an initial preliminary analysis prohibited exploration of such methods. There were also a few samples with iron (4 samples) and sulfide (3 samples) that were reported as below the detection limit. The iron and sulfide values for these samples were treated the same as sulfate (i.e., the values were set equal to the detection limit).

As identified in Section 2.3.2.3, two samples in the field study had extremely high porewater sulfide concentrations: Bean Lake in Becker County (sulfide concentration 16 mg/L) and Lady Slipper Lake in Lyon County (one measurement at 14.8 mg/L, other measurements below 10 mg/L). These samples were identified as outliers and were not included in the data analysis. Resolution of the above data issues ultimately resulted in 198 samples in the edited data set (i.e., n = 198).

Step 2 – Simple Regression

The most appealing and useful relationship for regulatory purposes is an estimation of sulfide (the suspected toxic constituent) as a function of sulfate (the constituent whose regulation is of

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95 LacCore_dataexport_updated_Jan_29_2014.xlsx
interest) and iron (the constituent known to mitigate the toxicity of sulfide). Using the edited lakes and streams data set (n = 198), the following linear regression was developed:

**Equation B-1**

\[ S = 0.354 + 0.00032 \text{ SO}_4 - 0.000012 \text{ Fe} \]

where \( S \) is sediment pore water total sulfide in mg S/L (0 to 10 cm depth), sulfate (\( \text{SO}_4 \)) is surface water sulfate in mg sulfate/L, and iron (\( \text{Fe} \)) is sediment pore water iron in \( \mu \text{g} \) iron/L. The relationship had an adjusted multiple \( r^2 \) = 0.070 and was statistically significant (\( \text{prob.} < 0.001 \)). Although the \( r^2 \) value is low, the regression coefficient for iron was nonetheless significant (\( \text{prob.} < 0.001 \)), whereas the regression coefficient for sulfate was not significant (\( \text{prob.} = 0.440 \)). The low explanatory power of the relationship is due to the large range of values. The relationship does indicate that sulfate is not a statistically significant contributor to sediment sulfide, and iron is a strong factor in reducing sediment sulfide concentrations.

**Step 3 Conduct simple regressions on log-transformed values**

Because the range of concentrations spanned several orders of magnitude, the variables were transformed to logarithms, and the following linearized relationship was developed:

**Equation B-2**

\[ \ln S = 0.839 + 0.161 \ln \text{SO}_4 - 0.407 \ln \text{Fe} \]

Equation B-2 had an adjusted multiple \( r^2 \) of 0.460, and was significant (\( \text{prob.} < 0.001 \)). The regression coefficients for iron and sulfate were both significant (\( \text{prob.} < 0.001 \)) indicating that both sulfate and iron are contributors to sediment sulfide concentration.

The higher explanatory power of the relationship occurs because the logarithms of the high concentrations do not contribute markedly to the error sum of squares, and because the underlying data distributions for porewater sulfide and surface water sulfate are lognormal. The data distribution for porewater iron is neither normal nor lognormal.

**Step 4 Estimate Power Law Equation**

The edited lakes + streams data were then fitted to a power function to explore the relationship between sulfate (in surface water), sulfide (in sediment pore water) and iron (in sediment pore water). We used the power function because it passes through the origin (i.e., if sulfate = 0, sulfide should = 0), and because it can approach and ultimately assume a linear shape (if the exponents =1). The result was Equation B-3.

**Equation B-3**

\[ S = 6.42 \times \text{SO}_4^{0.00427} \times \text{Fe}^{-0.445} \]

This relationship has an \( r^2 \) between observed and predicted = 0.595 and was significant (\( \text{prob.} < 0.001 \)), see Equation B-3.
Step 7 Explore an alternative method to address less than detect values

As an alternative to treating sulfate values < 0.5 mg/L as 0.5 mg/L (e.g., set the value equal to the detection limit), we repeated the analysis that resulted in Equation B-3 using a censored data set where all sulfate samples with a reported concentration of <0.5 mg/L sulfate (26 samples) were deleted from the analysis (n = 172). (Note: the iron and sulfide values that were included as equal to the detection limit were not eliminated from the data set.)

The resulting power law relationship (n = 172) was:

**Equation B-4**

\[ S = 6.68 \times \text{SO}_4^{0.00772} \times \text{Fe}^{-0.439} \]

This relationship had an \( r^2 \) between observed and predicted = 0.589 and was significant (prob. <0.001).

Deleting the less than values for sulfate had little effect on the explanatory power (\( r^2 \)) of the non-linear relationship as compared to that associated with Equation B-4. This analysis might be taken as a lower bound on any other methods of addressing less than detect values. Since including all values as equal to the detection limit and eliminating all less than detect values...
resulted in nearly identical relationships and with nearly identical $r^2$ and significance, exploring other more sophisticated techniques for addressing left-censored data were not considered. Figure 6-2.

Figure 6-2  Relationship between observed sediment pore water sulfide in lakes and stream sediments and that predicted by a non-linear relationship with surface water sulfate and sediment pore water iron as predictor variables, sulfate values less than detect removed.

However, noting the very small influence of surface water sulfate on the prediction of porewater sulfide, a test was run to determine whether a relationship between porewater sulfide and porewater iron only would have similar explanatory power. Using the data set that was used for Equation B-3 ($n=198$) resulted in Equation B-5 (note, this is the same as Equation 4-2):

**Equation B-5**

$$S \text{ (mg S/L)} = 6.51 \times (\text{Fe \mu g/L})^{-0.446}$$

The relationship had an $r^2$ between observed and predicted = 0.595 and was significant (prob. < 0.001).

The relationships described in Equation B-5 show that sulfide concentration in sediment pore water is dictated by the coincident iron concentration in sediment pore water almost regardless of the sulfate concentration in the surface water (Figure 6-3).
Figure 6-3  Relationship between observed sediment pore water sulfide in lakes and stream sediments and that predicted by a non-linear relationship with only sediment pore water iron as a predictor variable, n = 198 (1:1 line shown)