Summary Report of the Meeting to Peer Review MPCA's *Draft Analysis of the Wild Rice Sulfate Standard Study*

Saint Paul, MN
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Submitted to:
Minnesota Pollution Control Agency (MPCA)

Submitted by:
Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421

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Notice

This report was prepared by Eastern Research Group, Inc. (ERG), a contractor to the Minnesota Pollution Control Agency (MPCA), as a general record of discussion during the Peer Review Meeting on MPCA's *Draft Analysis of the Wild Rice Sulfate Standard Study*, held August 13 and 14, 2014, in St. Paul, Minnesota. This report captures the main points and highlights of the meeting. It is not a complete record of all details discussed, nor does it embellish, interpret, or enlarge upon matters that were incomplete or unclear. Statements represent the individual views of meeting participants.

Contents

Acronyms and Abbreviations

ACPR	augmented component plus residual plots
ANOVA	analysis of variance
AVS	acid-volatile sulfide
CFD	cumulative frequency distribution
DOC	dissolved organic carbon
EC	effect concentration
ERG	Eastern Research Group, Inc.
IAP	ion activity products
K_{sp}	solubility product constant
LC	lethal concentration
MN DNR	Minnesota Department of Natural Resources
mg/L	milligrams per liter
MPCA	Minnesota Pollution Control Agency
µg/L	micrograms per liter
NOEC	no observed effect concentration
ppb	parts per billion
ppm	parts per million
QA/QC	quality assurance/quality control
SEM	structural equation modeling
TOC	total organic carbon
TWA	time-weighted average
U.S. EPA	U.S. Environmental Protection Agency

1. Introduction

Background

To enhance scientific understanding of the effects of sulfate on wild rice, the Minnesota Pollution Control Agency (MPCA) contracted with Eastern Research Group, Inc. (ERG) in 2014 to organize an independent scientific peer review of the Agency's *Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review* (Analysis). The Analysis integrates the results of a five-component study (Study) funded by MPCA to gather information about the effects of sulfate and other substances on wild rice growth.

Conducted by scientists at the University of Minnesota Duluth and Twin Cities during 2012 and 2013, this research was intended to inform the Agency's evaluation of the state's sulfate water quality standard, adopted in 1973, of 10 mg/L applicable to water used for production of wild rice. The Study's main hypothesis was that wild rice is impacted by sulfate via the conversion of sulfate to sulfide dissolved in the water in the sediment, known as the sediment porewater (see Appendix A for a detailed overview of the Study).

During the first half of 2014, MPCA staff integrated the Study results, analyzed the data as a whole, received input from the Agency's Wild Rice Standards Study Advisory Committee, and reviewed existing monitoring data, other relevant scientific studies/information, and the original basis for the wild rice sulfate standard. Based on these inputs, MPCA developed the draft Analysis that was the subject of this peer review, conducted during summer 2014.

This peer review is one step in a multi-year effort by MPCA to clarify implementation of Minnesota's wild rice sulfate standard and determine if changes to the standard are needed. The enhanced scientific understanding gained from peer reviewer comments will inform MPCA's subsequent review of the wild rice sulfate standard and the development of a rulemaking proposal, if warranted, regarding that standard.

Peer Review

The peer review consisted of four stages: reviewer search and selection; development of reviewer individual pre-meeting comments; a 2-day peer review meeting; and development of this summary report, including reviewer's final post-meeting comments.

Reviewer Search and Selection

MPCA defined the expertise criteria needed for this review and established a nomination process, directing any interested parties to submit nominations to ERG. ERG also conducted a national and international search for qualified experts. From this broad pool of candidates, ERG selected seven experts (Appendix B) who, collectively, best met the required expertise criteria:

- Dr. Patrick Brezonik (discussion chair), University of Minnesota (retired)
- Dr. Gertie Arts, Alterra, Wageningen University and Research Centre, Netherlands
- Dr. Donald Axelrad, Florida A&M University

- Dr. Siobhan Fennessy, Kenyon College
- Dr. Susan Galatowitsch, University of Minnesota
- Dr. Mark Hanson, University of Manitoba
- Dr. Curtis Pollman, Aqua Lux Lucis, Inc.

Development of Reviewer Pre-meeting Comments

As soon as reviewers were selected, ERG provided them with:

- MPCA's charge to reviewers (Appendix A), which asked reviewers to respond to 10 specific and 3 general questions.
- MPCA's draft Analysis and associated references.
- The individual report for each Study component for reviewers to consult, as needed, to further understand and evaluate the MPCA Analysis.
- MPCA's wild rice sulfate standard web page for more background information about the Study and Analysis. This page also provided a link to an ftp site with all the Study reports and data.

ERG then held a briefing call with reviewers to ensure that they were clear about the review background, purpose, process, and charge. Working individually, each reviewer began preparing written pre-meeting comments in response to the charge questions. A few weeks prior to the peer review meeting, ERG also provided reviewers with stakeholder comments on the Analysis for their consideration as they finalized their pre-meeting comments. ERG forwarded the pre-meeting comments to all reviewers and MPCA a few days before the meeting.

Peer Review Meeting

ERG organized and facilitated a two-day peer review meeting, which took place on August 13 to 14, 2014, in St. Paul, Minnesota. Interested members of the public were invited to attend as observers and to provide oral comments at the beginning of the meeting. See Appendices C and D for the agenda and list of meeting observers, respectively.

Jan Connery, the ERG meeting facilitator, opened the meeting by welcoming the reviewers and observers and describing the peer review process. She noted that reviewers would be conducting a scientific review of the Analysis, but would not be discussing or recommending changes to the existing wild rice sulfate standard. She then facilitated an oral comment session in which 12 observers commented (Appendix E). After that, reviewers began their discussions, chaired by Dr. Brezonik, which proceeded in order of charge questions. Upon concluding their discussions, reviewers held a writing session during the afternoon of the second day to develop consensus recommendations and conclusions (Section 2 of this report).

Development of Meeting Summary Report

Following the meeting, ERG summarized the discussions and reviewers prepared their individual post-meeting comments (Appendix E). This report, prepared by ERG, provides a summary of the peer review meeting:

- Section 2 presents reviewer consensus conclusions and recommendations, and Section 3 summarizes the reviewer discussions.
- The appendices provide: the charge to reviewers (Appendix A), list of reviewers (Appendix B), meeting agenda (Appendix C), list of meeting observers (Appendix D), observer oral comments (Appendix E), reviewer post-meeting comments (Appendix F), slides provided by Dr. Pollman during the meeting (Appendix G), figures referenced or provided by MPCA during the meeting (Appendix H), and a figure provided by Dr. Arts during the meeting (Appendix I).

2. Reviewer Conclusions and Recommendations

The MPCA wild rice sulfate standard study (Study) had two major types of components: (i) experiments to evaluate wild rice responses to sulfate and sulfide concentrations and (ii) field observations. Two investigations were involved in each of these categories: for the wild rice response experiments, short-term bioassays on seeds and seedlings, which the MPCA refers to as “laboratory hydroponic experiment” and long-term, mesocosm-scale studies on wild rice plants; the field observations comprise a large field survey and a smaller study on root zone porewater profiles. An additional category of studies—controlled laboratory-scale experiments to further elucidate the biogeochemical dynamics of sulfide and the role played by sulfate in those dynamics—to date has played only a small role in the MPCA’s preliminary analysis document (hereafter referred to as the Analysis).

The panel agrees that these categories of investigations were appropriate as a general structure for the wild rice Study; their findings potentially support one another regarding the toxicity of sulfide to wild rice. Taken together, they provide support for the surface water sulfate, porewater sulfide, and iron paradigm that forms the basis for the response of wild rice to elevated sulfate levels. Additional controlled laboratory studies targeted at improving understanding of how sulfide affects wild rice physiology, and how that affects survival of wild rice at the population level, would be useful additions to the Study.

Short-term Bioassays—Laboratory Hydroponic Experiments

The laboratory study on seeds and seedlings made an important contribution by demonstrating that sulfide (not sulfate) is the chemical form that drives observed toxicity in wild rice and is detrimental to wild rice growth. This study also provided information on the concentrations of sulfide where toxicity to wild rice could be expected. These data could be used to inform the concentration range of concern for future studies. The treatment levels for sulfate and sulfide used in the laboratory studies did not cover the lower range of expected exposure and expected toxicity, which limits the precision of the analysis.

The use of EC20 as the no-effect concentration is not considered protective of wild rice. In order to compare the results of this study to other published research, the panel recommends that a more conservative threshold, such as EC10 or EC05, with appropriate uncertainty factors, including confidence intervals, be calculated using a nonlinear regression approach that accounts for the sigmoidal (i.e., logistic) nature of the experimental response data.

Use of measured initial sulfide concentrations in the analysis is not warranted given the declines in concentrations, some of which were quite large, that occurred during the experimental period. The panel recommends use of actual exposure concentrations computed by taking the time-weighted average or geometric mean of measured concentrations. In the current version of the MPCA’s analysis of the Study components (the Analysis), EC values as calculated are too high and cannot be relied upon.

If these experiments can be repeated, the panel recommends the following approach:

- Use of a split design, in which there is a root compartment separated from the shoot. This allows anaerobic conditions in the root zone to be maintained and exposure of the root (but not shoots) to the experimental sulfide concentrations.

- Use of an experimental period of 14 or 21 days, which is standard in ecotoxicology for aquatic macrophytes. Response measurements should be collected at regular intervals.
- To the extent possible, use of the same biological endpoints in the laboratory study as used in the mesocosm and field studies. Decisions on biological endpoints for all the field and laboratory studies in turn will feed into the modeling approaches that can be used. This should be part of the conceptual framework and design for the overall Study and will allow better integration of the study components.
- A larger sample size. A power analysis should be done to determine the number of replicates and treatment levels needed.
- We anticipate that a minimum of six exposure concentrations should be used, with several treatment levels bracketing the current water quality standard.
- Maintaining the exposure concentrations throughout the experimental period. This will be easier if roots are separated from shoots.
- The outcome of these tests can be used to help validate the field studies.

Mesocosm Studies

Mesocosm studies were conducted over three years, with four treatment levels (50, 100, 150, 300 mg/L nominal sulfate) plus a control (approximately 7 mg/L measured sulfate) to characterize the effects of sulfate over the full life cycle of wild rice plants. Each treatment was replicated six times, and some water quality parameters were monitored over the course of the mesocosm study, including sulfate in the water column. A number of parameters relevant to population dynamics also were monitored over multiple years. The mesocosm study is of value because it allowed for characterization of effects across all life stages of wild rice. The statistical analysis in the report relied primarily on linear regression for each of the monitored responses.

The selected nominal concentration range for dosing the mesocosms started at five-fold the current sulfate water quality standard of 10 mg/L (the lowest treatment was 50 mg/L), which limits the ability to interpret possible effects at and below current protection goals. The statistical analysis of effects relied solely on nominal concentrations of sulfate, and not the actual concentrations measured during the studies. The presence of sulfate at approximately 7 mg/L in the controls also presents a significant weakness to the utility of the Study and interpretation of effects related to sulfide exposure. Based on thresholds for wild rice estimated from the field surveys, we conclude that the sulfide values observed in the controls are not toxicologically insignificant. These findings imply that the mesocosm study did not have an effective control for sulfate and sulfide. What constitutes an effective control level for sulfate should be defined.

The panel has the following recommendations regarding the mesocosm studies.

- Clarity regarding the measured responses should be improved; for example, when, how, and why responses occurred should be described in the report.
- Plant responses as they relate to measured, not nominal, sulfate concentrations should be described and modeled.
- The demographic data should be used to develop a population model in order to understand factors influencing population persistence, within the limits of the study conducted. This should help elucidate whether specific measured responses can be linked to population persistence, which could inform assessment endpoints for field monitoring.

- Plant responses as they relate to measured porewater sulfide concentrations should be modeled, similar to what is recommended above.
- If possible, the mesocosm study should be repeated with an effective control for sulfate, and with more treatment levels bracketing the current water quality standard. This could be achieved by reducing replication, but should be done with caution following power analysis.
- The performance of wild rice and water quality conditions in the controls need to be compared to that expected under natural conditions in order to validate the test systems themselves.
- Other, more powerful statistical approaches, for example, mixed-level (hierarchical) modeling, should be explored when analyzing the totality of the dataset.
- Rooting zone profiles should be incorporated into the interpretation of plant response data.

Field Survey

The field survey study was comprehensive and provides a rich data set that can be used to address the question of how, and at what level, sulfate, via sulfide, affects wild rice. The field survey encompassed a wide range of sites that varied in their concentrations of sediment water and porewater sulfate, sulfide, and iron. The panel agrees that the field survey provides some of the best data that the MPCA has available to investigate the relationship between wild rice and surface water sulfate levels. These data also offer a means of determining sulfide levels that are protective of wild rice. Much more analysis should be done on this data set. For instance, additional statistical analysis of the field data, such as (i) determining whether thresholds (relative to sulfate/sulfide or other water quality variables) occur with regard to wild rice cover and (ii) the probability of wild rice occurrence versus porewater sulfide levels, would be instructive. Further, while trends in the data are important, the variability in the data can be equally instructive and should be considered more carefully in the Analysis.

The Study would have been improved if more wild rice sites in the two areas with high surface water sulfate levels in Minnesota were sampled. In addition, further discussion is warranted of field locations where wild rice was absent but that were nonetheless potentially suitable for wild rice growth.

Trends may be clarified if thorough and separate analyses were done on the data based on hydrologic type. The field survey data currently distinguishes between three different types of ecosystems—lakes, rivers, and paddies. Insofar as they support rooted hydric vegetation (such as wild rice), all three categories are considered jurisdictional wetlands. The further separation of sites to include palustrine wetlands (e.g., marshes), to the extent that they occur in the database, as an additional category of wild rice sites may prove useful. The hydrology, chemistry and biota of these ecosystems can vary dramatically; classifying these sites for analysis may reduce variability in the data and reveal differential responses to sulfate.

Initial modeling of the field data using a limited subset of variables accounted for only about 20 percent of the variance in wild rice cover. Further analysis with a more expanded set of variables needs to be conducted. For instance, N and P are important factors in wild rice growth and are affected by sulfate concentrations. Expanding the complexity of the models should help elucidate the interactions of the environmental variables that determine wild rice distribution. The integration of a biogeochemical model using a suitable statistical modeling framework (e.g. structural equation modeling [SEM]) into a demographic model on the wild rice life cycle also is needed to better link the role of sulfate as a driver of wild rice population declines.

Combining the field data with the sulfide concentration-response results from the short-term laboratory (hydroponic) and mesocosm experiments, and the finding of no-effect of sulfate in the former experiments provides weight of evidence that sulfide is the cause of toxicity. If possible, analyses of specific sites focused on the decline of wild rice cover over time with associated water quality variables would provide support to link field results to the cause of declines.

The field study data (Figure 17 in the Analysis) support a working hypothesis of 75 µg sulfide/L in sediment porewater as a threshold for significant toxic effects, although this needs to be confirmed. Preliminary statistical analysis by the panel shows that the threshold level may be as low as 20-50 µg/L. Overall, the panel concurred that the MPCA data support the preliminary finding of a threshold of $\leq$ 75 µg/L, more so than the proposed level of 300 µg/L.

The lack of more quantitative ecological data on wild rice in the field sites hinders use of an otherwise rich data set. Better definition is needed by MPCA of a wild rice beneficial-use endpoint that could be measured in future field surveys. Percent wild rice cover was a semi-quantitative measure; however, stems per m^2, biomass per m^2, or reproductive metrics such as the number of flowering stems could be considered instead as they are more quantitative biological endpoints that may more fully document the vigor of wild rice populations.

Sulfide Toxicity to Wild Rice: Evidence from the Hydroponic Studies, Mesocosms and Field Survey Data

Although the panel agrees that (1) sulfide levels in sediment porewater above 300 µg/L can be toxic to wild rice and (2) the field survey and mesocosm data support this conclusion, the panel does not agree that the three types of studies point to 300 µg/L as the starting point for toxicity effects. The field survey data show negative effects at much lower sulfide concentrations, and results of the short-term bioassays indicate deleterious effects on wild rice seedling growth when sulfide exceeded a range of 150-300 µg/L. Establishing a level of 300 µg/L as a threshold for the onset of effects is not supported by the data in the Analysis and would likely result in ecologically significant adverse effects to wild rice.

Modeling the Control of Porewater Sulfide by the Availability of Sulfate and Iron

The application of quantile regression to model sulfide was predicated on the fact the relationship between sulfide and sulfate is described by a response envelope that suggests that maximum and upper higher quantile ranges of sulfide can be predicted by sulfate concentrations. Quantile regression is useful if the goal is to predict upper limits of sulfide that can occur for a given sulfate concentration. The boundaries can be progressively improved with greater model *specificity*. By including other variables in addition to sediment iron, such as sediment total organic carbon (TOC), quantile regression thus could yield better and less restrictive limits on the amount of sulfide that sulfate is ultimately responsible for producing. This is useful for helping to ensure that unduly low sulfate concentrations are not imposed on sites that are not expected to produce high sulfide levels (*e.g.,* sites with low sediment TOC).

The field survey data indicate clearly that porewater sulfide and porewater iron concentrations are interrelated, and that the relationship is an inverse relationship. This inverse correlation is wholly consistent with the thermodynamics of iron-sulfide equilibria and precipitation chemistry.

If MPCA is interested in better quantifying the relationship between sulfate and sulfide, rather than simply trying to predict the nature of the upper boundary of the response envelope, then the MPCA should consider using structural equation modeling. SEM has the ability to model complex variable interactions, including indirect or mediated pathways.

Preliminary application of SEM to model porewater sulfide indicates that a model that considers sediment iron, sediment TOC, and sulfate as driving variables, and that includes a feedback loop between porewater sulfide and porewater iron is quite successful at explaining the complex link between sulfate and sulfide. By considering both direct and indirect interactions, the SEM model indicates that sulfide concentrations are more sensitive to variations in sulfate than indicated by the quantile regression models.

None of the modeling in the Analysis considers the effect of sulfate on internal loading of phosphorus or ammonium, which in turn has the potential to further amplify the sulfate-sulfide response. Nor does the modeling consider the effect of nitrate loading, which should be considered. SEM is a useful framework for modeling this issue, and the preliminary SEM model could be used to explore and potentially quantify the significance of this issue. The modeling done to date also does not explicitly consider the effects of hydrologic type (i.e., lake, river, wetland and paddy), although the SEM model does implicitly consider this issue through the inclusion of sediment TOC. This question needs to be further evaluated.

Regarding the suitability of acid-extractable iron (rather than porewater iron) in the multiple quantile regression modeling, the panel believes that the larger question is whether multiple quantile regression is the method that best suits MPCA's needs with respect to establishing the relationship between sulfate and sulfide. We conclude that it is not. SEM modeling indicates that the effect of sediment iron on porewater sulfide is mediated by its effect on porewater iron. The effect of sediment iron is therefore *indirect* rather direct, and thus techniques such as quantile regression or traditional multiple linear regression are not as well suited for this modeling problem as SEM.

The panel also concludes from the initial SEM modeling that, consistent with thermodynamics, porewater iron exerts the greatest amount of control on sulfide. This is not to say that sediment iron is not important, as the model shows that sediment iron is important in controlling the supply of porewater iron. In aggregate, these results indicate that both porewater iron and acid-extractable iron need to be included in a model framework that attempts to model sulfide biogeochemistry and that SEM (or other model frameworks that allow for indirect and feedback relationships) would be a preferred approach to quantile regression. The panel recommends that MPCA consider the use of SEM both to model the complex biogeochemistry of porewater sulfide and to explore competing effects of iron and sulfide, as well as other variables on wild rice response.

The panel also has the following comments and recommendations regarding the use of modeling in analyzing the relationships among sulfate, sulfide, and iron in relation to wild rice:

- Model validation is important to demonstrate the ability of the model to robustly predict "out-of-sample" sulfide concentrations. Approaches for validation can include *k*-fold cross validation, and jackknife cross validation.
- Analytical (diagenetic) models offer a more deterministic, process-driven approach to simulate sulfide. The implementation of such models requires large amounts of data, plus many degrees of freedom (coefficients to adjust), and the ability to apply such an approach broadly across a

number of sites is problematic. As a result, the consensus is that such an approach is of limited value.

- The question can be asked whether wild rice modifies the sediment porewater chemistry through oxygen transport. This question can potentially be addressed through SEM.

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

The Synthesis section of the Analysis needs to be strengthened by providing an overview of the Study and its goals, components, and key findings. A true synthesis of the findings of the studies needs to be developed to point out where commonalities exist between Study components, where the studies differ, and where weaknesses in the laboratory and mesocosm studies limit the linkage of results from these studies to the field survey results. More discussion of the ecology of wild rice plants in relation to the issue of exposure to sulfate/sulfide is needed.

A sulfide level of 300 µg/L as a threshold for the onset of effects to wild rice is not supported by the data in the Analysis document. MPCA needs to reconsider this value in the Synthesis based on the weight of evidence from the hydroponic, mesocosm, and field survey studies.

Although the conceptual model described in the Synthesis is qualitatively correct, the current Synthesis goes too far in implying that sulfide concentrations in sediment can be predicted accurately by the multiple quantile regression model based on sulfate concentrations in the overlying water and acid-extractable iron in sediments. Prediction uncertainties are related at least in part to uncertainties about the dynamics of key biogeochemical processes involved in sulfate-sulfide-iron interactions, including the dissolution kinetics of iron oxyhydroxides and precipitation kinetics of solid-phase FeS. Linkage of the sulfate/sulfide iron biogeochemical processes to other processes that may affect wild rice (e.g., phosphorus cycling) and the feedback loops among these processes also need to be developed in the Synthesis. In addition, the panel expressed concern that the role of iron oxide plaques on plant roots received more focus in the Synthesis than merited from the very limited observational data on plaques in the component studies.

The panel recommends that data for lakes, rivers, paddies and possibly palustrine wetlands undergo separate analysis because of the different characteristics of these types of water bodies. If a more comprehensive model can be developed that accounts for the differential behavior of these water body types (e.g., flow characteristics, sediment organic content), a single model ultimately may be sufficient to explain the effects of sulfate on wild rice. Should separate models be required, the Synthesis section likely will need to treat these water body types separately as well.

Further Research

The panel recommends that the MPCA first re-analyze their current data-set in light of the peer reviewers' comments, especially around the studies that examine the role of sulfate and sulfide in wild rice responses (laboratory bioassays and mesocosms) and for occurrence in the field as related to water quality parameters. This re-analysis should include the two studies that received little attention in the MPCA's analysis thus far—the rooting-zone porewater profile study and laboratory experiment on temperature effects. Re-analysis should improve the overall value of the current datasets in understanding the concentrations at which sulfate and sulfide act adversely on wild rice. In light of this re-analysis, further studies can then be considered to fill data gaps, including those proposed by the review panel, as well as others.

3. Summary of Reviewer Discussions

3.1 Laboratory Hydroponic Experiments

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.

Before discussing the charge questions related to short-term bioassays (Charge Questions 1-3), reviewers requested the following clarifications from MPCA:

> ***Question:*** What was the basis for using EC20 and not EC10 or EC05 in analyzing the bioassay data?
> ***MPCA Response:*** EC20 represents the concentration at which growth is reduced by 20 percent relative to the control. EC20 is sometimes used as an estimate for No Observed Effect Concentration (NOEC) in aquatic ecotoxicity tests. MPCA is open to suggestions regarding the use of EC20 or other NOEC levels.
>
> ***Question:*** Were hardness and alkalinity of the test exposure waters determined for any of the assays? If not, do the raw data exist that would allow hardness and alkalinity to be determined?
> ***MPCA Response:*** MPCA took samples of test waters used in the short-term bioassays and submitted them to a lab for analysis, but those data have not yet been analyzed.
>
> ***Question:*** What was the justification and ecological significance for requiring that the mesocotyl length be at least 2 cm?
> ***MPCA Response:*** Scientists performing the experiment found that a seed would sometimes sprout, grow to 1 cm, and then die. If the mesocotyl elongated to 2 cm, there was a high level of assurance that the plant would be viable.
>
> ***Question:*** If EC20 and EC50 sulfide concentrations are determined, is the range between these concentrations viewed as protective or inhibitory to wild rice?
> ***MPCA Response:*** The sulfide concentrations between EC20 and EC50 bracket a zone of transition of effects to seedlings exposed to sulfide. MPCA is interested in hearing the panel's view on this question.

Reviewers then began their discussions. Several reviewers noted that the hydroponic test method represents the worst-case exposure scenario among the studies conducted, since the seedlings were submerged in sulfate and sulfide solutions. Reviewers generally agreed that the physical design of the hydroponic experiment should be improved to more accurately reflect natural wild rice conditions during seed germination and seedling growth. Seeds and roots should be physically separated from the shoots, perhaps using some sort of semi-permeable membrane sealed with a gel. This would allow the separation of seeds and shoots and enable the experimenter to limit exposure of shoots to sulfide solutions, which would more closely mimic natural wild rice conditions. A reviewer clarified that, with the split experiment design, (1) the shoots should be submersed, rather than exposed to air, since wild rice is aquatic for much of its life cycle, and (2) the design enables roots to be exposed to sulfide solutions while limiting the exposure of shoots to the sulfide solution. All reviewers agreed that a troubling component of the experimental design was the fact that the sulfide concentrations decreased during the course of the experiment. This made the results much less valuable. Reviewers decided to defer discussion of alternative approaches until their discussion under Charge Question 2, below.

Reviewers then asked MPCA another question:

> ***Question:*** Did MPCA try to grow plants in a way that would allow the roots and shoots to be separate? If so, what happened?
> ***MPCA Response:*** Researchers spent many months trying to grow plants to about a foot tall so that roots could be immersed in an anaerobic sulfide solution and stem and leaves would be aerial, but were not successful in a sufficient quantity to perform the assays. Researchers never attempted to separate roots and shoots with a semi-permeable barrier sealed with gel, because they did not have enough time.

One reviewer noted that the hydroponic study was the main study of the three that developed effect concentration values, though it is also the least useful due to experimental design flaws and the fact that it did not cover the full wild rice life cycle. In the hydroponics study, 150 to 300 parts per billion (ppb) sulfide was considered to be an effects concentration, though effects were observed below 150 ppb sulfide; this warrants further investigation. Another panelist noted that MPCA made a statement in the Analysis that the lowest sulfide concentration level of 134 micrograms per liter (µg/L) was not toxic, but felt that this statement was too strong; at most MPCA could claim there was insufficient evidence to conclude this concentration was toxic.

A reviewer noted that even though sulfide EC values were identified in the hydroponic study, she did not have much confidence in the numbers because the experimental conditions were not maintained. A second reviewer agreed that the sulfide concentrations identified were not reliable. The assays may give a sense of the range in which effects on plant growth begin, but more research is needed. Also, the assay has only been used a few times and is not standardized, which makes it difficult to have confidence in the results. He recommended that the Analysis provide more transparency about dataset quality assurance/quality control (QA/QC) and the consistency of the observed responses. Another reviewer noted that, even if the sulfide toxicity concentrations were not reliable, sulfide toxicity was clearly demonstrated, which is still an important result. This result allows MPCA to focus on sulfide toxicity as a general paradigm and to view the toxic effects of sulfate toxicity as a mediated response through its effects on sulfide.

There was consensus among reviewers that the hydroponic study should have had more treatment concentrations for sulfate and sulfide and more replicates. One reviewer was disappointed that short-term bioassays included no treatment levels lower than the current sulfate standard of 10 mg/L, or any in small multiples of concentrations above the standard (e.g., 20 or 30 mg/L). Adding these treatment levels would improve understanding of the uncertainty in the results, even if plant growth was not affected. He also recommended that MPCA add a table with the sulfate and sulfide data to the Analysis document.

A reviewer noted that the small number of replicates does not give the study much statistical power. Adding replicates would be easy to accomplish in small laboratory-based experiments. Another reviewer responded that it would be preferable to see more data, not necessarily more replicates. He also wanted to see more treatment levels, especially in the area of greatest response on the curve, since the area of greatest response currently is in the middle of a large treatment range with few data points, and the shape of the response curve is highly influenced by the data in this range. It would also be helpful to perform some form of power analysis to establish a level of confidence in the number of replicates. He suggested that there be more balance between the number of treatment levels and the number of replicates. A third reviewer agreed that the number of treatment concentrations was too low, especially on the lower end, since there are examples in the literature of lower sulfide concentrations (e.g., 10 µg/L) that might be toxic to macrophyte roots. She recommended that, if the hydroponic study is redone, researchers should distinguish between germination and growth.

Reviewers agreed that it would be useful for the hydroponic study to consider wild rice population dynamics when determining effects concentrations. Two reviewers commented that MPCA's use of EC20 and EC50 is not necessarily protective of wild rice. Compounded annually, the effects at EC20 exposure levels could be anticipated to cause a dramatic decline in wild rice populations over several years. The field survey and mesocosm studies are more useful, since they consider all life stages of wild rice, as opposed to only germination and seedling growth as in the hydroponics study. The field study provides more insight into the mechanisms of sulfide toxicity in wild rice plants and considers sulfide moderation by total organic carbon (TOC), iron, and other chemical processes.

A reviewer noted that the hydroponic study results cannot be extrapolated to the field for several reasons: The experiment only takes place over 11 days and, while the seeds can germinate under anaerobic conditions, it is not clear whether these conditions would persist for the plant shoots once the seed has germinated. Another panelist responded that it may not be as important to focus on the life cycle, since wild rice is an annual. She thought that the focus on regenerative dynamics for this test was appropriate; that the 2 cm mesocotyl standard used was reasonable; and that the ten-day window was also reasonable, since this is the period in which the seedling relies on the endosperm. A third reviewer added that, because wild rice is an annual, it is important to consider whether the plant is producing seed. This cannot be determined from the hydroponics results. Seed production carries the population forward from year to year.

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

Brezonik noted that, in their pre-meeting comments, reviewers agreed that it was inappropriate to use the initial exposure concentrations as the operative exposure concentrations for the test. As one reviewer stated: Using the initial concentration is not conservative and will produce an effects concentration that is not protective. Reviewers provided a range of suggestions for what should or could be used instead:

- A reviewer noted that if one were to look at the initial and final concentrations, one would expect to see an exponential decay in concentrations. If this is the case, a useful approach would be to use the geometric mean concentration between the initial and final values and use the geometric mean to conduct a statistical analysis.
- One reviewer suggested that use of the time-weighted average (TWA) would also be appropriate; however, another reviewer said that, because sulfide declined up to 90 percent over the experimental period, the higher concentrations could have been acutely toxic (leading to plant mortality), in which case a TWA would underestimate the lethal concentration 50th percentile (LC50) or LC90 etc. value. A third reviewer commented that the initial concentration did not kill the plants in the hydroponics study; the effect on wild rice was more inhibitory than lethal. He also noted that using the TWA is a standard approach in ecotoxicology. A fourth reviewer agreed that the TWA should be derived from several concentration measurements

over time, and that using the TWA would result in lower values for EC20, EC10, etc. She also noted that a NOEC could be deduced from this type of test, using the ANOVA test, for example.

- One reviewer advocated use of the lowest sulfide concentration measured in order to produce the most conservative effects concentration and be most protective of wild rice, since the plant is an annual.

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?

Brezonik summarized reviewer pre-meeting comments by noting that reviewers were in general agreement that regression analysis is an appropriate approach to derive the EC20 and EC50, but most panel members had some suggestions and concerns. Dr. Pollman had considerable feedback on this charge question.

Pollman analyzed MPCA data to see if he could duplicate MPCA's results and quantify uncertainty. He presented his findings using four slides (see Slides 1 to 4 in Appendix G). The regression model based on the sigmoidal logistic equation is satisfying and appropriate, since an inherent idea in the model is that, at very low concentrations, there is a range where there is no response, then a concentration range where a rapid change is observed, followed by sufficiently high concentrations where no further effects are observed. As shown in slide 1, the regression approach meets these expectations when modeling the change in plant mass as a function of initial sulfide concentrations, and the data fit well. Dr. Pollman's analysis included confidence limits on mean predictions which, for greater transparency, he would like to see in MPCA's statistical analyses.

Slide 2 addressed the question of whether to use initial or final sulfide solution concentration by using the rangefinder study results. The blue curve shows responses from initial exposure concentrations, and the red curve shows the same data using final exposure concentrations. The observed effects are quite different depending on which concentration is used.

In the next slide (Slide 3), the model was used to define the region of maximum response to changes in sulfide additions. This analysis examines the incremental change in plant mass as a function of incremental change in sulfide concentration, which is essentially a derivative of the response curve. The solid black curve is based on initial sulfide concentration and indicates that 300 µg/L sulfide is the area of most rapid response. If this analysis is redone using the final concentration, however, the sensitivity curve is very different. How sensitivity is defined is greatly influenced by the data used in the analysis. Also, an important consideration in logistical regression is the confidence level ascribed to model coefficients generated by the statistical analysis. In this case, the model fits the data well, but there is not a high level of confidence in the model coefficients. Pollman was not confident in using these models for predictive purposes unless more data are collected at more concentration intervals.

Pollman then presented Slide 4 to further illustrate differences between the initial and final sulfide concentrations that occurred during the hydroponics study, using the rangefinder experiment as an example.

Other reviewers agreed that non-linear regression is appropriate, and added a number of comments:

- A reviewer agreed that non-linear regression is standard in these ecotoxicity studies, but was disappointed that confidence intervals were not provided, since this is also a standard practice and would allow greater information about how much confidence to have in the EC20 and EC50 values determined in the study. He noted that in Figure 6 in the Analysis (page 16), which examines weight change, it is unclear how control data were incorporated into the data. Range-finding tests with definitive testing were included, which is not normal practice. The more than 20 percent decline in performance between some of these tests did not give this reviewer much confidence in the EC20 value used to set the concentration at which no effects were observed on wild rice plants. He said that he typically uses R to generate a concentration response with confidence intervals. He also commented that the Analysis needs more data transparency, since it describes a variety of endpoints with responses, but only includes calculations for a few. In particular, he suggested that MPCA include in the Analysis a table showing whether there was a concentration response.
- A reviewer agreed with other panelists that it was difficult to place faith in the models as presented because the actual sulfide concentrations are not known, and it is not conservative to use the initial concentrations. She pointed out that different values were provided for EC20 and EC50 than in other components of the Analysis. More integration is needed between different Analysis components to explain these differences. Extrapolating these data beyond the seedling stage is also problematic, because lower concentrations may have effects later in the life cycle (e.g., seed production).
- Another reviewer noted that there is no basis for using germination and seedling growth as the best endpoints in the Analysis. Without knowing the mechanism of toxicity, it is not possible to know whether the time-weighted average is the most appropriate sulfide concentration or not. As mentioned earlier, if the higher concentrations during the experimental period were acutely toxic, then a TWA would underestimate the lethal concentration value.

Reviewers discussed whether EC20 is an appropriate level to use and what methods are appropriate to calculate effect concentrations.

- One reviewer asked if there are accepted conventions when selecting effect concentrations. Another reviewer responded that toxicology researchers typically accept effects on 5 percent of the population. The relevant question is “What do you want to protect?”
- A reviewer noted that both the endpoint and ecological relevance are important considerations in determining effect concentrations, because there will be a range of sensitivities depending on what endpoint is measured. It is also unclear in this case whether the NOEC speaks to the variability in the test. Researchers typically conduct at least six concentration exposures to determine an accurate NOEC. EC05, which would protect 95 percent of the population, has been used in lieu of a NOEC in Canada. Before setting an effect concentration, researchers need to know the statistical power of the test and what changes can be detected. Another reviewer expressed concern that the Analysis seems to assume that EC20 is equivalent to NOEC, though an effect on 20 percent of the population seems high.
- A reviewer had different thoughts about how to determine the effect concentrations. In combination with other datasets in the Analysis, researchers could conduct a population analysis with life table transition data and then model the wild rice population to look at the sensitivity of the population to different life stages. If population viability is highly sensitive to

this life stage, it makes sense to adjust from EC20 to a more protective level, such as EC10. Researchers would need to determine the consequence of 10 or 20 percent mortality on the likelihood of extinction of a population over a long period, such as 50 years. A reviewer responded by noting that EC20 or EC50 are not of importance in this experiment, since it is difficult to extrapolate the data to other life stages. The important result of this study is demonstrating unequivocally that sulfide exerts toxicity on wild rice.

- Another reviewer responded that wild rice plants are exposed to sulfate and sulfide concentrations during their entire life cycle, making it difficult to extrapolate lab results to the field. In his opinion, regulators should use the more conservative EC to account for uncertainty between lab and field conditions.
- A reviewer emphasized that a sulfide concentration between EC20 and EC50 (hydroponics study) is not suitable as an endpoint for wild rice protection. Instead, referring to Figure 17 on page 37 of the Analysis, he suggested that MPCA consider what the agency would define as success regarding wild rice protection based on percent wild rice cover at sites. The agency could then define an appropriate endpoint based on a specified proportion of sites (or proportion of Minnesota waters) where the wild rice exceeded the desired percentage cover (e.g., 5 percent, 10 percent, etc.).
- Finally, a reviewer asserted that modeling can be used to help decide an appropriate EC level. For example, modeling the decline of a population over years will allow researchers to determine a sustainable EC level. Specifically, researchers can use a Williams test, which is a type of ANOVA analysis, to determine a NOEC if the experimental design meets the proper criteria. The current study would need a greater range of concentrations.

3.2 Utility of the Field Survey Data

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.

Reviewers began this section of the meeting by asking MPCA to provide clarifications in five areas:

Question: Did MPCA consider how sulfate loadings from groundwater may influence sulfate and sulfide concentrations in surface waters and sediment porewaters?
MPCA Response: MPCA recognized that groundwater movements into or out of surface waters may influence surface water and sediment porewater concentrations of sulfate and sulfide, but did not have a reliable method of assessing groundwater movement, especially for sulfate and sulfide. MPCA tried to investigate groundwater flow in 2013 by collecting temperature profiles of the top 50 cm of sediment at five levels. MPCA has not completed analysis of the data. The data are of good quality, but there is low confidence that these data will help determine the groundwater flow at field survey sites. MPCA was operating on the hypothesis that sulfate was diffusing down from surface water into the sediment, which was supported by the quantile regression analysis.

Question: Why were non-parametric methods used for correlation analysis? Why was Spearman correlation used, rather than Pearson correlation?

MPCA Response: Initially MPCA was working with many variables and some were not able to be transformed to normal distribution. MPCA used Spearman correlation for expediency, but is open to suggestions on how to proceed.

Question: What was the seasonal variation in the physical-chemical parameters and how did this affect interpretation of data collected at different times across the various sites?
MPCA Response: MPCA recognizes that seasonal variation may be important and addressed the issue in 2013 by sampling 15 field survey sites monthly in June through September and testing trends of parameters. There were some significant seasonal trends in obvious parameters, such as wild rice cover and floating leaf cover. In surface water parameters, the significant trends were in temperature, alkalinity, pH, magnesium, and sodium. The significant porewater parameter trends were in pH and potassium. There were no significant seasonal trends in parameters important to the Analysis, such as sulfate in surface water, sulfide in porewater, porewater iron, sediment iron, or the acid-volatile sulfide (AVS). MPCA did not adjust the data in any way due to the lack of significant seasonal trends in these parameters.

Question: Did you examine the magnitude of the seasonal parameter trends?
MPCA Response: MPCA graphed out all the season parameter trends and examined them visually. For some parameters, such as temperature, the trend was significant, but trends were very subtle for surface water chemistry parameters.

Question: Some porewater data were collected in situ from a number of lakes. Examining these data would help determine whether there was groundwater excursion of sufficient sulfate to influence porewater chemistry. Do these data exist? Can these data be examined?
MPCA Response: These data (the peeper profiles) were quantified for a number of parameters, including sulfate, sulfide, reduced iron, and pH. MPCA has not fully analyzed the data and interpreted it. Sites where samples were tested were near mining operations, so sulfate seeping through groundwater may have been higher than typical. These field sites may not be typical of the overall experiences of the sites, so would likely not be representative of all sites.

After receiving these clarifications, Brezonik kicked off discussion of Charge Question 4 by summarizing the reviewer's pre-meeting comments. He noted that reviewers generally agreed it was not a problem that MPCA did not use a probabilistic survey when choosing sample collection sites. Reviewers also agreed that the field survey dataset collected is robust and of good quality, with many variables, though most reviewers expressed disappointment that many additional analyses that could have been done with the data were not. Reviewers then began their discussions.

- A panelist explained that the field survey did not need to be probabilistic, because the goal of the study was to capture the entire range of sites available, with enough sites on the extreme ends to determine relationships between water chemistry variables and wild rice effects. Further studies may benefit from a probabilistic survey if the purpose is to characterize the range of Minnesota waterway conditions and the condition of rice in them. The survey collected a large amount of data, including acid-extractable iron, AVS, porewater concentrations of sulfide and iron, and surface water variables. The data are well suited to determining relationships between chemicals; however, MPCA does not appear to have examined toxicity mechanisms. She would like to have seen consideration of other variables, such as eutrophication issues, since sulfate loading tends to exacerbate eutrophication.

- Another reviewer agreed that a probabilistic approach in the field survey would not have been valuable, but mentioned that it may be valuable to examine historical records of wild rice species in Minnesota. This would allow MPCA to eliminate geographic areas where there never were wild rice paddies, which would have cleaned up the dataset. The reviewer was interested in the idea of using discriminant function analysis to examine areas occupied versus unoccupied with wild rice and determine if sulfate/sulfide concentrations, hydrology, or water level fluctuations were important. She agreed that the robust field survey dataset could be further analyzed to gain more insights.
- The next reviewer was impressed by the amount of data collected across a wide swath of parameters, but thought the analyses performed were initial and rudimentary. Although alkalinity, magnesium, and calcium were measured, it does not appear that hardness was measured, despite the fact that Moyle's initial report mentions water hardness as a factor for wild rice performance. (Another reviewer later responded that researchers can readily calculate hardness using the existing magnesium and calcium data.) The field data helped MPCA interpret the lab and mesocosm data; however, the mesocosm data are of limited utility, since the mesocosm was already at 10 mg/L sulfate in the controls.
- A third reviewer agreed that the field survey data were valuable and of high quality. He noted that, to address whether the data are representative of where wild rice occurs, MPCA looked at cumulative frequency distribution (CFD) plots of sulfate and at interquartile ranges to determine how the range in the field survey compared with sites where wild rice was known to occur. The reviewer expressed concern with using interquartile ranges because this approach provides a narrow perspective. He suggested using the 90 and 10 percent quantiles or the 95 and 5 percent quantiles, since the response curve is influenced by values near the upper and lower ends as well as values in the middle. He believed the field survey data were representative, but recommended that MPCA do more work to properly demonstrate this.
- Pollman raised a concern about the field survey data. In Slide 5 in Appendix G, he had attempted to reproduce the CFD provided for the field survey data, but his CFD does not match up with the CFD included in the figure that MPCA generated for lakes. Also, he noted that panel B ("Streams") of Figure 9 in the report includes a CFD based on a survey conducted by the MPCA and Minnesota Department of Natural Resources (MN DNR). The text caption implies this survey was for lakes only, and thus the caption needs to be clarified, or the figure corrected.
- A reviewer noted that other variables (e.g., phosphate, nitrate, etc.) in the dataset may interfere with what happens to sulfate. When analyzing sulfate fate and transport, the full geochemical cycles of sediment porewater and surface water should be considered. The data are included in the appendices, so the analysis can be extended. One concern about the field survey data was that samples were taken in habitats dominated by other aquatic plants, such as water lilies, when wild rice was absent. Water lilies prefer different habitats than wild rice. For example, they grow in muddy habitats and have roots that can grow in anaerobic sediments. The most appropriate habitat for wild rice is covered only by a subset of the current data.
- A panelist commented that the field survey study simply presented the data without any discussion. In contrast, Drs. Pastor and Johnson analyzed and interpreted their data in their reports. The scientists who collected the data likely could have provided MPCA with a useful service by analyzing those data. The reviewer also had a few concerns about figures in the Analysis. It was difficult to discern the negative relationship between sulfide and iron in the two sets of maps in Figure 8A and 8B. He wondered why MPCA did not simply plot sulfide and iron concentration data against each other on a bivariate plot and show the concentration, as was done later in the Analysis. Also, in Figure 9 it was not clear whether the "e" on the X-axis was the

"natural number" 2.718 or if it was supposed to be "E," representing a power of 10. MPCA responded that this should have been "E" for log10(x).

- A reviewer suggested that, though correlation does not prove sulfide toxicity, MPCA may be able to be confident about the effects of sulfide in the field survey data by combining dose-response relationships, eliminating other causes, and using other statistical analyses. Pollman's suggestion to use SEM could help with this. Another panelist agreed, noting that field data are important because correlation cannot prove causation in simple laboratory experiments, and laboratory experiments cannot mimic field conditions.
- A panelist suggested that it would be helpful for MPCA to take anaerobic field samples to understand more about the porewater in order to further study sulfide.

Reviewers then discussed the utility in separating lake and river data in the field survey:

- A reviewer suggested separating lake and river data in order to eliminate variability between the two datasets. She noted that Figure 9 separates lake and river data, using the U.S. Environmental Protection Agency (U.S. EPA) national probabilistic surveys as a comparison dataset to demonstrate that the range of concentrations in the field have been covered. Rivers and lakes behave differently and have different hydrologies. Streams are higher in oxygen, for example, which affects wild rice growth and sulfide generation. Analyzing lake and river datasets separately will allow MPCA to identify common threads in the two ecosystem types.
- Another reviewer suggested that it would also be useful to aggregate the stream and lake data to generate a model that works for both. The biogeochemical variables collected allow researchers to account for differences in hydrologic type. For example, TOC in sediments is different between lakes and streams and is an important variable in determining variations in sulfide concentrations.
- Another panelist agreed that there is utility in separating lakes and streams data, but suspects that it is sometimes difficult to determine whether a body of water is a lake or a stream. Some wide river sections, for example, may behave more like lakes than other rivers. Another panelist responded that paddies also behave differently, so it may be worthwhile to separate rivers, lakes, and paddies.

Reviewers sought clarity from MPCA about what iron analysis was used in the field survey study and when the field survey data would be available. MPCA responded that acid-extractable iron was always used, and said they intended to make this clearer when revising the Analysis. Also, the field survey contractor did not include data analysis in the report because the data were not available at the time the report was due. MPCA had a follow up contract with Amy Myrbo and Daniel Engstrom (Science Museum of Minnesota) to provide this analysis, which was delivered as a consultation (rather than a formal report).

3.3 Mesocosm Experiment

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.

Before starting the discussion of Charge Question 5, reviewers asked MPCA several questions of clarification.

Question: Were oxygen profiles done in mesocosms and were the depths of oxic and anoxic zones measured?
MPCA Response: Yes, measurements were taken at two depths, 5 centimeters below the surface and a few centimeters above the sediment, on a weekly basis. The water was only 25 cm deep. Data were collected but have not been fully analyzed. Oxygen concentrations were similar at the two depths but varied over the growing season. Measurements were almost always below saturation (e.g., 2.5 mg/L in mid-July).

Question: Were any oxygen concentrations measured in the porewater?
MPCA Response: Nate Johnson used voltammetry to measure oxygen concentrations along with other chemical species. A probe was lowered at 1 mm intervals into the water. Starting at 1 mm below the sediment, there was no oxygen.

Question: What were the conditions in the mesocosms over the winter?
MPCA Response: Before winter, the water was drained from the tanks to the level of the ground, since the tanks were buried in the ground. Tanks were covered in dark plastic sheets supported by wire before ice formation. The water froze completely down to the sediment in the winter and there were no animal incursions, ensured by a deer-proof fence with a locked gate.

Question: How was thinning accomplished? How were plants selected for follow up studies? Were the sediment layer and sand layer mixed during thinning?
MPCA Response: Thinning of wild rice plants was done to ensure an even distribution of plants in the tanks. An ideal plant density, 30 plants per tank, was taken from existing literature. This density minimizes root zone overlap and competition for nutrients. Plants chosen were representative plants from six quadrats in the tanks. Thinning began when plants emerged, continued until the floating leaf stage, and was stopped once plants started putting out aerial shoots. Plants were not big when they were thinned, so sediment disturbance was minimal.

Question: How did MPCA settle on the 30 plants left in the mesocosms after thinning?
MPCA Response: The 30 plants were established by trying to thin so that plants were evenly distributed across the mesocosms. Once the plants became aerial they no longer needed to be thinned.

Question: How did wild rice perform (i.e., plant growth and seed production) compared with wild rice populations in the field?
MPCA Response: MPCA did not attempt to compare the wild rice performance in mesocosms with field populations.

Question: Did water quality parameters reflect those in the field?
MPCA Response: Mesocosm exposures were designed to mimic those in the field, but MPCA did not attempt a comparison.

Question: Did repeated plant sampling change sediment conditions?
MPCA Response: Each year six plants were sampled at the end of the growing season after the plants were harvested. A total of 24 plants remained in the tanks.

Question: What was done in the following spring? Were new seeds planted in mesocosms?
MPCA Response: Seeds that were available to germinate in mesocosms were provided by the plants. MPCA made no effort to manipulate this.

Question: Were the 24 plants that remained, after six plants were removed, cut down or left in the tanks?
MPCA Response: The plants were cut off at the roots, keeping the roots in the sediment. The plants were then weighed wet in a lab, with a subset being dried and weighed. All plant matter was returned to the tanks three days after being removed. *(Note: This response was provided a little later in the discussion after MPCA has checked the answer.)*

Question: Had the seeds already fallen off these 24 plants into the mesocosm?
MPCA Response: The plants were removed after seeds were mature and had already fallen off the plants.

Question: Did other plants grow in the mesocosm as a result of seeds in the sediment? If so, what was done with the other plants?
MPCA Response: A few other macrophyte species sprouted, but were weeded out on a regular basis as soon as they were noticed. The weeded plants were removed from the tanks.

Question: How was the identification of the wild rice species confirmed?
MPCA Response: MPCA consulted with Welby Smith, a plant taxonomist with the MN DNR, who follows the taxonomy of Edward Terrell as presented in *Flora of North America*.

Question: In Appendix 2, seeds heads were reported as "eaten." What does this mean? Were they eaten by insects? Mammals? How did this affect the interpretation of the data?
MPCA Response: Seed heads were not actually eaten. Individual plants that had any evidence of grazing by rice cutworms were noted. Only 27 of the 380 plants showed any signs of grazing, and these plants were not correlated with any treatment differences in height or seed production. These data were included with other data in the analyses.

Question: Considering that wild rice is wind pollinated, did treatment placement influence responses?
MPCA Response: Five treatments were randomly laid out in each of six rows, so treatments were in a stratified random placement to minimize bias.

Question: Only 2013 data are included in the appendices. Where are the 2011 and 2012 data?
MPCA Response: The 2011 and 2012 data were sponsored by another entity and MPCA did not have access to them while writing the report. The data are now available and are posted on MPCA's ftp site.

Reviewers then started their discussion of Charge Question 5. Three reviewers agreed that the mesocosm study was an important component in the suite of studies, but that the data collected could have been more fully analyzed and interpreted, since the analysis of the mesocosm data is currently preliminary. A reviewer commended MPCA, noting that mesocosm experiments are difficult to set up and that they are typically considered a success when a plant life cycle can be completed during the experiment. Another reviewer noted that the Analysis discusses sulfide levels in porewater and its links to iron and AVS, but more could be done to link these soil chemistry data with the biological response of wild rice. A third reviewer said that mesocosm studies are useful when looking at demographic parameters that can help develop population models. A strength of this mesocosm study was that it measured seed viability and weight, allowing MPCA to compare seedling growth and survival data from the mesocosm studies and hydroponic experiments.

Reviewers suggested how the mesocosm experimental design could have been improved:

- Two reviewers noted that the lowest sulfate concentrations in the mesocosm study were 7 mg/L, just below the current standard of 10 mg/L. Without a real control concentration, it is difficult to interpret the data, since effects on wild rice may start at lower sulfate concentrations. It may have been better to use a typical sulfate concentration found in natural streams in the region as a control, for example.
- A reviewer noted that sulfide concentrations were measured monthly in 2012 and 2013. Because sulfide sediment levels can fluctuate, it would have been beneficial to measure sulfide levels more frequently.

Reviewers then discussed whether it would make sense for MPCA to focus on sulfide concentration, since sulfide is the toxic agent that inhibits wild rice plants:

- A reviewer suggested that it would be valuable for MPCA to plot mesocosm wild rice growth and seed parameters against mesocosm porewater sulfide. This is because there is a closer correlation in the field study between porewater sulfide concentration and wild rice cover than for surface water sulfate concentration and wild rice cover. Further, Li et al., 2009[1] did a mesocosm study and never achieved a steady state regarding sulfide in porewater after adding sulfate at various concentrations to mesocosm surface water. The amount of sulfate in surface water may thus not be meaningful for mesocosms; the sulfide concentration in mesocosm porewater is, however, meaningful. He also suggested calculating EC10 values from the mesocosm data, since they are more representative of actual conditions than the hydroponic studies.
- Another reviewer responded that the relationship between sulfate and sulfide is very complex. Modeling would help MPCA understand the relationship between certain sulfate concentrations in surface water and sulfide concentrations in porewater.
- A third reviewer pointed to Figure 13 (on page 31 of the Analysis). In this figure, AVS at the highest sulfate concentration is covering the entire vertical axis, indicating that many factors are involved in relating sulfate and sulfide concentrations.
- A reviewer responded that porewater sulfide is the best correlate with seed production and growth. He noted that Armstrong and Armstrong (2005)[2] conducted a study on another type of rice and concluded that the influence of sulfide on root growth can be correlated with a number of physiological parameters. He thought that it would be helpful to understand the mechanism of toxicity on wild rice so that researchers could examine the roots of wild rice in the field and find an effect to correlate with physiological problems.

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[1] Li, S., I.A. Mendelssohn, H. Chen, and W. H. Orem. 2009. Does sulphate enrichment promote the expansion of Typha domingensis (cattail) in the Florida Everglades? Freshwater Biology 54: 1909-1923.

[2] Armstrong J. and W. Armstrong. 2005. Rice: sulfide-induced barriers to root radial oxygen loss, Fe^{2+} and water uptake, and lateral root emergence. Annals of Botany 96: 625–638.

- A reviewer offered an analogy about the relationship of sulfate to sulfide using the relationship between mercury and methylmercury. No one proposes a methylmercury standard, even though this is the hazardous agent. Mercury is what is actually regulated. Sulfide is harmful, but sulfate is what has to be regulated.
- A reviewer responded that quantile regression may be useful to figure out the relationships between the biogeochemical variables rigorously and quantitatively, in order to determine the "outer envelope" concentrations of sulfate that produce acceptable levels of sulfide in porewater.

Reviewers discussed the assumptions about iron in the Analysis and its role in sulfide concentrations. One reviewer noted that page 37 of the Analysis concludes that the variability of sulfide concentrations in mesocosms cannot be explained by variability in available iron, since mesocosm sediments all came from a single source. The reviewer did not think this was an accurate statement, since differences in exposure to oxygen would influence the available iron. Also, page 7 of the Analysis claims that containers may not have reached equilibrium for sulfide, sulfate, and iron reactions (meaning there may be excess iron available to buffer the elevated sulfate, but once the iron is used, a toxic effect may be seen at lower sulfate concentrations). This statement implies that there is a fixed amount of iron in the system, but a continuous source of sulfate, which is not true; there is a continuing source of iron to the sediment just as there is a continuing source of sulfates to surface water. New sediment develops from plants that grow up and die, and dust and other materials fell into the mesocosms from the atmosphere during the summer when they were open.

Another reviewer agreed that the available iron is not fixed, and noted that an important source of iron in the field sites is groundwater. She gave an example of clean water in the Netherlands that had a groundwater iron supply, which precipitated phosphorus. The low nutrient concentrations resulted in a very good quality of water, but iron from groundwater would not counteract immense concentrations of sulfide and is not an endless supply. Another reviewer mentioned the well-documented phenomenon of iron plaques on roots, which may help protect wild rice plants from sulfide toxicity. However, the Analysis based the discussion of iron plaques on roots on a single plant that was analyzed. The sample size is much too small to allow researchers to reach conclusions about the effects of iron plaques on root systems.

Pollman presented some concerns about the ways in which regression modeling was conducted. He thought the data could have been analyzed to produce more useful and powerful results. Currently, the analysis is based on three sets of data, each representing a different year. There were five treatment levels and six replicates for each treatment level. The regression was done by averaging replicates across each treatment level, meaning MPCA constructed a regression model for each year based on five data points, which results in a lower capacity to detect significant effects. MPCA could use all the data in a framework called "hierarchical modeling," which allows the researcher to isolate fixed effects (e.g., relationship between sulfate and a plant growth metric) and random effects imposed by experimental design (e.g., treatment years). Pollman conducted this mixed level modeling with these data, which resulted in a higher degree of confidence about the relationships between variables. He agreed with another reviewer that it would have been better to use actual concentration data, rather than nominal concentrations. He also thought that conducting power analysis would allow MPCA to quantify the level of change that the analysis is capable of detecting in future studies.

Pollman also suggested that the figures in the Analysis that were derived from the Pastor study should mention that these are adjusted R-squared values, which are a more conservative representation of the correlation coefficient determination, rather than the strict R-squared values. He concluded by noting that there are many data that could be used as a separate dataset to validate field study modeling, but have not yet been.

Other reviewers suggested additional ways that MPCA could analyze the mesocosm data or modify the Analysis:

- One reviewer suggested that Table 7 in the Analysis could be modified to highlight where there were differences between years at various treatment levels.
- A few reviewers suggested that MPCA should consider the effects of eutrophication on sulfate and sulfide. Sulfate could potentially accelerate eutrophication because of its ultimate effect of liberating phosphorus. Another reviewer added that it would be important to consider the biogeochemical influences of phosphorus if MPCA would like to use mesocosm data to validate field survey data.
- One reviewer suggested focusing more on wild rice population viability. The mesocosm study allows researchers to observe whether differences observed in seed viability result in changes that lead to extinction or reduced population viability. MPCA should compare the vigor of mesocosm plants (i.e., plant biomass and seed production) to wild plants. The vigor of wild rice plants is likely available in the literature. Another reviewer concurred, and suggested that MPCA use life table-based population simulations. A third reviewer agreed that it would be helpful to see a comparison between mesocosm plants and plants in the field.
- Another reviewer suggested that MPCA pay more attention to outlier results and large variances associated with certain trends rather than simply focusing on the trends. For example, Figure 13 on page 30 in the Analysis claims that sufficient iron was available to precipitate sulfide as AVS, as it was produced yielding an increase in AVS with increasing sulfate treatment concentrations. However, in the figure the range of AVS production at a sulfate level of 300 mg/L encompasses almost the entire response range, and the interquartile range was about half the response range. The text was correct about the trend of increasing AVS, but noticing the trend only tells part of the story.
- A reviewer suggested that it would be useful for MPCA to sample porewater anaerobically. MPCA responded that they already sampled porewater anaerobically by inserting 10 cm rhizomes into the sediment vertically in a way that oxygen was not introduced to the sample.

3.4 Wild Rice in Relation to Sulfate, Sulfide, and Iron

Charge Question 6: Do you agree or disagree with 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?

During this discussion, all reviewers expressed concern that 300 µg/L sulfide may not be protective of wild rice. Several reviewers did not believe that this sulfide concentration was derived in a way that was meaningful or transparent. Reviewers made the following comments:

- One reviewer noted that Figure 17 in the Analysis clearly shows a wild rice response starting at 75 µg/L sulfide and sublethal effects starting as low as 30 µg/L.

- Another reviewer thought that the field survey data were more important than the hydroponics study. He also did not accept that EC20 is a useful value for determining a NOEC. He commented that this charge question included an inherent assumption that 300 µg/L was acceptable.
- A reviewer noted that if data are analyzed using actual concentrations, instead of the initial concentrations, the concentrations in which effects are observed will be lower.

Pollman prepared some brief remarks on the mesocosm modeling and more extensive remarks about the field survey data, and he illustrated his remarks using slides (shown in Appendix G). He noted that the regression modeling results in the mesocosm study included 12 analyses related to response in seed production, eight of which indicated a decline in seed production. The inconsistencies between the results of these analyses need to be explained more fully.

Pollman commented that Figure 17 in the Analysis was a nice analysis of the field survey data. MPCA developed a series of histograms depicting sites with at least 5 percent wild rice cover plotted as a function of porewater sulfide concentrations. Figure 17 shows a sulfide effect on wild rice cover, but Pollman asserted that this assesses the upper limit response, since it ignores declines in rice cover occurring at lower sulfide levels that are not sufficient to drive wild rice cover below the 5 percent threshold. He generated a plot by analyzing the observational data in a different manner, as seen on Slide 6. This plot shows the cumulative percent cover as a function of ambient sulfide concentrations. This was done by sorting the data by sulfide levels and then taking the mean percent cover at each sulfide level. The cumulative percent cover is weighted by the number of observations at each sulfide concentration. There are two ways to construct this curve: displaying the cumulative percent cover from low sulfide concentrations to high, or running the analysis in the opposite direction. The *forward* analysis shows whether there is a general change in percent cover moving toward higher sulfide concentrations. This plot shows that high percent cover is associated with low sulfide concentrations. As the curve moves past 20 µg/L, which is much lower than the 300 µg/L suggested in Charge Question 5, there is a rapid decline in percent cover. The decline evens out by about 50 µg/L.

Pollman ran the same analysis backward (reverse), recalculating cumulative percent cover as a function of sulfide concentration grading from high to low. The reverse curve (also shown in Slide 6) shows that percent cover starts dropping around 50 µg/L sulfide, and that the two curves (forward and reverse) combined suggest a sulfide threshold for inducing reductions in percent cover ranging between 20 and 50 µg/L sulfide. He also ran the same analysis using sulfate concentrations, expecting to see more scatter (Slide 7). This analysis suggests that cumulative percent cover starts to decline around 2 mg/L sulfate. In Slide 8, he analyzed the data using logistic regression, a more conservative approach that models the likelihood of occurrence or absence of wild rice cover. This analysis is only concerned about whether wild rice cover is absent or present (and thus less sensitive to showing effects), plotted against sulfide concentrations. He found that at 500 ppb sulfide the probability that there would be no cover was approximately 50 percent. Referencing Slide 9, he explained that there is a statistical modeling technique that is more appropriate for dealing with the complex biogeochemical relationships involved here (e.g., sediment iron, sediment TOC, sulfide, sulfate, etc.). He extended this model to predict wild rice cover as a function of a complex biogeochemical model, and the model does predict a significant relationship. It is also clear, however, that other factors are important in determining cover as well. (See later discussion for more details about the model.)

Reviewers then discussed what was meant by the word "toxic" in the Analysis. One reviewer noted that "toxic" implies lethal effects, which would mean a loss of individuals from the population. However, because some of the most important effects are sublethal, she suggested that MPCA use another word when describing sulfide effects on wild rice plants. She also recommended that MPCA take a population-level approach in the Analysis, because examination of various life stages is important to understanding the mechanism of toxicity. Cover is an appropriate level of ecological response, but greater mechanistic understanding will come from mesocosm studies. Two reviewers responded that "toxic" often means "injury" as well as "lethal," and therefore thought it was appropriately used in the Analysis. Another reviewer suggested that the term "inhibitory" might be a better choice in this context. A fifth reviewer recommended that MPCA better define all important terms used in the Analysis.

3.5 Control of Porewater Sulfide by the Availability of Sulfate and Iron

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?

In their opening remarks for this charge question, several reviewers commented that multiple quantile regression seemed to be an appropriate method for analyzing the data. One reviewer noted that multiple quantile regression seems suitable for a multi-variable analysis, but had concerns about how the statistical power of the analysis was reported. She noted that researchers will typically include a pseudo R-squared, which was not provided in this Analysis. Another reviewer commented that she agreed with this approach in theory, but thought that MPCA may need to add more variables when running the regression. She also suggested that MPCA compare several statistical approaches to see which is the best fit. A third reviewer commented that multiple quantile regression is an innovative approach to a difficult problem, and has been used widely in economics and ecology when the relationship between two variables is complicated by other factors, which is the case with sulfate and sulfide. A fourth reviewer noted that, while he had written in his pre-meeting comments that multiple quantile regression was appropriate, he had changed his view in light of Pollman's description of structural equation modeling.

A reviewer asked about Figures 22 and 23 in the Analysis. The line in Figure 22 appears to be the same as the red line in Figure 23, but this is not clearly stated. If the lines are not the same, this should be described in the text. If the lines are the same, the statement in the legend of Figure 22 is not correct because the line in Figure 23 represents the 95^{th} percentile, not the maximum.

At this point, reviewers, deferred to Pollman, as the primary expert on the panel in this area. Pollman presented his insights about Charge Question 7 using several slides (shown in Appendix G). He noted a philosophical question that needs to be considered to determine the best modeling approach: Do we care about variables other than sulfate and sulfide in terms of how they affect the relationship between sulfate and sulfide? Sulfide is the toxic agent and there is a relationship between sulfate and sulfide, although it cannot be accurately described by a conventional bivariate regression model. The other approach is to model the outer envelope, so that researchers can be confident that the level of sulfide will be within the envelope predicted by a given sulfate concentration. Quantile regression is a useful modeling approach when the goal is characterize the distribution or boundaries of the dependent variable. Upper quantile values are more meaningful from a toxicity perspective than characterizing variations and mean distribution. Quantile regression modeling allows one to model the separate effects of the independent variable on the distribution of the dependent variable. For example, if one were concerned about the effects of sulfate on low levels of sulfide concentrations, one would find essentially no relationship. However, if interested in characterizing the upper boundary, one would see a clear relationship between sulfate and sulfide concentrations

Pollman introduced some points concerning the mechanics of conducting quantile regression modeling. First, quantile regression is less sensitive to leverage from other variables. When conducting least squares regression modeling involving log-transformed variables, the researcher is making certain assumptions about the distribution of the error term, and introduces bias when back-transforming the model to predict an appropriate sulfate level that would result in a certain sulfide level. To overcome this bias, researchers would need to use generalized linear modeling or smearing factors. Quantile regression modeling makes no assumptions about the distribution of the error terms, therefore bias is not introduced when back-transforming the model.

Slide 10 is based on a bivariate model that examines the relationship between sulfate and sulfide. If there is undue leverage, quantile regression modeling may be more appropriate. Pollman made a leverage plot based on the least squares regression model (Slide 10) and found that two sites introduce undue leverage in the model. Slide 11 uses Cook's D, which is an estimate that determines whether points are putting undue weight on the modeling. He found two outlier points, which would need to be mitigated in order to use least squares regression modeling. These slides support the idea that quantile regression would be more appropriate than least squares regression modeling.

Pollman compared the performance of three models: least square regression, quantile regression (both median and 75th percentile), and robust regression models. He compared these three models for sulfate, sulfate and sediment iron, and sulfate, sediment iron, and sediment TOC. As the variables were expanded, the three models started to converge, resulting in similar slopes to predict sulfide from sulfate levels, although the models did not completely converge. The comparison shows that quantile regression is the most appropriate analytical framework of these three. He noted that quantile regression is useful for determining sulfate limits when other factors (e.g., sediment TOC) are unknown. However, other variables clearly affect sulfide concentrations and MPCA should try to account for these effects in the model. He pointed out a mechanical issue in Appendix G on page 91, which evaluates a model that includes the ratio of log sulfate to log sediment iron, along with log sulfate and log sediment iron as independent variables. His concern is that inclusion of the ratio along with variables in the model violates the assumption that independent variables are truly independent.

Pollman also noted that MPCA needs to be aware of the heteroscedasticity of the error term when conducting least squares or quantile regressions. When calculating the significance of model coefficients, the assumption is made that error terms are independent of the predicted response variable concentration. The plot in Slide 12 shows residual error vs. predicted log sulfide concentration. As values of predicted sulfide concentration increase, the error terms increase, which violates this underlying assumption. This issue can be addressed by using a boot-strapping technique. This will not change the values of the coefficients, but will change the significance of the coefficient terms.

Pollman then discussed issues related to model specificity. Porewater iron is a critical variable in determining porewater sulfide concentrations. The problem is that in traditional regression modeling approaches (e.g., least squares and quantile regression) porewater iron is not truly independent from the dependent variable. Quantile and least squares regression are not the most appropriate models to understand the biogeochemical relationship between sulfate and sulfide. Structural equation modeling allows researchers to model relationships between dependent and independent variables when the relationships are complex. SEM has been used by social scientists and ecologists in recent decades. The dynamic between sulfate and sulfide is complex. Sulfate influences sulfide through a mediated effect by interacting with other variables (e.g., sediment total sulfur). There is also a dynamic feedback relationship between porewater iron and porewater sulfide. SEM allows researchers to look at direct, indirect, and total effects in aggregate.

Slide 13 shows an SEM schematic for porewater sulfide and porewater iron concentrations based on field survey data and includes as forcing functions sediment TOC, sediment iron, and sulfate (independent variables). The model shows a mediated response between sulfate and sediment total sulfur, which leads to an effect on porewater sulfide concentrations. Sediment TOC has a mediated effect on porewater sulfide by interacting with sediment total sulfur and porewater iron. There is a direct relationship between sulfate and sulfide and a reciprocal relationship between porewater sulfide and porewater iron. The model fits the data well. The slope of the direct relationship between sulfate and sulfide is 0.16, but the true influence of sulfate is much greater when its mediated effects are considered. Slide 14 shows how the SEM schematic performs when predicting porewater sulfide and porewater iron concentrations. The observed and predicted data points match up well. Slide 15 shows a bar graph that quantifies the influence of each variable on sulfide. The red bar represents direct effects, the yellow bar represents indirect effects, and the blue bar is the aggregate effects (i.e., sum of direct and indirect effects). The graph shows that the most important variables that affect sulfide are porewater iron, followed by sulfate, sediment iron, sediment TOC, and total sediment sulfur to a lesser extent. Sulfide also exerts a large positive, feedback effect on its own concentrations by driving lower porewater iron concentrations.

Slide 16 shows a plot that compares slopes from three modeling approaches: the SEM schematic, 50th percentile quantile regression, and 75th percentile quantile regression. This plot shows that MPCA would be underestimating the effect of sulfate on sulfide concentrations by using either quantile regression method.

Following Pollman's slides, other reviewers sought clarification on some points. One reviewer asked about the relationship between TOC and sulfide and whether it is directly chemical in nature, or related to bacterial interaction. Pollman responded that this is mainly a biological issue. TOC serves as a substrate for bacterial activity that helps drive the process of sulfate reduction. With less TOC, there is less carbon in the sediment, and less reduction of sulfate to sulfide. Another reviewer asked if there are software packages that produce the SEM schematic. Pollman responded that LISREL is a popular software package, but he uses Stata. Although software is available for SEM, generating the model is still an iterative process. Another reviewer commented that MPCA has a powerful dataset to figure out the complex relationship between sulfate and sulfide. A reviewer asked whether the SEM schematic could be improved with more data. Pollman responded that this is certainly possible, because MPCA's dataset has not been exploited fully to determine which data would be useful. It is very possible that sulfate's relationship with eutrophication could be an important factor to include.

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?

Summarizing reviewers' pre-meeting comments, Brezonik noted that reviewers had mixed responses to this question, and that five reviewers had stated it was appropriate for MPCA to use acid-extractable iron, instead of porewater iron. Reviewers then began their discussions.

A reviewer noted that the acid-extractable iron represents the portion that is potentially available in the system. A second reviewer agreed, adding that MPCA should be most interested in the potential iron available to bind sulfide. She added that Figures 22 through 24 in the Analysis were difficult to evaluate because the legends and text were inconsistent when identifying whether the quantile regression used whole soil iron or acid-extractable iron (or whether these terms were used interchangeably). A third reviewer noted that ecotoxicology studies typically use acid-extractable iron to determine the worst-case scenario for toxicity.

Pollman suggested that it would be more appropriate to use porewater iron, instead of acid-extractable iron. He noted, however, that quantile regression and other single equation regression models generate biased parameter estimates if an independent variable is also influenced by a dependent variable in the model; porewater iron and sulfide concentrations are inversely related. It is clear from the SEM schematic that porewater iron is one of the most important factors influencing sulfide concentrations. In order for quantile regression analysis to be run properly, porewater iron would need to be left out because porewater iron and sulfide are inversely related. Traditional modeling, such as quantile regression, is not appropriate in this situation. To predict accurate concentrations of porewater sulfide, it is important to have accurate concentrations of porewater iron. This indicates that a model framework is needed that can incorporate porewater iron in a way that will not generate biased parameter estimates; SEM has this capability.

Another reviewer commented that he had written his response to this question before he knew about SEM. He thought of two main approaches to addressing the question. The first approach is a simple empirical model. The second approach is to develop a series of linked diffusion-reaction differential equations to describe all the variables in the system (e.g., sulfate, sulfide, TOC, sediment iron, etc.). The second option is completely unrealistic for regulatory purposes because there would be too many coefficients with high levels of uncertainty.

The same reviewer noted that Johnson (2013) conducted a porewater data analysis with data he had gathered. His analysis showed that iron and sulfide were in quasi-equilibrium with an amorphous iron sulfide phase. He found that high sulfate treatments were significantly oversaturated, which suggests that sulfide was being produced in the system at rates faster than solid phase iron sulfide could form. Equilibrium between solid phases and dissolved phases cannot be assumed since precipitation of solid phase crystalline forms is not instantaneous. The reviewer encouraged MPCA to extend Johnson's analysis, examining the extent to which equilibrium conditions are maintained between dissolved iron and dissolved sulfide. One additional complexity is that a portion of the ferrous ions in water may not be free ferrous iron, but maybe be complexed to dissolved organic matter. This type of further analysis would be complementary to Pollman's suggestion of using SEM.

One reviewer expressed the opinion that Charge Question 8 seemed post hoc in that MPCA had not conducted an explicit experiment to address this question. A reviewer replied that he was not sure what a logical choice would be for another experiment because the current field survey data have not been exhaustively analyzed. The first reviewer responded that data describing the mitigating effects of iron on wild rice growth are completely missing. It would be useful to have an experiment that examines whether iron would mitigate the ecological effects on wild rice of added sulfide levels. Additionally, current models do not account for the effects from oxygenated rhizospheres and iron plaques on root systems. MPCA needs to understand the mechanism of toxicity better before claiming to understand how iron mitigates sulfide stress. A reviewer responded that there is a substantial amount of literature about interactions between sulfate, sulfide, and iron. Another reviewer noted that these studies are on perennials, and wetland annuals have not been studied in any detail. For a regulatory standard it would be inappropriate to extrapolate from other species. A reviewer noted that, in his literature review before the meeting, he noticed studies that mentioned iron toxicity in other rice species. He wondered whether iron toxicity could show up in SEM schematics. Pollman responded that the data are not currently available to use SEM to address physiological questions in wild rice. This is one weakness in the field survey dataset. He pointed out, however, that the Pastor mesocosm data address porewater chemistry and sediment particulate chemistry, and could be useful in further testing the accuracy of the SEM model.

MPCA clarified that their interest in iron was not post hoc. They collected iron data in the porewater, surface water, and sediment. However, they did not conduct experiments using these data because they were not sure which experiments would be appropriate at the time and time was very limited.

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

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?

At this point in the meeting, reviewers referred to Figure 17 from the Analysis document to clarify the presentation of histograms relating to plant cover. In Figure 17, porewater sulfide is on the x-axis, although it was noted that plant cover could be graphed against other variables on the x-axis (e.g., sulfate concentration in surface water, concentration of iron in porewater). MPCA staff later provided three such histograms for distribution to the peer reviewers: one of plant cover against surface water sulfate and two of plant cover (greater than 2 percent and 5 percent, respectively) against porewater iron (see Appendix H). With respect to the other variables, as shown in the histograms, plant cover decreases as sulfate increases, and plant cover increases as porewater iron increases. One percent cover corresponds to two to four stems per square meter.

Reviewers then began discussing Charge Question 9. Summarizing reviewers' pre-meeting comments, Brezonik noted that there was general agreement that it was appropriate to focus on sulfide although some reviewers suggested additional variables to consider. Reviewers then began their discussions.

- One reviewer noted that there should have been a more concerted effort to include other variables in the Analysis. SEM modeling incorporates sulfide interactions with iron. Any focus on sulfide should include its relationship with iron. Also, the Analysis includes a reference to Moyle's hypothesis about magnesium. It would be useful to analyze the effect of magnesium on wild rice, which could be done using the extensive field survey data. Another reviewer supported the idea of including magnesium in the Analysis.
- A third reviewer agreed with the focus of sulfide in porewater, but noted that the complexity of the system should be addressed more fully, especially regarding the sediment system. For example, sulfate plays a role in driving eutrophication through increased rates of decomposition of organic matter and the liberation of nutrients. Also, sulfide binding iron can lead to phosphate release by iron which becomes bio-available in the system. She also suggested examining the mercury cycle. The methylation activity of sulfate-reducing bacteria generates methylmercury, which may be important.
- Another reviewer commented that the emphasis on sulfide in porewater was right in general, although he would have liked to see pH dependency recognized and does not think that the various acid-base forms of sulfide should be considered equally toxic. For example, the sulfide

water quality criterion for protection of fish and other aquatic life in U.S. EPA's 1986 Gold Book[3] is 2 ug/L undissociated H_2S (pH dependant). The effect of pH on reducing variance in percent rice cover could be tested statistically. Nitrogen is another factor that should be considered. A study by Sims et al. 2012[4] shows that increased nitrogen results in increased stem production, flower production, seed production, seed biomass, and more seedlings in wild rice.

- Dr. Arts agreed with the focus on sulfate and iron and how they interact with sulfide, but wanted to see an overview of the processes that play a role in wetlands. She presented a figure (see Appendix I) that showed all the chemical processes that are part of the decline of the water soldier (a macrophyte in the peatlands) in the Netherlands. Iron is mainly supplied through the groundwater in this part of the Netherlands, and the sediments are anaerobic. Sulfate is reduced to sulfide and bicarbonate is generated, which stimulates mineralization. Once mineralization occurs, nutrients become available, including phosphate and ammonium, and iron is bound to sulfide. If there is a deficiency in iron, free sulfide is produced, which is toxic to the roots. If iron is bound to sulfide, then phosphate is generated and will diffuse throughout the overlying water, leading to eutrophication. Minnesota wetlands may be different, but it would be helpful to develop a diagram that describes what happens to sulfate and sulfide in relation to all biogeochemical cycles. Another reviewer responded that this conceptual diagram shows that phosphate release is stimulated by sulfate, and there is a feedback loop that reinforces this process. Phosphate stimulates primary production, which stimulates increased accretion of organic matter in sediments, which stimulates higher rates of microbial activity, which stimulates faster rates of sulfate reduction. Another reviewer added that a German limnologist named Einsele and an American limnologist named Hasler published a paper in the late 1940s relating increasing sulfate concentration to increased internal loading of phosphorus, but that this relationship had been largely ignored during the era of major eutrophication research in the decades of the 1960s and 1970s.
- A reviewer commented that iron may be a limiting nutrient in wild rice growth, which is one interpretation of Figures 15 and 16 in the Analysis. He said that this is consistent with the histograms that MPCA provided earlier. The histograms plateau, suggesting that iron is a limiting agent, but is not toxic to the plants.
- A reviewer responded that nitrate may also be limiting in the system. Another reviewer noted that the reviewers should use caution when considering the histograms, because they do not represent a change in biomass and focus simply on presence or absence of wild rice.
- A reviewer commented that he thought it was appropriate that MPCA did not include mercury in this study, because there is no indication that mercury plays a role in wild rice growth. He had spoken with one of the co-investigators previously, Dan Engstrom, who is involved in another study by the MN Department of Natural Resources that is studying the question of whether sulfate is relevant to methylmercury cycling. MPCA responded that they had found funds separate from those for the wild rice study to collect mercury data in the mesocosm study, but not the field study. MPCA has not yet analyzed the data.

Two reviewers discussed the need for more studies relating sulfate to biological effects on wild rice. The variable is currently wild rice cover, which is a crude variable to use to understand the actual effects on plants. Cover does not describe population viability, and other variables could be more sensitive. A

[3] USEPA, 1986. Quality Criteria for Water. Office of Water Regulations and Standards. EPA 440/5-86-001. U.S. Environmental Protection Agency, Washington, DC.

[4] Sims L., J. Pastor, T. Lee, and B. Dewey. 2012. Nitrogen, phosphorus, and light effects on reproduction and fitness of wild rice. Botany 90: 876–883.

reviewer agreed with this point, adding that the field survey and mesocosm studies include the full life cycle of wild rice, and so are likely more relevant than the hydroponic study, which does not deal with distribution of wild rice per square meter or seed production. He also suggested that MPCA develop an endpoint related to the beneficial use of wild rice, such as seed production, or to the percent of state waters where wild rice cover exceeds a certain amount (e.g., 5 percent, 10 percent, etc.) related to human and wildlife use of this natural resource.

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.

Before starting discussion of Charge Question 10, reviewers asked MPCA for two clarifications:

> ***Question:*** Have nitrogen and phosphorus been ruled out as factors affecting the distribution of wild rice?
> ***MPCA Response:*** No, MPCA collected nutrient data in the field survey. These nutrients clearly influence distribution of wild rice, either directly or indirectly, but the Spearman correlations for the field survey data did not show a correlation between phosphorus or nitrogen and surface water sulfate concentration or plant cover.
> ***Question:*** Relative to the availability of iron at a site, were groundwater inflows taken into consideration?
> ***MPCA Response:*** When iron concentration was high at a site, a leading hypothesis was that this was due to groundwater flow, but there were very few data to address this question. MPCA collected a temperature profile in 2013 from the top 50 cm of sediment in an attempt to detect groundwater flow, but the agency has not fully analyzed the data. The data seem to be of high quality, but MPCA is not sure whether detection of groundwater flow will be successful.

Then, summarizing reviewer pre-meeting comments, Brezonik noted that, while reviewers generally agreed that the Synthesis (i.e., pages 51 and 52 in the Analysis) needed more work, they had a wide variety of comments.

Reviewers discussed the order of the Synthesis and the integration of the three studies. A reviewer noted that the response of wild rice is the key endpoint. The field survey study gives a broad sense of the variables that affect the presence and absence of wild rice in the field. She suggested that it would make sense to describe the field survey first and then describe the hydroponic and mesocosm studies to examine how plants are affected at individual sites and individual plants. This would facilitate integration between the studies that is currently lacking in the Synthesis. She also noted that the Synthesis currently examines effects on wild rice in the same way in all three studies, though there are different logical aims for the three studies.

A second reviewer agreed that MPCA needs to integrate the studies and suggested that two key findings in the studies could be used to shape this integration: (1) sulfide toxicity related to wild rice growth was in the range of 200-300 µg/L in the hydroponic study; and (2) the mesocosm and field survey data are consistent with these results, though wild rice seems to be more sensitive to sulfide than indicated in the hydroponic study. One way to integrate the study components more logically would be to make a schematic diagram that shows the basic tenets of the conceptual model. He also advocated for a second figure that shows how the experimental pieces fit into the conceptual model and inform the various conceptual model components. Another reviewer suggested questions for MPCA to address when integrating the studies:

- What did we learn from these studies?
- How can we draw conclusions from the study results and use them to inform a reasonable sulfate standard?

Reviewers also commented that the Synthesis relies too much on the hydroponics study and is too focused on biogeochemistry, as opposed to biological effects on wild rice. One reviewer reiterated that he thinks MPCA should analyze the field survey data in more depth. Another reviewer was surprised by the lack of discussion of effects on wild rice plants. If MPCA is going to make site-specific standards based on iron and sulfate concentrations and compare these conditions with the hydroponics study to determine a NOEC, the sulfate concentration determined will not be protective. He noted that the panel has already discussed issues concerning the hydroponic experimental design and suggested that MPCA use the Bradford Hill criteria when writing the Synthesis.

Some reviewers mentioned the utility of separating rivers, lakes, and paddies in the Analysis, because these are different systems under different conditions, especially relating to eutrophication. For example, higher levels of phosphorus may be needed for growth in the paddies than in lakes and rivers.

One reviewer presented ten concerns he had with the Synthesis:

1. Toxic effects above EC20 need to be modified. MPCA should be careful not to imply there is a NOEC at EC20.
2. The statement in the Synthesis that the responses of wild rice from the field survey and mesocosm study are consistent with hydroponic study is misleading at best. The field survey results show effects starting at 75 µg/L sulfide.
3. The statement that sulfide immediately reacts with iron and precipitates out is wrong or at least not substantiated by data. There is a disequilibrium between solid phase iron sulfide and the ionic constituents, ferrous ion, and hydrosulfide ion. The literature on precipitation kinetics of minerals shows that these types of reactions are slow to occur in the natural environment, and so it cannot be assumed that sulfide will immediately react with iron.
4. Oxygen leakage in the roots is viewed as a mitigative phenomenon that could cause sulfide reoxidation. He did not know the importance of oxygen leakage, but data from Nate Johnson's report indicates it is not important. There was some ambiguity in these results, however, and more research is needed.
5. Sufficient data are lacking to draw conclusions about the effects of iron plaques on root systems.
6. Line 1237 uses the word "immediate," which is inappropriate because of the slow kinetics of solid phase dissolved interactions.
7. The statement on line 1239 says that ferric oxyhydroxides are available to bacteria to reduce to dissolved iron. If sulfide is present, the oxidized iron should be used first by microbes. One could argue that, if sulfide is present, there should be no ferric oxyhydroxides. This may be a simplistic view, since natural systems are not homogenous, and there are reservoirs of oxidized iron.
8. The discussion of the kinetics of iron sulfide formation needs to be modified to consider slow kinetics.
9. The last paragraph goes too far in implying that multiple quantile regression model can accurately predict concentration of sulfide in porewater, because the kinetics of formation of amorphous ferrous sulfide are uncertain.

10. The conceptual model seems qualitatively correct, but it presents an overly optimistic impression about our ability to predict whether toxic sulfide levels will occur in a given wild rice stand from the sulfate concentrations in surface water and acid-extractable iron in sediment.

A reviewer commented on iron limitation. He said that the current conceptual model precludes the joint occurrence of high porewater iron and high sulfide concentrations. The caveat to this is iron limitation. The model indicates that the availability of iron jointly with sulfate dictates the amount of AVS that can form in the sediments. At low levels of iron, low concentrations of AVS will form, unless there are high sulfate concentrations to drive the reaction forward. However, there are currently no data to suggest that iron reservoirs will be depleted or overwhelmed in the presence of high sulfate concentrations.

Pollman disagreed with the type of analysis conducted in Figures 20 and 21 in the Analysis, in which the ratios of sediment AVS to iron and TOC are compared as a function of sulfate. He thought that this was an indirect way to analyze a potentially important relationship. He suggested that MPCA can construct better models by modeling sediment AVS directly and using sediment iron or sediment TOC as an independent variable, instead of as a component of a dependent variable. The results from this model would be more interpretable. To demonstrate this concept, Pollman constructed a linear regression model where he regressed AVS as a function of sulfate and sediment iron concentrations. The figure shown in Slide 17 (see Appendix G) shows some results. The left panel compares observed vs. predicted values. The lack of curvilinearity in the model (except at the very far left portion) means that relationships between observed and expected values are linear using these log-transformed variables. The results in this panel alone suggest that iron is not limiting[5] of AVS based on existing data. This point is further underscored by constructing augmented component plus residual plots (ACPR), shown in the next two panels. These plots support the assertion that there is no evidence of iron depletion limiting the formation of AVS, although the model does indicate that AVS formation is a joint function of the availability of sulfate and sediment iron.

Reviewers also discussed the importance of oxygen loss from the roots into the sediment. One reviewer did not see any evidence that this process would help protect wild rice from sulfide toxicity. Another reviewer pointed to studies in the Florida Everglades (Li et al. 2009[6]) that found oxygenated sediments around root systems to be an important factor in predicting whether a plant would be tolerant to sulfide. In particular, researchers compared native sawgrass and invasive cattail species. Cattails are much better at oxygenating sediments and thus detoxifying sulfide (via promoting oxidation of sulfide to sulfate) and were about a third as sensitive to sulfide as sawgrass. Another reviewer supported this view and added that there are macrophytic plants that can live in anaerobic conditions because of their ability to release oxygen to the sediment. A third reviewer noted that wild rice is an annual plant, with a small root system and an emergent shoot for only a short period in its life cycle. These factors make it doubtful that oxygen release from the roots would be as important as in cattails. Cattails are good at pumping oxygen to their roots partially due to their massive system of rhizomes. The physiology of wild rice plants may make them more vulnerable to being outcompeted by other plants as eutrophication increases. Another reviewer responded that wild rice needs to grow from seeds every year, and that the seed germination stage may be vulnerable to eutrophication. She recommended that MPCA consider this sensitivity in any future physiological studies.

[5] It is important to note that the reviewer is using the term "limitation" to indicate whether or not the supply of iron has become wholly depleted or exhausted.

[6] Li, S., I.A. Mendelssohn, H. Chen, and W. H. Orem. 2009. Does sulphate enrichment promote the expansion of Typha domingensis (cattail) in the Florida Everglades? Freshwater Biology 54: 1909-1923.

3.7 General Questions

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

Reviewers generally agreed that the study components chosen were appropriate and satisfying, while also recommending specific changes to some of the studies or resulting data analyses.

- One reviewer noted that it is not easy to design experiments or get approval to conduct them, and congratulated MPCA for accomplishing both. He has conducted similar studies in Florida, and even though he thinks MPCA should redo the hydroponics study, he noted that MPCA's field survey data were very strong.
- Another reviewer commented that the field and mesocosm studies were the most useful, though MPCA may be able to expand their use of the root zone profiles and sediment incubation studies. The hydroponic study was useful to show sulfide toxicity, but MPCA should not use the NOEC values found in the study. She also noted that it is very difficult to plan which studies will be more informative ahead of time, and that the panel appreciated the challenges faced by MPCA.
- Another reviewer commented that the scope of MPCA's work was sound. She emphasized the need to make use of the mesocosm and hydroponic data to start getting a handle on population dynamics of wild rice, even if the panel recommends making changes to those studies. She noted that larger population sizes in wild rice have been associated with lower outbreeding and higher reproductive output. The reverse is also true; smaller populations are associated with more inbreeding and less reproductive output. This is why it is important to understand the vulnerability of wild rice populations and whether certain sulfate concentrations may result in population decline. She said that using presence/absence of wild rice occurrence is not the best system, and that a population system would allow for earlier detection of wild rice decline.
- One reviewer said that she supports the approach of moving from simple laboratory experiments to higher ecosystem experiments. She suggested that if questions remain in the Analysis about linking sulfide toxicity to a plant response, MPCA should return to the laboratory and conduct focused experiments to answer these questions.
- A fifth reviewer commented that the five study components were appropriate, though in retrospect some additional experiments would have been beneficial to MPCA (e.g., effectiveness of wild rice plants at oxygenating surrounding sediments, plant physiology studies, etc.). Scientific investigation often leads to the need for additional experiments. This is simply the nature of science.
- A sixth reviewer noted the logical progression of the experiments chosen. The hydroponics study isolated the sulfate and sulfide effects, the mesocosm study introduced sediments and showed a relationship between increasing sulfate concentrations and a plant response to sulfide, and the field survey helped determine the biogeochemical link between sulfate and sulfide. The field results also showed that it is possible to link wild rice occurrence with sulfide and sulfate. From a statistical modeling perspective, the field data were robust and had a large number of samples collected, which allows for the development of reasonable models and the use of SEM to bear on a complex problem.

Reviewers discussed the importance of choosing a logical endpoint. One reviewer noted that a sulfate concentration water quality standard would be highly dependent on which wild rice endpoint was chosen. Another reviewer suggested that each component in the study series should be designed to help MPCA achieve the chosen endpoint. A third reviewer responded that choosing an appropriate endpoint has to consider the cultural, aesthetic, and economic value of wild rice, which might require the involvement of social scientists and/or other experts. He thinks that the general desire is to see sufficient seed production and wild rice cover, and that MPCA can make an effort to quantify this. The U.S. EPA's framework for risk assessments involves setting an endpoint and then knowing the effect measures. That information is not provided, which makes it difficult to determine whether any proposed change to the current standard is of high quality. At this point, MPCA clarified that wild rice production was not limited to human use, and did also include wildlife use. MPCA noted that the agency is making an effort to gain more specificity in the rule about beneficial use, so that permits do not have to be evaluated on a case-by-case basis, as is currently the case.

Reviewers discussed the idea of taking sulfate samples at all the sites in two high sulfate regions in Minnesota. One reviewer noted that there are only eight such sites in southwestern Minnesota. It would be useful to find out if there are individual sites in this region that do not have high sulfate concentrations, and whether these sites have wild rice cover. He suggested that it would be useful to examine specific sites with high sulfate concentrations that do have wild rice cover. There are about 40-50 sites in south central, southeastern, and west central Minnesota where sulfate concentrations are 12-34 mg/L. It is important not only to evaluate trends, but also to examine the exceptions to those trends. Another reviewer added that there are two subspecies of wild rice in Minnesota, and so it would be worthwhile to determine whether one subspecies is better at living in high sulfate environments than the other. MPCA then stated that the field survey data include subspecies data, and thus it would be possible to code this as a variable in their analysis.

Reviewers offered a few ideas for additional analysis. One reviewer suggested that commercial production statistics may be useful in evaluating long-term trends in wild rice production, which would be geographically specific**.** Another reviewer suggested that these data could help inform an investigation of historical wild rice decline. A third reviewer responded that this may not be reliable, because a decline in production may be related to over harvesting, since wild rice is an annual that self seeds every year. Another reviewer suggested looking at the wild rice seed bank to inform investigations into the sustainability of wild rice populations.

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

Reviewers began the discussion of Charge Question 12 by commenting on the two study components by Nate Johnson, the root zone profiles and sediment incubation experiments, which were not featured in the Analysis. One reviewer commented on Johnson's use of thermodynamics to determine whether supersaturation of iron sulfide phases is occurring. He thought that the relationship between ion activity products (IAP) and K_{sp} and how these translate into under-saturation or super-saturation could be better described. The text concludes that super-saturation is occurring, but he is not convinced about that for two reasons. First, depending on the actual FeS solid phase modeled, K_{sp} values vary by approximately two orders of magnitude. Second, porewater contains dissolved organic carbon (DOC) which complexes tightly with Fe^{2+}; thus, the free concentration of Fe^{2+} is not known, which is a necessary component in IAP calculations. Another reviewer commented that sulfide concentration data in the root zones should be incorporated into the evidence to determine causality and to link to effects data. These data can be used to show the concentrations at which plant effects are seen in relation to surface sulfate concentrations.

After this discussion, reviewers were asked for any additional comments. One noted that MPCA should be very strict in the QA/QC of the data they use when setting standards. Confidence in the numbers is very important, so the QA/QC of the data should be transparent. There is also nothing wrong with acknowledging that not all the data support the hypothesis. It is good to recognize the limits of the studies. He also suggested that the Analysis should engage much more extensively with existing literature. Confidence in MPCA's findings would be increased by comparing it to similar work. Another reviewer wanted to address a point about the panel's interpretation of the data. She pointed to a study by Pillsbury and Maguire (2009)[7] which examined historic wild rice wetlands. The study found that the greatest losses in wild rice were associated with higher levels of nutrients, and thus more nutrients are not always beneficial to wild rice growth. A third reviewer suggested that MPCA use data from commercial paddy production to determine if relationships between sulfate concentrations and overall productivity exist. He noticed in Figure 20 that there is a wide range of sulfates in the water from low natural to high natural. MPCA clarified that most commercial paddies are not in natural wetlands, but are constructed on an upland. Commercial operations are under no obligation to report data to MPCA, but MPCA can always ask. This may be a useful analysis.

A fourth reviewer suggested that it would be beneficial to include a map in the Analysis that shows the distribution of field sites and sulfate concentrations. This would help interpret Figure 1. He pointed out that this map exists in Myrbo (2013), but not in the Analysis itself.

Pollman made several recommendations about how MPCA could improve their modeling and analysis:

- Figure 13 on page 31 of the Analysis gives the results of the mixed model used to test whether seasonality was important. The caption indicates that the sample period was modeled as a fixed effect and the treatment was modeled as a random effect. He suggested that these effects be reversed.
- There is value in using mesocosm data in conjunction with field survey data as a validation exercise.
- On page 17 of the Analysis, the 2011 field survey data were separated from data collected in 2012 and 2013. MPCA should incorporate these data together. To test whether use of different labs in 2011 affected the data, he used a dummy variable to separate the 2011 data and then incorporated this variable into some sulfide regression modeling. No significant effects were observed.
- Page 38 of the Analysis provides a discussion of oxygen dynamics at the sediment-water interface. The hypothesis given is that oxygen concentration is higher at the sediment-water interface because of groundwater delivery of oxygenated water, but there is no evidence to support this. Groundwater should actually have depressed oxygen levels. The elevated oxygen could be due to turbulence effects or sediment oxygen demand. Pollman displayed his point on Slide 18 (see Appendix G), which shows a box plot of sediment organic carbon content at lakes, streams, and paddies. Stream sediment has lower organic content. This translates to lower sediment oxygen demand, which is consistent with higher oxygen content at the sediment-water interface in streams.

[7] Pillsbury R.W. and M.A. McGuire. 2009. Factors affecting the distribution of wild rice (Zizania palustris) and the associated macrophyte community. Wetlands. 29(2):724-734.

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.

Reviewers reiterated their general agreement that it would be beneficial to more fully analyze the field survey data, to change the experimental design of the hydroponic studies, and to understand the effects of sulfide on physiology to determine a mechanism—all topics they had discussed in more detail in response to previous charge questions.

One reviewer emphasized the importance of separating the lake and river data for analysis and reviewing existing literature in more depth. Seed mass in rivers is 41 percent lower than in lakes for wild rice. Wild rice may behave differently in lakes and rivers, or there could be genetic differences. Additionally, added another reviewer, Figure 6 of the Analysis shows wild rice growing in streams with high concentrations of sulfate. A third reviewer responded that there are differences in hydrologies, loadings, chemistry, biological responses, and other factors in lakes, rivers, and paddies. She noted that these differences are the reason the U.S. EPA collected data independently for rivers and lakes in their field surveys.

A reviewer suggested redoing the hydroponic studies and growing plants to 30 cm, in order to keep the roots and leafs separate. Another reviewer offered that it would be useful to conduct laboratory experiments during a full life cycle of the plant to determine sulfide effects on seedling germination, seed production, seed biomass, root zones, etc. These laboratory experiments could be based on sulfide data from the mesocosm experiments. A third reviewer noted that it would be very difficult to get a wild rice plant to grow for an entire life cycle in the laboratory; the mesocosm approach is the most controlled environment in which to examine a full life cycle. Growing the plants in a greenhouse would still not make them viable for laboratory experiments. Growing a plant large enough to use is constrained by the space required, since it would require a large quantity of media flow. Another reviewer suggested sinking a tank in a paddy to obtain a long root zone. This would also allow the plants to seed at the rate they naturally grow. A reviewer responded that, taking this approach in the Netherlands, she had been able to manipulate nutrient levels and other variables and to get the plants to reproduce, which allowed researchers to monitor the life cycle. Another reviewer suggested that it might not be necessary to grow long plants because seedlings could be kept in an anoxic layer separated by a film and a water layer above that. He thought that it would not be insurmountable to maintain a constant flow in order to keep concentrations constant.

A reviewer reiterated that it was important for MPCA to have a logical endpoint in mind when designing further studies. The endpoint should be both measurable in the field and determinable from experiments. An endpoint would not be meaningful if it could not be measured in the field. A reviewer responded that one logical endpoint would be to have positive, or at least stable, wild rice populations; any endpoint would need to ensure sustainable seed production and seedling germination. A reviewer asked if the mesocosm data could be used to make these types of evaluations for endpoints, and a reviewer responded that the mesocosm data would be a nice start, but MPCA should consult the literature for other additional values, which would then need to be field verified.

Reviewers also discussed additional field survey considerations. One suggested that additional field data plus the weight of evidence could allow MPCA to establish causality of sulfide on plants. Another reviewer noted that it would be important to compare concentrations of sulfate in surface and groundwater to determine the source of sulfate; establishing the source of sulfate will be important for conceptualizing the whole system. She also wanted MPCA to extend its focus on eutrophication, which may be an important factor in the reduction of wild rice in Minnesota.

A reviewer suggested that MPCA conduct power analysis when conducting any further studies, in order to optimize the usefulness of their experimental design and results. He recommended that the agency consider how to treat the data, either using a traditional ANOVA test to look for treatment level effects or by trying to model a response. If MPCA chooses the latter and uses a non-linear model (i.e., regression fit to the logistic equation), it will be important to obtain more data points in the treatment levels at the critical area of response, as opposed to more replicates. Also, it is possible to account for only about 20 percent of the variance observed in the field survey data, which indicates that other variables are important in wild rice occurrence. MPCA can exploit their field survey measurements to find other variables that could explain some of this variance. It will also be important for MPCA to conduct model validation (e.g., jackknife analysis) to ensure that results are able to withstand close scrutiny in statistical analysis.

Appendix A

Charge to Reviewers

Charge for Peer Review of “Analysis of the Wild Rice Sulfate Standard Study”

Introduction

The Minnesota Pollution Control Agency (MPCA) has contracted with Eastern Research Group, Inc. (ERG) to convene and facilitate a scientific peer review of the Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review (Analysis). This peer review is the next step in MPCA’s ongoing efforts to enhance scientific understanding of the effects of sulfate on wild rice. This enhanced scientific understanding will inform MPCA’s review of the wild rice sulfate standard and the development of a rulemaking proposal, if warranted, regarding that standard.

Background and Current Standard

Water quality standards are fundamental tools under the federal Clean Water Act (CWA) and Minnesota Statutes, designed to help protect and improve the quality of the state’s lakes, streams, wetlands and groundwater. Minnesota water quality standards consist of three components:

1. The beneficial use(s) for which a water body is to be protected,
2. The narrative and/or numeric criteria that specify what conditions in the water are protective of the beneficial uses, and
3. Antidegradation provisions to minimize the lowering of water quality that is better than the minimum level needed to protect beneficial uses.

Under the CWA, states and federally authorized Indian Tribes are required to identify the beneficial uses for which their waters are to be protected, then to adopt criteria and antidegradation provisions to protect those beneficial uses. The CWA also requires that water quality standards be regularly reviewed to solicit public input and incorporate new science. MPCA is the state agency responsible for this work in Minnesota.

One of the beneficial uses identified in Minnesota’s water quality standards rules is “water used for production of wild rice” (Minnesota Rules, Part 7050.0224, subpart 2). Wild rice is an important plant species in aquatic environments in parts of Minnesota, particularly northern Minnesota. It provides food for waterfowl, is economically important to those who harvest and market wild rice for human consumption, and is also a very important cultural resource for many Minnesotans.

This recognition of the importance of wild rice in Minnesota, and an observed relationship between the presence of wild rice in waters with lower sulfate levels (and its absence in waters with elevated sulfate), led to the adoption of a 10 milligrams per liter (mg/L) sulfate standard in 1973 applicable to water used for production of wild rice. Based on testimony presented at public hearings leading to the adoption of the sulfate standard, it was intended to apply both to waters with naturally occurring wild rice and to waters used for paddy rice production.

The standard was based on field observations and water chemistry correlations made by Dr. John Moyle primarily in the late 1930s and early 1940s, who concluded that "No large stands of rice occur in water having sulfate content greater than 10 ppm [parts per million, or mg/L], and rice generally is absent from water with more than 50 ppm. "

The existing wild rice sulfate standard was developed based on correlations of Dr. Moyle's observations and water chemistry data. However, the specific mechanism by which sulfate appears to be impacting wild rice was not the subject of Dr. Moyle's study. This, along with questions that have arisen regarding the implementation of the current standard, led to interest in further understanding the effects of sulfate on wild rice to inform a review of the wild rice sulfate standard.

Overview of the Wild Rice Sulfate Standard Study

In 2010, MPCA initiated a multi-year effort to clarify implementation of the state's wild rice sulfate standard. As part of this effort, the state legislature funded a study to gather additional information about the effects of sulfate and other substances on the growth of wild rice. This research was intended to inform an evaluation of the existing wild rice sulfate standard.

Following the development of a detailed research protocol in 2011, in 2012 the MPCA contracted with groups of scientists at the University of Minnesota Duluth and Twin Cities to undertake the Wild Rice Sulfate Standard Study. The Study's main hypothesis is that wild rice is impacted by sulfate via the conversion of sulfate to sulfide dissolved in the water in the sediment, known as the sediment porewater. The Study consists of five components:

1. **Controlled Laboratory Hydroponic Experiments:** Controlled laboratory hydroponic experiments determined the effect of elevated sulfate and sulfide on seed germination and early stages of wild rice growth and development.
2. **Field Survey of Wild Rice Habitats:** A field survey of wild rice habitats was conducted over two field seasons (2012-2013) to investigate physical and chemical conditions correlated with the presence or absence of wild rice, including sulfate in surface water and sulfide in the sediment porewater of the rooting zone. The survey crews also sampled the mesocosms (below) on a monthly basis during 2013. This field survey was supplemented by the data collected during a pilot field survey in 2011.
3. **Mesocosm Experiment:** An outdoor container wild rice growth experiment conducted in 2013, using natural sediments, determined the response of wild rice to a range of sulfate concentrations in the surface water, and associated sediment porewater sulfide concentrations in the rooting zone. This mesocosm experiment continued an experiment conducted by the principal investigator during 2011-2012 under separate funding.
4. **Collection and Analysis of Rooting Zone Depth Profiles:** Depth profiles of dissolved chemicals were collected in the mesocosms and at two field sites to characterize sulfate, sulfide, and iron.
5. **Sediment Incubation Laboratory Experiment:** Sediment was incubated in the laboratory, without wild rice, to explore the difference that ambient temperature has on the rate at which elevated sulfate concentrations in water enter underlying sediment and convert to sulfide, and to what degree sulfate is later released back into the overlying water.

Each of the Study components has a specific purpose and associated strengths and limitations. Data collection was completed in December 2013 and is documented in individual reports from the researchers.

Overview of MPCA Analysis

During the first half of 2014, MPCA staff integrated the Study results; analyzed the data as a whole; gained input from the Wild Rice Standards Study Advisory Committee; and reviewed existing monitoring data, other relevant scientific studies/information, and the original basis for the wild rice sulfate standard to develop an analysis of the Study results (the Analysis). The MPCA first developed a preliminary analysis and then refined the analysis based on feedback received and MPCA scientists' continued data analysis and interpretation. MPCA's Analysis draws most heavily from the data collected in the hydroponics experiments, the field survey of wild rice habitats, and the mesocosm experiment. The sediment incubation experiment provides some insight into sulfate-sulfide conversion dynamics, and results from the rooting zone depth profiles can be used to characterize the effect of elevated sulfate concentrations on rooting zone geochemistry and to define seasonal differences in the rooting zone geochemistry from both mesocosm and field sites. However, though the results from the rooting zone depth profiles and sediment incubation experiment are briefly summarized, further analysis and possibly additional study are needed before general findings can be drawn from these two Study components.

Purpose of the Peer Review

Independent scientific peer review is the next step to ensure that MPCA's scientific work is technically sound, and therefore can be relied upon to inform future work or decision-making. Any change to the water quality standard must be based on a sound scientific rationale; any change may also be controversial. Normally, MPCA relies on published peer-reviewed scientific studies during the development or revision of water quality standards. In the case of the Study, MPCA is analyzing the Study results prior to publication of those results in scientific journals. Therefore, MPCA is undertaking this peer review to receive independent scientific feedback on MPCA's Analysis.

It is important to note that this is not a review of the existing wild rice sulfate standard, or any recommendations for changes to the standard. MPCA has not yet developed policy recommendations or a proposal regarding any changes to the wild rice sulfate standard. Rather, MPCA will consider the peer reviewers' comments as the agency further refines MPCA's Analysis of the effects of sulfate on wild rice. If warranted, a Technical Support Document will be developed to describe the scientific basis for any proposed changes to Minnesota's water quality standards.

This peer review is a step in the larger process in which MPCA will consider available information to determine if changes to the wild rice sulfate standard are needed. MPCA will also seek informal and formal public comment on any recommendations and rulemaking proposal that are developed. Any proposed change to the wild rice sulfate standard would be adopted into Minnesota's water quality standard rule (Minnesota Rules Chapter 7050) in accordance with the procedural requirements of the Minnesota Administrative Procedures Act and would require the approval of the US Environmental Protection Agency.

Charge to Reviewers

ERG is conducting this peer review under contract to MPCA. The review questions consist of 10 specific questions, followed by three general questions. In conducting the peer review, reviewers are asked to refer particularly to the following sources:

- MPCA Analysis, and associated references.
- The individual report for each Study component, as needed, to further understand and evaluate the MPCA Analysis.
- The MPCA's wild rice sulfate standard web page for more background information about the Study and Analysis. A link to an FTP site (ftp://files.pca.state.mn.us/pub/tmp/wildRice) with all the Study reports and data is also available on this page.

Laboratory Hydroponic Experiments (see Analysis, pp. 13-16, 38-39)

The hydroponic experiments involved a series of aquatic toxicity tests designed to evaluate the relationship between a controlled exposure of wild rice plants to a dilution series of sulfate or sulfide concentrations, and the biological responses observed in the plant's growth and development. The sulfide seedling experiment involved immersing photosynthesizing seedlings in an anoxic sulfide solution. Over time, sulfide was oxidized by the oxygen the seedlings produced. This led to a decrease in the sulfide exposure concentration between each renewal of the test solution. MPCA staff relied on the initial sulfide concentration, rather than the lower sulfide concentrations that developed between renewals, as the operative exposure concentration in this Analysis, as this was the highest, and presumably most toxic, concentration to which the plants were exposed. It is uncertain if the leaves of seedlings would ever be exposed to sulfide in a natural setting (see pages 38-39 of the Analysis).

> *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.*

> *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?*

Utility of the Field Survey Data (see Analysis, pp. 21-25, 35-36, 41-47)

Statisticians recommend that surveys be probability-based when the point of the survey is to characterize the population being sampled. Probability-based surveys allow the survey results to be extrapolated back to the larger population. The field survey site selection was purposefully not probability based, in that the point was not to characterize the population of wild rice production waters but rather to explore the effect of elevated sulfate on the chemistry of the porewater of actual and potential wild rice habitat.

> *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.*

Mesocosm Experiment (see Analysis, pp. 26-32)

Wild rice was grown from seed in 100-gallon polyethylene containers containing sediment from a natural wild rice bed. Concentrations of sulfate in the overlying water were maintained at desired experimental treatment levels, and wild rice was allowed to grow and self-propagate for three seasons (2011-2013). An unusual rate of wild rice mortality occurred in all the containers at the beginning of the 2013 growing season. The mortality may be related to an unusually cold spring and subsequent late start to the growing season. Statistical analysis of the mesocosm experiment data shows significant differences in biological endpoints, such as seed weight, associated with increased sulfate exposure concentrations. The mesocosm experiment provides data on the interactive effects of sulfate, sulfide, and iron on wild rice in a system that is less controlled but more natural than the hydroponic experiments, and more controlled but less natural than the field survey.

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.

Wild Rice in Relation to Sulfate, Sulfide, and Iron (see Analysis, pp. 35-39)

The MPCA Analysis uses the results of the hydroponic experiments to demonstrate that elevated sulfide concentrations are toxic to wild rice seedlings. These data showed deleterious effects of sulfide on seedling plant growth when sulfide exceeded the range of 150 to 300 micrograms per liter (µg/L). In addition, this effect is corroborated by data from the field survey and the mesocosm experiment. A histogram of field survey data (Figure 17) shows that 69 to 80% of the sites had wild rice present (above a threshold of 5% cover) when porewater sulfide was less than 75 µg/L, and, as porewater sulfide increased, a more-or-less continuous decline in the percent of sites with wild rice present was observed. The Analysis also draws on data from the mesocosm experiment, in which 300 mg/L sulfate treatments exhibited a median sulfide concentration of 778 µg/L that would be predicted to impair wild rice growth based on the EC50 of 383 µg/L estimated from the hydroponic experiments. Seed weights were significantly lower in the highest sulfate treatment.

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?

Control of Porewater Sulfide by the Availability of Sulfate and Iron (see Analysis, pp. 21-25, 45-47)

The MPCA Analysis suggests that a quantile regression analysis of the field survey data is an appropriate way to estimate the relationship between sulfate in surface water and the potential concentration of sulfide in porewater, and that the uncertainty in the estimate can be reduced by incorporating the concentration of available iron in the sediment (as measured by an acid extraction).

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?

Synthesis: How Sulfate, Sulfide, and Iron Interact to Affect Wild Rice (see Analysis, pp. 51-52)

Elevated sulfate concentrations in surface water are a concern because bacteria convert sulfate to sulfide in the sediment where wild rice seeds germinate and develop roots and leaves. The Analysis demonstrates that the availability of iron mediates the accumulation of sulfide in the porewater of the sediment, and hence the exposure of wild rice seedlings to potentially toxic levels of sulfide. Therefore, based on the Analysis, the potential toxicity of sulfide is jointly controlled by sulfate in surface water and iron in the sediment.

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.

General Questions

Charge Question 11: Please state your overall assessment of the five Study components. Did MPCA choose appropriate Study components to meet Study objectives 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.

Appendix B

Peer Reviewers

Minnesota Pollution Control Agency

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

List of Reviewers

Gertie H.P. Arts, Ph.D.
Senior Aquatic Ecologist and Ecotoxicologist
Alterra, Wageningen University and Research Centre
The Netherlands

Donald M. Axelrad, Ph.D.
Assistant Professor
Institute of Public Health
Florida A&M University
Tallahassee, Florida

Patrick L. Brezonik, Ph.D. (Panel Chair)
Professor Emeritus
Department of Civil Engineering
University of Minnesota
Minneapolis, Minnesota

M. Siobhan Fennessy, Ph.D.
Jordan Professor of Biology and Environmental Studies
Biology Department
Kenyon College
Gambier, Ohio

Susan Galatowitsch, Ph.D.
Professor and Head,
Department of Fisheries, Wildlife, and Conservation Biology
University of Minnesota
St. Paul, Minnesota

Mark L. Hanson, Ph.D.
Associate Professor
Department of Environment and Geography
Faculty of Environment, Earth, and Resources
University of Manitoba
Winnipeg, Manitoba
Canada

Curtis D. Pollman, Ph.D.
CEO, Aqua Lux Lucis, Inc.
Gainesville, Florida
and
Adjunct Research Professor
Department of Geological Sciences
University of Florida

Appendix C

Meeting Agenda

Minnesota Pollution Control Agency

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Final Agenda

WEDNESDAY, AUGUST 13, 2014

MORNING

7:45 – 9:30 a.m.	Registration/ Check-In
8:30 – 8:50 a.m.	**OPENING REMARKS** *Jan Connery, Facilitator, Eastern Research Group, Inc. (ERG)* • Introductions, Meeting Purpose, Process and Agenda
8:50 – 10:00 a.m.	**OBSERVER COMMENT PERI*OD*** – *Moderated by Jan Connery, ERG*
10:00 – 10:15 a.m.	BREAK
10:15 – 11:30 a.m.	**REVIEWER DISCUSSION: LABORATORY HYDROPONIC EXPERIMENTS** *Moderated by Patrick Brezonik, Chair* • Charge Questions 1, 2 & 3 • Other Comments
11:30 a.m. – Noon	**REVIEWER DISCUSSION: UTILITY OF THE FIELD SURVEY DATA** • Charge Question 4
Noon – 1:30 p.m.	LUNCH (on your own)

AFTERNOON

1:30 – 2:00 p.m.	**REVIEWER DISCUSSION: UTILITY OF THE FIELD SURVEY DATA (cont.)** • Other Comments
2:00 – 2:50 p.m.	**REVIEWER DISCUSSION: MESOCOSM EXPERIMENT** • Charge Question 5 • Other Comments
2:50 – 3:10 p.m.	**REVIEWER DISCUSSION: WILD RICE IN RELATION TO SULFATE, SULFIDE, AND IRON** • Charge Question 6
3:10 – 3:25 p.m.	BREAK
3:25 – 3:40 p.m.	**REVIEWER DISCUSSION: WILD RICE IN RELATION TO SULFATE, SULFIDE, AND IRON (cont.)** • Other Comments
3:40 – 5:00 p.m.	**REVIEWER DISCUSSION: CONTROL OF POREWATER SULFIDE** • Charge Question 7 • Charge Question 8
5:00 p.m.	ADJOURN

THURSDAY, AUGUST 14, 2014

MORNING

9:00 – 9:10 a.m.	Review of the Day's Agenda, Format, and Goals – *Jan Connery, Facilitator*
9:10 – 9:40 a.m.	**REVIEWER DISCUSSION: CONTROL OF POREWATER SULFIDE (cont.)** *Moderated by Patrick Brezonik, Chair* • Other Comments
9:40 – 10:45 a.m.	**REVIEWER DISCUSSION: SYNTHESIS** • Charge Question 9 • Charge Question 10 • Other Comments
10:45 – 11:00 a.m.	BREAK
11:00 – 11:45 a.m.	**REVIEWER DISCUSSION: GENERAL QUESTIONS** • Charge Question 11
11:45 a.m. – 1:15 p.m.	LUNCH (on your own)

AFTERNOON

1:15 – 2:00 p.m.	**REVIEWER DISCUSSION: GENERAL QUESTIONS (cont.)** • Charge Questions 12 and 13 • Other Comments
2:00 – 3:30 p.m.	**WRITING SESSION**
3:30 – 3:45 p.m.	BREAK
3:45 – 4:15 p.m.	**WRITING SESSION (cont.)**
4:15 – 5:00 p.m.	**CLOSING REMARKS** • Reviewer final comments • ERG closing remarks
5:00 p.m.	ADJOURN

Appendix D

Meeting Observers

Minnesota Pollution Control Agency

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

LIST OF OBSERVERS

First Name	Last Name	Organization
Kurt	Anderson	ALLETE/Minnesota Power
Leonard	Anderson	MPCA Wild Rice Sulfate Standard Advisory Committee
Sara	Barsel	MPCA Wild Rice Sulfate Standard Advisory Committee
Chrissy	Bartovich	US Steel
Brian	Beck	Wenck Associates
Rob	Beranek	Cliffs Natural Resources
Michael	Berndt	Minnesota Department of Natural Resources
Jeffrey	Brenner	Minnesota Department of Health
Andrew	Chelseth	Stinson Leonard Street
Peter	Clevenstine	Minnesota Department of Natural Resources
Betsy	Daub	Friends of the Boundary Waters Wilderness
Diadra	Decker	Water Legacy
Jon	Dockter	Minnesota Cultivated Wild Rice Council
Paul	Eger	Global Minerals Engineering
Daniel	Engstrom	Science Museum of Minnesota
Lea	Foushee	NAWO
Mike	Hansel	Barr Engineering
Stephanie	Hemphill	MinnPost, KAXE-FM
Brian	Hiti	Iron Range Resources & Rehabilitation Board (IRRRB)
Meghan	Jacobson	Emmons & Olivier Resources, Inc.
Thomas	Jamar	Jasper Engineering
Jaime	Johnson	ArcelorMittal Minorca Mine Inc.
Myra	Kunas	Minnesota Department of Health
William	Latady	Bois Forte Band of Chippewa
Peter	Lee	Lakehead University
Paula	Maccabee	WaterLegacy
Molly	MacGregor	
Howard	Markus	
Joseph	Mayasich	Western Lake Superior Sanitary District
Craig	Pagel	Iron Mining Association
John	Pastor	University of Minnesota
Jane	Reyer	Save Lake Superior Association
Robin	Richards	ENVIRON International Corp.
Nancy	Schuldt	Fond du Lac Band of Lake Superior Chippewa
David	Sherek	Biwabik Public Utility
Robert	Shimek	MPCA Wild Rice Sulfate Standard Advisory Committee
David	Smiga	US Steel
Pat	Tammen	Wetlands Action Group
Robert	Tammen	Wetlands Action Group

LIST OF OBSERVERS (continued)

First Name	Last Name	Organization
Michael	Twite	Magnetation, Inc.
Darren	Vogt	1854 Treaty Authority
Christine	Wagener	US EPA Region 5
Rachel	Walker	Barr Engineering Co.
Bob	Whipple	
Eric	Williams	US Steel

MPCA STAFF

First Name	Last Name	Organization Name
Gerald	Blaha	MPCA
Patricia	Engelking	MPCA
Rebecca	Flood	MPCA
Liz	Kaufenberg	MPCA
Katrina	Kessler	MPCA
Shannon	Lotthammer	MPCA
Philip	Monson	MPCA
Adonis	Neblett	MPCA
Emily	Peters	MPCA
Cori	Rude-Young	MPCA
Jeff	Smith	MPCA
Edward	Swain	MPCA

CONTRACTOR STAFF

First Name	Last Name	Organization Name
Jan	Connery	ERG
Steve	Gillard	ERG
Kate	Schalk	ERG

Appendix E

Observer Oral Comments

Comments Presented by Robert Shimek

Boozhoo Indinawemaganinaanig. Hello, all my relatives. First of all, I don't come with any credentials. I noticed a lot of people lining up and stating their credentials. I am just a regular reservation Indian, a hand-harvester from the White Earth Indian Reservation. I want to say this about the science: the science is the science and it plays an important role in so much of what we do; the things we use on a day-to-day basis, whether it has something to do with agriculture, or aerospace engineering, or, in this case, mining. We do know that science, or lack of it, has played an important role in turning some segments of our landscape, not only here in Minnesota, but nationally, into this huge geochemical experiment. We know we have mining sites nationally that have become Superfund sites. We know that 70 percent of the headwaters in western states here in America have been polluted by mining. We know that in a few cases entire landscapes have become these huge science projects.

In other words, how do we clean up the pollution on the Coeur d'Alene River and Lake Coeur d'Alene, and places like that, where you have tens or hundreds of thousands of miles of massive problems? Science, or lack of it, has led to a lot of these problems. Here, on a more local level, we already know we have some problems with the mining industry in Minnesota. We know that the mining industry has already decimated some beds of naturally occurring wild rice. As you make your deliberations and statements and come to a conclusion, I would urge you to take the highroad as you go through the information, the science, and the questions and the way they are asked.

At the end of the day, is 10 ppm of sulfate protective of wild rice? Does it need to be more? Does it need to be less? That's the key question here. There are many ways to skirt, or talk around that question, but as someone who has been out on those rice beds for over 40 years, it's not only a way of life; for some of us, the rest of the year does not go right unless we get to go ricing. Miigwech niibowa. Thank you for your time.

Comments Presented by Mike Hansel

Testimony
Peer Review Meeting
MN Chamber of Commerce

Good morning. My name is Mike Hansel. I'm a Senior Chemical Engineer at Barr Engineering Company and I'm here today to represent the MN Chamber of Commerce. The Chamber represents more than 2,400 businesses of all types and sizes across Minnesota. Chamber members are the only dischargers who have been required to comply with the existing 10 mg/L sulfate water quality standard, either through site specific standards, compliance schedules to meet the 10 mg/L standard or seasonal discharge limitations to protect wild rice. As such, Chamber members are already impacted by the current standard, and will continue to be impacted by the current standard or, if the standard is modified, whatever the new standard will become.

Before I go further, I would like to thank you all for taking the time and applying your expertise to review the research conducted by the MPCA and its contractors and others. This is and will continue to be a critical water quality standard, and such a standard must be based upon sound science and with the rigor that is necessary to support development of water quality standards.

The current standard is for sulfate, but it is quite clear from the research that sulfate is not all that toxic to wild rice. Both the hydroponic testing by the University of Minnesota and by Fort Environmental Labs demonstrates that sulfate is not toxic at concentrations up to 1,600 to 2,500 mg/L.

The MPCA's hypothesis is that sulfide <u>in the sediment porewater</u> is toxic to wild rice. In Charge Questions 1, 2, 4, 6, 8 and 9 MPCA asks the Panel to opine on "sulfide in the porewater" or "sulfide in the sediment porewater". However, the hydroponic tests don't show toxicity to those parts of the plant <u>exposed to porewater</u> – germinating seeds, mesocotyl growth or roots – at levels of 2,400 µg/L. In the field survey only 2 lakes showed such high levels – located in western and southern Minnesota in the Des Moines glacial lobe. The mesocosm study also showed such high levels at high and medium sulfate treatment, but only after depletion of the iron (as reported by Dr. Johnson) in a non-renewing environment. The toxicity of sulfide with which the MPCA is concerned is due to toxicity to parts of the plant which are not exposed to sulfide. Leaves and shoots grow in the overlying water, and all of the data points to oxygenated water immediately above the sediment as well as, in some cases, in the upper portions of the sediment.

MPCA other hypothesis is that iron mitigates the toxicity of sulfide by precipitation and removal from solution, as noted in Charge Questions 6, 8 and 9. MPCA is now concerned that iron may be depleted and sulfide, at some point in time, may reach toxic levels. This concern is not supported by the facts.

The only place where iron depletion appears is in lakes in southern and western portion of the state (in Des Moines glaciation lobe) and in mesocosm studies where iron was depleted by non-renewal of solution. In the field surveys, in all but two cases, the concentration of iron in the porewater was at least twice the concentration of iron in overlying surface waters. In many cases porewater iron was several orders of magnitude higher than overlying surface water, indicating that iron is diffusing from the porewater into the surface water. The source of this iron, in the Chamber's view, is inflowing groundwater. Except for the southern and western portions of the state, there is abundant naturally occurring iron which is constantly replenishing sediment porewater by the inflow of groundwater to the littoral zone of lakes and into most streams (gaining streams) in Minnesota. There is and will continue to be more than sufficient iron to precipitate sulfide, and the MPCA's concern seems unfounded and unsupported by the data.

With regard to Charge Question 5, the Chamber has a number of other concerns with the mesocosm studies. First, 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. It is quite likely that the test design contributed, at least in part, to the observed effects in the mesocosm. Second, the high plant mortality across all treatments in 2013 greatly complicates the interpretation of the mesocosm results. Although MPCA hypothesizes that a late and cold spring are responsible for the high mortality, additional information should be used 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 throughout Minnesota. Also were the growth rates and other metrics observed in the mesocosms suppressed as compared to natural rice, indicating that the rice in the mesocosms was already stressed by the experimental design? And therefore the results of the sulfate treatments may not be representative of sulfate toxicity in natural conditions?

The MPCA has attempted to correlate the concentrations of sulfide in porewater with sulfate in the overlying surface water. With regard to Charge Questions 7, 8 and 9, the Chamber notes two things:

1. The statistical analysis used to develop this correlation is highly suspect, depending largely upon a single outlier value. Others will speak more to this and other issues regarding the MPCA's statistical treatment of the data.
2. Correlation, as you know, does not equal causation. In order to set water quality standards, the MPCA needs to know whether or not sulfate, whether transformed to sulfide or as sulfate, <u>causes</u> toxicity to wild rice.

In conducting your review of the research, and in regard to Charge Questions 10, 11, 12 and 13 the Chamber asks the Peer Review Panel to consider four overarching concerns:

1. Does the research adequately mimic natural conditions? If so, than it ought to be accorded higher weight. If not, for example the exposure of the non-rooting zone portions of the plants to anoxic conditions with sulfide, and the non-renewal of the mesocosms, than it ought to be accorded less weight. In the Chamber's view, the sulfate hydroponic tests, the sulfide hydroponic tests on plant materials exposed to porewater and the field surveys ought to be accorded the highest weight. The sulfide hydroponic tests on plant materials not in the root zone and the mesocosm studies ought to be accorded much less weight.

2. Does the research meet the US EPA standards for regulatory research? Again, if so, it ought to be accorded higher weight (e.g., Good Laboratory Practices). If not, it ought to be accorded less weight.

3. A critical issue that has yet to be addressed by the agency is the role which groundwater inflow to the sediment porewater in the littoral zone of lakes and in most streams (gaining streams) in Minnesota. Rather than depend upon extraction of iron from sediment minerals, it seems more likely that inflowing groundwater is a more likely and abundant source. As noted above, in all but two cases from the field survey, porewater iron concentration was at least twice as high as surface water iron concentration and in some cases several orders of magnitude higher.

4. Sulfate is not the only know stressor on wild rice. According to the Great Lakes Indian Fish and Wildlife Commission (GLIFWC), multiple stressors exist, including water levels, fluctuations in water levels, competitive plant species, invasive plant species, invasive animal species, disease and global climate change. All of these variables are independent of water chemistry, and need to be taken into consideration in determining whether rice in any given water body is stressed by sulfate or sulfide concentrations.

The Chamber appreciates the fact that the MPCA provided the Panel with the research conducted by Fort Environmental Labs and the Chamber’s analysis. While I’m not sure of the protocol for this meeting, I would be happy to answer any questions about those documents.

Thank you for the time to make these comments prior to your discussions. And thank you for taking the time to peer review the research in this important consideration of a critical water quality standard.

Comments Presented by Robert Tammen

I am Bob Tammen from Soudan, MN, an old mining town. I worked in several of the mines when I was younger. I appreciate being here for this scientific process; we have been reminded many times that it is, indeed, a scientific review process. In 2011 my wife, Pat, and I spent considerable time at the Legislature, and I think it's fair to say that this scientific process is actually an appendage of Minnesota's political process. We were in the hearings when the legislators introduced legislation to change the sulfate standard to 50 mg/L, 250 mg/L, and legislation to eliminate it completely. We know their intent was to legalize the degradation of Minnesota's public waters. Of course that was not successful due to input from the EPA.

Then, they funded this study, and perhaps rightly in a scientific manner the study is restricted to a narrow part of the sulfate standards. We were forbidden to discuss the methylation of mercury and the documented toxicity with which some of our children are born. We cannot study that topic in this particular process. As someone who lives on the range, I would like to point out that we have invested a substantial amount of Minnesota's resources in an effort to weaken our discharge standards when we could have taken all the intellectual horsepower in this room and started cleaning up sulfates.

Our surface water and a lot of our groundwater is low in sulfates. Where my wife and I live, sulfate levels are in the single digits. We object to the degradation of those public waters, and I do not think we should get caught up in the idea that if they can find a stand of wild rice that will tolerate high sulfate levels, we should start dumping high sulfates into the waters in mining country. I would like to say, as a Minnesota citizen living on the iron range, we are not your sacrifice zone, we are not your lab rats, and we want the sulfates cleaned up. Thank you.

Comments Presented by Paula Maccabee

WaterLegacy
Protecting Minnesota's waters and the communities who rely on them

Comments of Water Legacy to Peer Review Panel (August 13, 2014)

Wild Rice Sulfate Standard Studies for Minnesota Pollution Control Agency

WaterLegacy is a non-profit organization formed to protect Minnesota's waters and the communities that rely on them. We believe this Peer Review Panel must consider the questions prescribed by the Legislature in setting up the wild rice studies:

1) Is Minnesota's existing water quality standard limiting sulfate to 10 milligrams per liter in wild rice waters needed to protect wild rice?

2) Should this sulfate limit be applied year-round or only during specific times?

We count on your expertise to apply science and analyze these key questions.

First Question: Do the field surveys, mesocosm experiments and hydroponic experiments taken together support the existing 10 milligrams per liter sulfate standard?

Answer:
The most striking result of Minnesota's wild rice studies is that all study modalities support Minnesota's existing 10 milligrams per liter sulfate standard.

Before the Pollution Control Agency withdrew its February 2014 summary in the face of political pressure, the Agency concluded, "**The 10 mg/L sulfate standard is needed and reasonable to protect wild rice production from sulfate-driven sulfide toxicity.**"

In fact, were Minnesota to seek protection of 95 percent of the resource, as suggested in some federal guidance, a *more stringent* limit on sulfate would be required.

Second Question: Do data on sulfide and incubation studies of conversion of sulfate to sulfide support any limit on the time of the year when the sulfate standard should apply to protect wild rice?

Answer:
The studies do not support any temporal limit on the application of Minnesota's wild rice sulfate standard.

The primary mechanism by which sulfate discharge becomes toxic to wild rice is through conversion to sulfide. Dr. Nate Johnson's research shows that, after about 80 days, a great majority of the sulfate that diffuses into sediments will react to form sulfide, even under cold conditions.

There is no time of year when discharge of sulfates will not harm wild rice.

Should amendments to wild rice rulemaking come out of this process, decision-makers will assume that peer reviewers have analyzed these two basic questions about the wild rice sulfate standard. To exclude these questions from your discussion would undermine and marginalize the work of this Panel.

A third basic question has been posed to this Panel. If iron levels are high, should there be an exception to the sulfate standard?

From our perspective, the question whether iron in porewater mitigates the effects of sulfate and sulfide on wild rice is a “Hail Mary” pass thrown by industry once they realized the studies support retaining Minnesota’s wild rice sulfate standard of 10 milligrams per liter. But it is still a worthwhile question

Third Question: Are the wild rice sulfate studies sufficient to support a theory that high concentrations of iron in porewater prevent sulfate from harming wild rice?

Answer:
The wild rice studies are insufficient to support this theory:

1) There are no experiments varying iron and sulfate to assess effects on sulfide or wild rice.
2) The only basis for a claim that iron “mitigates” effects of sulfate on wild rice are correlations; and these field correlations are from sampling that was intentionally biased.
3) The field data suggesting wild rice may survive in higher sulfate waters doesn’t apply to lakes, and river systems may have confounding factors.
4) Mesocosm experiments suggest iron sulfide plaques on wild rice roots may interfere with nutrient uptake.

Proposing “site-specific” standards to permit more sulfate discharge where there is high iron may seem like good politics, but it is not good science.

In closing, WaterLegacy asks that the Peer Review Panel analyze the two basic questions that the wild rice sulfate studies were designed to answer and that the Panel find the iron theory premature.

Science seeks simplicity in the pursuit of truth, while politics seeks exceptions and deviations in the service of powerful interests. Help Minnesota use science, not politics, to determine what rules should protect wild rice.

Thank you.

Paula Goodman Maccabee
Advocacy Director/Counsel for WaterLegacy
phone: 651-646-8890
cell: 651-775-7128
e-mail: pmaccabee@justchangelaw.com

Comments Presented by Lea Foushee

NORTH AMERICAN WATER OFFICE

PO BOX 174 LAKE ELMO, MN 55042

Phone: 651-770-3861

August 13, 2014

My name is Lea Foushee. I am the Environmental Justice Director for the North American Water Office. As an Indigenous woman, I must entreat you on behalf of the wild rice, a sacred food that grows on water.

Potsherds, a broken piece of pottery, encrusted with wild rice char dug up by Western scientists even date Indigenous Peoples eating wild rice in Minnesota 1,830 years ago. Oral Tradition talks about 20,000 or more years. This deep history of relationship with wild rice demonstrates the intrinsic harm possible from an abusive manipulation of nature for the benefit of a few.

These wild rice standards under review should not be designed to perpetuate another generation of extraction based mining standards. A wild rice dead zone exists due to a mining history in Minnesota. Virgin mining development proposals from companies like Polymet, Twin Metals and some 30 other pollution permits currently pending in the shadows, will expand the wild rice dead zone from historical mining damage that already exists in the Partridge River/St Louis River. There is no protocol for wild rice as an identity food. No documentation has been/or can be provided that states no additional loss of wild rice habitat will occur as a result of proposed mining actions, and emissions from coal burning power plants.

More destruction of wild rice habitat is not a benefit for Minnesotans or Minnesota. NAWO recommends that a metals Reclamation based standard be established that would require all metals be optimally recovered from solid waste and recycled for repurpose. New mines of base extraction should only be allowed for make up metal necessary for essential use. New mines must have site-specific standards that recognize site-specific characteristics in order to ensure protection.

The sacrifice of additional wild rice habitat and contaminated Minnesota water for the benefit of a few at the expense of all cannot be allowed. That would be genocide.

www .nawo.org MINNESOTA Environmental Fund email: gwillc@nawo .org

Comments Presented by Rob Beranek

Subject: *Public Comment for the Minnesota Pollution Control Agency Commissioned Peer Review Panel on Sulfate and Wild Rice Studies*

Commentator: *Rob Beranek, Manager Water Regulatory Strategy and Planning, Cliffs Natural Resources*

Good Morning. My name is Rob Beranek. I'm the Manager of Water Regulatory Strategy and Planning for Cliffs Natural Resources (Cliffs). Cliffs is the operator and owner or partner at three of the operating taconite iron mines in Minnesota. The 130th anniversary of iron mining was celebrated last month and Cliffs is proud to be an important part of that heritage that exemplifies a balance between protection of our natural environment and utilizing our natural resources in a mindful way that contributes to the economy and livelihood of so many in Northern Minnesota. We are and will remain affected by the sulfate wild rice standard as we continue the long tradition of mining in an area where both iron ore and wild rice have coexisted for over a century.

On behalf of Cliffs, please first let me express my gratitude for the careful thought and consideration you will be giving this matter. I appreciate you providing your expertise in the review of the studies and preliminary analysis and offer the following comments.

In regards to Charge Question 1, portions of the sulfide hydroponics experimental design were flawed because leaves and shoots of the wild rice plants were exposed to elevated concentrations of sulfide which is unrepresentative of normal conditions in nature. We are concerned this bias has resulted in an overly conservative value being identified as a sulfide toxicity threshold to wild rice. When the appropriate portion of the wild rice plant such as the germinated seed, mesocotyl growth and roots were exposed to elevated sulfide we do not see evidence of toxicity up to levels of 2,400 ug/L. Please see pages 15 through 23 of the MN Chamber's Technical Analysis of Wild Rice Research dated February 2014 (February 2014 MN Chamber Analysis).

With regard to Charge Question 4, the lake and stream field survey data provides compelling evidence that elevated sulfate and porewater sulfide are not of concern for the vast majority of the natural range of wild rice in Minnesota; and further to our comments on Charge Question 1 this is additional evidence that the sulfide hydroponics test design is not reflective of the natural environment. We are compelled by the MN Chamber's analysis of underlying geological factors, as well as ecosystems and climate, which aligns well with Moyles isopleth of sulfate and wild rice. Please see pages 47 through 58 of the February 2014 MN Chamber Analysis for additional information. I'd like to highlight that there is a location in the data set I'm personally familiar, a location where the sulfate present in the surface water is at nearly 1,000 mg/L where there is a healthy population of wild rice present which underscores why we think there are issues with experimental design which do not match what is happening in the natural environment.

With regard to Charge Question 5, we have similar concerns to those I've already outlined regarding the sulfide hydroponics testing, specifically we are concerned that the mesocosms are not reflective of the natural environment. Most importantly is the lack of an inflow of nutrients to replenish the mesocosm system. Among the most significant missing nutrient is iron because of its relative abundance in nature and the mitigating effects on sulfide accumulation. We are also concerned that if the test acceptability criteria established for the sulfate hydroponics test of at least 90% of control juvenile seedlings living at test termination is applied to the mesocosm studies it would fail with only a 15% survival rate. We think extreme caution should be used with the extension of any of the mesocosm experiment data to setting a water quality standard. Pages 25 through 28 of the February 2014 MN Chamber Analysis are reflective of our concerns.

Our comments on Charge Question 6 combine our thoughts from Charge Questions 1 and 4. We think that the flawed sulfide hydroponics experimental design coupled with the disregard for geological, climatic, and ecological reasons for wild rice variability in the field studies result in a faulty conclusion regarding evidence of sulfate vis-a-vis sulfide as causal of wild rice toxicity in the natural environment. Further, we think a better reasoned approach is equation 4-1 of the February 2014 MN Chamber Analysis supported by Appendix B, the equation and discussion of it can be found on pages 38 through 40.

My single comment on Charge Question 7 has been informed by discussions with professionals familiar with statistical analysis of these types of data sets. Our understanding is that this data set does not meet the criteria to make multiple quantile regression an appropriate tool. I look forward to you lending your expertise to this matter and reviewing for yourselves.

And my final comments for the remaining Charge Questions 10 through 13 are a synthesis of observations from the entire collection of studies. The sulfate hydroponics studies conducted by the University of Minnesota Duluth and Fort Environmental Labs support a sulfate standard of 1,600 to 2,500 mg/L. When the sulfide hydroponics study is corrected for a flawed experimental design, it demonstrates a no observable effects concentration of at least 2,400 ug/L sulfide. These findings coupled with the observations from the field studies of wild rice present at sulfate concentrations up to 2 orders of magnitude above 10 mg/L sulfate should weigh heavily on the outcome of your review of the studies and analysis.

Thanks again for your time and expertise. We are looking forward to the results of your review.

Comments Presented by Nancy Schuldt

Water Projects Coordinator, Fond du Lac Band of Lake Superior Chippewa
Statement to MPCA Wild Rice Sulfate Standard Peer Review Panel
August 13, 2014

Wild rice or manoomin is of profound cultural significance to the tribes; this significance was not articulated in the "Background and Current Standard" section. It is an integral part of their way of life and is used for subsistence, and it is of grave concern to the tribes that is has declined so substantially from its widespread historic distribution across the upper Midwest – in fact, it once thrived throughout the US from the eastern seaboard to the Rockies.

What was also left unsaid in MPCA's analysis is that the 10 mg/l sulfate criterion for the protection of wild rice is also an approved criterion for the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa in our federally approved water quality standards; the Bad River and Lac du Flambeau Bands in Wisconsin are also contemplating including this criterion in their next review. While the 10 mg/l sulfate criterion is certainly unique to this region and this specific natural resource, the **USEPA determined that it was scientifically defensible** and approved it in 1973 for the state, in 2001 for Fond du Lac, and in 2005 for Grand Portage. Dr. Moyle's extensive observational dataset from the mid-twentieth century on Minnesota's aquatic resources and insightful analysis is a valid foundation for the existing standard. It has been infuriating to hear industry and some legislators continually disparage and malign the science that the standard is based upon.

We have extensively monitored our reservation wild rice waters and sediments for the past sixteen years in implementing our standards. Other tribes and tribal agencies have monitored healthy, harvestable wild rice waters across the region, confirming that **regardless of interannual variability in yield, the water chemistry in these natural, productive wild rice beds is consistently low in sulfate.** We have also collaborated and continue to fund our own research on a broad range of wild rice-related questions, including cumulative effects of sulfate loading over time.

Tribes expressed serious concerns to the MPCA regarding the hydroponics testing from the very beginning because they are very short-term and limited in scope. Even when germination occurs and seedlings begin to grow, the hydroponics studies cannot determine whether or not the seedlings will continue to grow for a full season at the exposure concentrations and produce viable seeds for the next season. At best, they may serve to support **the working hypothesis that sulfate loading to a reducing environment leads to generation of hydrogen sulfide, which is known to be toxic to virtually every living thing.** They cannot possibly be as ecologically relevant as the field studies and the experimental mesocosms in answering the overarching questions posed by the MN Legislature that directed this research:

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. It is disturbing to see a preponderance of charge questions to this peer review team on the issue of iron's role in the effects of sulfate on natural, variable wild rice ecosystems. Iron may indeed precipitate sulfide in anoxic sediments, but iron sulfide also coats the roots of wild rice plants when sulfate is elevated; this was unequivocally demonstrated in Dr. Pastor's work. The impacts of that biochemical activity is not yet fully understood; therefore it is premature to conclude that 'sufficient iron' (whatever that may be) in the sediments will mitigate the effects of high sulfate loadings to wild rice waters. The science simply is not there.

I look forward to a rigorous technical review of all of the current research program components. I will leave you with the questions I am most interested in hearing your expert opinions on:

Does the 10 milligram per liter sulfate standard adequately protect at least 80% of natural wild rice stands? And is protecting only 80% of natural wild rice stands (a diminishing resource) sufficient from a population ecology perspective? EPA's science review board findings on other criteria suggests that the 95th percentile is the endpoint typically selected by EPA when deriving numeric aquatic life criteria under section 304(a).

Is there a scientific basis for the seasonality of the wild rice sulfate standard?

How can the tribes use the results of this research to better protect our wild rice waters?

Thank you.

Jane Reyer 218.248.1349
1625 E. First St. ☙ Duluth, MN 55812 jane.reyer@gmail.org

Peer Review Meeting for the Minnesota Pollution Control Agency's Draft "Analysis of the Wild Rice Sulfate Standard Study"
August 13, 2014

Statement on behalf of Save Lake Superior Association

Thank you to MPCA for the opportunity to observe this meeting and to provide input, and thank you to the researchers and peer reviewers for your work and whatever part you may be able to play in bringing some objectivity to the thorny thicket of political views on a subject that is so important to Minnesota.

Most of our questions and concerns about the MPCA's Draft Analysis document are reflected in the charge questions and in the submissions by Advisory Committee members, and I will not go into them in great detail. However, I do want to touch on a couple of these. Relating to Charge Question 2, the Draft Analysis states that EC20 is "sometimes used to characterize a no-effect concentration." I am not a scientist, but what this says to me is that the EC20 level is sometimes not used in that way. I am curious as to the health and productivity effects over the long-term (hundreds and thousands of years) that might be expected from a persistent 20% reduction in growth. To state this another way, is the assumption that the EC20 level is equivalent to no effect warranted?

Second, Charge Question 4 asks whether it is appropriate to base the conclusions MPCA has drawn on a field study that was not representative. I just want to make sure that this question is applied to conclusions regarding iron as well as those regarding sulfide. It seems to me that in deliberately including high sulfate waters that support wild rice and then measuring the iron in those waters, the experiment deliberately fails to identify high sulfate waters that also contain high iron but do not support wild rice.

Our next issue does not seem to be covered by a charge question. It has to do with the import of the presence of plaque on the roots of wild rice plants in high-sulfide waters in the mesocosm experiment. MPCA reaches a conclusion about this that is not supported (or even discussed) by Dr. Pastor's paper. In its discussion of the issue, MPCA cites only an article that supports its theory; although it refers to other theories, it provides no citations for them. They may be included in Dr. Pastor's references, but because he does not discuss this point, the trail is difficult to follow.

My understanding based on a recent presentation given by Dr. Pastor is that he is currently conducting research with a working hypothesis that runs counter to MPCA's apparent position that this plaque is benign, and its conclusion that the presence of sufficient iron mitigates the impact of sulfate. My understanding is that Dr. Pastor is testing the hypothesis that the plaque formed by iron sulfide precipitate causes or contributes to reductions in viable seed production over time. MPCA appears ready to conclude that the presence of iron allows wild rice to thrive in higher sulfide waters; we believe this conclusion would be premature in light of the current uncertainty regarding the role that root plaque formed by iron sulfide precipitate plays in the health and productivity of wild rice stands over time. We hope that you will address this issue.

Comments Presented by Robin Richards

Good morning. My name is Robin Richards. By training I am a plant physiologist, by profession I am a consultant focused on toxicity and ecological elements of the Clean Water Act. I am in charge of ENVIRON's water resources sector, which includes an Ecotoxicity testing lab. I am part of the Wild Rice Standards Study Advisory Committee and am here today representing my opinion on two specific items that are intertwined in the charge questions: Data Quality, and Statistical Analysis of Data.

ENVIRON has actively participated in state rulemaking to either develop or re-develop water quality criteria for numerous chemicals including sulfate, chloride and potassium. Our participation has included generating or validating toxicity test data on these substances. ENVIRON's Ecotoxicity laboratory is NELAP certified, certified in numerous states, and generates Good Laboratory Practices (GLP) complaint data. In generating toxicity test data for rulemaking that results in enforceable water quality standards, we have experienced firsthand the level of detail, documentation, transparency, and rigor required to meet stakeholder's, particularly USEPA's, expectation for data quality. We have come to appreciate the additional effort required to develop water quality standards; a high degree of confidence is needed in every aspect of generating data that will be used in establishing a state rule under the Clean Water Act. There is a stark contrast between experimental design, execution, and documentation between studies done for publication versus those done to be used in support of a regulatory program, which is enforceable, and is focused on protecting water.

Our most recent work involved toxicity tests on potassium using many non-standard USEPA species. As expected, the review of the quality assurance program plan (QAPP) for toxicity testing and for the concurrent analytical testing required revisions to assure regulators that its execution would generate valid data. In addition, as was a challenge for MPCA, there was little toxicity literature to go by for how best to investigate potassium, and a number of hypotheses on interactions amongst chemicals that could factor into the response of invertebrates to potassium. Hence, it took a year to finalize the QAPP, but execution of the detailed QAPP did generate valid data with documentation to allow stakeholders to be confident of the results. The attention to detail included repeating toxicity tests three times, providing reference toxicant results for test organisms, providing the daily toxicity testing laboratory bench sheets for every test, conducting daily analytical testing on several parameters, providing the analytical laboratory reports for every sample, conducting numerous duplicate samples for confirmation of analytical performance as well as split samples between two different analytical laboratories, and providing all statistical calculations.

I believe this investment by the stakeholders in the QAPP prior to toxicity testing, the attention to detail, the rigor and discipline exercised during execution, and the transparency in results and data analysis, is necessary to assure confidence in developing a rule that results in monitoring waters to enforce compliance with a newly developed water quality standard.

The Advisory Committee did review draft QAPPs for some of the studies and did attempt to engage MPCA on improving elements of those QAPPs. Some studies, however, were started without input to improving the QAPPs. I know I encouraged paying attention to detail, keeping records, and sharing any and all data and calculations. However, the final results of the studies shared with the Advisory Committee, while perhaps sufficient to provide an indication or inform a decision about the suitability of the10 mg/L sulfate standard, do not reflect the rigor and discipline one would expect of data to be used to develop a water quality standard in Clean Water Act rulemaking.

Development of the wild rice water quality standard entails generating data to understand the biological response of wild rice to a chemical (sulfate or sulfide). As such not only does there have to be confidence in the data quality related to the response (or toxicity endpoints) of the wild rice, but there has to be confidence in the concurrent chemical analytical results. I will highlight a concern with the data quality of the wild rice response to sulfide, which in this case has to do with the experimental design for the sulfide hydroponics testing. The hydroponics test system exposed the entire plant to sulfide. This exposure condition, with sulfide exposure to the vegetative growth stage, would not occur in nature and introduced highly variable sulfide test concentrations. Specifically, the fully anaerobic test system exposed post-germination plant tissues to sulfide in anaerobic water that would not be present in overlying waters where wild rice naturally grows. During the hydroponics test, as plant photosynthesis occurred, oxygen was released, resulting in decreased sulfide concentrations and highly variable exposure conditions.

Independent of concerns with the sulfide hydroponic test design conditions, the lack of documentation addressing the quality of the sulfide analytical data calls into question the attention to detail and discipline paid to this extremely important component of developing a water quality standard. Enforcement of a water quality standard will be evaluated based on analytical data for a chemical, not on biological response of wild rice. Quoting Dr. Robert Hare: The key is measurement. Science cannot progress without reliable and accurate measurement of what it is they're trying to study. Simple as that.

A few concerns undermining my confidence in the quality of the spectrophotometric sulfide analytical data include:

- The instrument was calibrated using a 1 inch flow path cell, while the samples were measured using a 1 cm flow path cell.
    - It is not acceptable under NELAP or GLP programs to use different equipment for the calibration versus the actual sample analysis
    - Though an adjustment was made to concentrations reflecting use of different equipment (2.5 initially, now to be 2.54), sulfide concentrations were sometimes

ported uncorrected in the excel file. Documentation of uncorrected vs. corrected is lacking leading to a high risk of error.

- The lab did not document many critical aspects in executing this method:
  - The condition (clean and scratch-fee) and the type of cuvettes (plastic, glass, etc.) used in the calibration and used for analysis of samples was not identified
  - The frequency that the instrument was calibrated was not specified
- The referenced Method 8131 uses a wavelength of 665 nm rather than 660 nm cited in the SOP. No documentation is provided as to use of another wavelength.

I look forward to the Peer Review panel's comments on the quality of wild rice toxicity data and the concurrent analytical results in the context of the confidence needed to enforce a water quality standard.

Shifting to my second item – statistical analysis of data - also ties into the charge questions 7 and 8 on the use of quantile regression analysis.

Although quantile regression does provide one statistical method that could be used to predict sulfide at a site based on sulfate or sulfate and iron concentrations, considerable care must be taken when applying this approach. These concerns are particularly important if a rarely used technique, such as quantile regression, is used in preference over more conventional methods such as multivariate regression analysis, which is more widely understood. In my view, MPCA's application of quantile regression is invalid.

Some specific factors affecting the validity of the quantile regression are:

- As discussed by Cade and Noon (2003), quantile regression is most applicable in predicting the importance of limiting factors (e.g., sulfate and iron) on the response variable (e.g., sulfide) when unknown factors are important. Thus, a plot of the data should reveal a wedge shape, such as that presented in MPCA's 2014 Appendix C. However, Figures 23 and 24 do not exhibit a wedge shape that would be indicative of sulfate functioning as a limiting factor for porewater sulfide. Thus, the primary assumption of the quantile regression, that sulfide is limited by sulfate, is not supported by the data. This indicates that the quantile regression is invalid for this application.
- By design, quantile regression attempts to "model around" unknown or unmeasured factors. As discussed in Cade and Noon (2003), quantile regression "is not a panacea for investigating relationships between variables, it is even more important for the investigator to clearly articulate what is being studied and why." Given the low quality of the regression fits and the weak relationships between the measured variables in the field data, I am very concerned that MPCA's analysis is inappropriate. By focusing on the weak relationship

between wild rice, pore water sulfide, iron, and sulfate; other factors that could be far more important could easily be ignored. Furthermore, the analysis does not seem able to provide meaningful predictions regarding the expected benefits to wild rice populations associated with any proposed change to the standard. Quantile regression is seemingly ready-made to ignore the importance of other factors in this analysis.

Once confidence in data validity is established, there are other statistical methods and approaches available that are more appropriate and I look forward to the Peer Review panel's suggestions.

Thank you for your consideration of my comments.

Comments Presented by Leonard Anderson

ADVISORY COMMITTE FEEDBACK TO PEER REVIEWERS

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

Comments Presented by Meghan Jacobson

My name is Meghan Jacobson and I am a limnologist at Emmons & Olivier Resources. I have a PhD in biogeochemistry, and a bachelor of civil engineering from the University of Minnesota – Twin Cities. My professional work focuses on modeling the response of lake water quality to phosphorus loading from the watershed and lake sediments, and developing Total Maximum Daily Load studies. Hundreds of lakes are impaired for eutrophication from excess phosphorus in Minnesota, many with high rates of internal phosphorus loading from the sediments.

While I understand that this study was focused on the mechanisms that control wild rice growth in high sulfate waters, and specifically the interaction between sulfate, sulfide, and iron in sediment porewater, I would like to highlight that in lakes high rates of sulfide production in sediments also play a role in eutrophication which can affect wild rice growth.

When iron is bound to sulfide in the sediments, there is less available iron to bind with phosphorus. Low available iron to phosphorus ratios in lake sediments can result in greater rates of internal phosphorus loading, increased algal growth, reduced water clarity and reduced plant growth. In shallow lakes, increased algal growth from high internal phosphorus loading can flip a lake into a turbid stable state, further propagating high internal phosphorus loading, high concentrations of algae, reduced water clarity, and reduced plant growth.

Therefore in addition to the direct effects of the interaction between sulfate, sulfide, and iron on wild rice growth, I would encourage MPCA to also consider the indirect effect of increased sediment sulfide production on increased internal phosphorus loading, reduced water clarity, and reduced wild rice growth. One suggestion would be to see if there is a correlation between Secchi depth transparency (a measure of lake clarity) and porewater sulfide concentration. (T-tube transparency was included in Table 5 of the report, but this is a measure of stream clarity). And in the development of a method to predict porewater sulfide concentrations based on lake sulfate and sediment iron concentrations (as suggested in line 1124 near the end of the report), it would also be important to account for the effects of reduced available sediment iron on internal phosphorus loading and lake eutrophication.

Thank you for the opportunity to comment.

Comments Presented by Bob Whipple

Boozhoo. Indawaymaganag. Wabashki inzhinikaz. Nindoodaim migizi. (*Hello. My relatives. White Eagle is my name. My clan is the eagle*.) My English-given name is Bob Whipple and I do not have any information to submit to this panel. I am used to speaking from the heart and I just want to say what Paula Maccabee said and thank her for those remarks. I did not have much time to take a look at the information here, but I believe the standards set back in 1940 work just fine. On a personal note, I just want everyone to see that I am a human being, and my granddaughter is a human being. She is also Ojibwe, and whatever results come from this peer review are not only going to impact me, but her and those who follow after her. So, again, the water quality standard for sulfate works just fine at 10 mg/L.

Appendix F

Reviewer Post-Meeting Comments

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Responses to Charge Questions from Dr. Gertie H.P. Arts

Laboratory Hydroponic Experiments (see Analysis, pp. 13-16, 38-39)

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 pore water can be toxic to wild rice.

The hydroponic study demonstrates that sulfide is the compound that is toxic and detrimental to wild rice growth. That is an important result from the hydroponic study. The hydroponic experiments have shown effects of sulfide on both weight and length of stems and roots of juvenile seedlings. The hydroponic study also shows the toxicity ranges which are of concern with respect to the effects on wild rice. However, the concentration test range as used in the hydroponic experiments did not cover the low range of expected toxicity. From this point of view, and for the aim of deducing effect concentrations such as EC10 values – which are recommended here as these toxicity endpoints are common use in ecotoxicology – the experiments are not appropriate.

The submergence of the full wild rice seedling in a sulfide solution was considered as a worst-case approach. It is advised to use an experimental set-up where the roots are separated from the shoot and exposed to sulfide, while the shoots are not.

The results of the hydroponic experiments show that toxicity to the juvenile seedlings starts at concentrations lower than reported in the analysis. The hydroponic experiments show that toxicity starts at concentrations between 150 µg/L and 300 µg/L. These thresholds are in agreement with values in the international literature (Smolders and Roelofs, 1996: 5 µM/L, i.e., 160 µg/L).

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?

The use of the initial exposure level is not appropriate in my view. Due to renewal of the test solutions every two days and a decline of sulfide concentrations during the period in-between de test solution renewal dates (probably because of O_2 production by the wild rice seedlings), the actual exposure concentrations are lower than the initial exposure concentrations. Therefore, a time-weighted average (TWA) concentration would have been more appropriate for calculating the thresholds and effect concentrations of the sulfide experiment. In addition, the variability in initial sulfide concentrations at each sulfide exposure level, also supports the use of a TWA approach.

At page 16 of the MPCA report at line 397 the authors conclude that sulfide is toxic at concentrations above 300 µg/L. However, in my view sulfide is already toxic at 300 µg/L and below. Therefore, the correct conclusion should be that sulfide toxicity starts at concentrations between 150 and 300 µg/L. Based on the test range in the experiments, a NOEC can be deduced of 150 µg/L, or based on average initial sulfide concentrations: 134 µg/L. Please note that taking the Time-weighted-average (TWA) concentrations, this value might be different and probably lower, as the sulfide concentrations decrease during the periods between renewals. TWA concentrations are considered more appropriate in this case and will probably lead to effect concentrations lower than reported in the analysis and presented in Table 1.

Table 1: Effect concentrations (EC50 and EC20) for plant biomass as reported in the analysis

Test	EC_{50}	EC_{20}
Range finder	239	459
Test 1	210	326
Test 2	322	365
Average	257	383

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?

I agree with the regression analysis to derive EC_x values for sulfide toxicity. It is an adequate approach and it is scientifically accepted to derive effect concentrations from these type of tests. However, I do not agree with deriving an EC_{20} as a value where no effects are to be expected. It is more appropriate to derive an EC_{10}. Secondly, I advise to take the Time Weighted Average (TWA) concentrations for calculating effect concentrations, as the experiments have been performed as renewal tests. Therefore and in summary, I suggest to derive an EC_{50} ànd EC_{10} value from the sulfide seedling hydroponic data by means of log-logistic regression analysis and based on TWA concentrations. Deriving an EC_{50} and EC_{10} is common use in ecotoxicology and enables the comparison of results with values published in literature.

Utility of the Field Survey Data (see Analysis, pp. 21-25, 35-36, 41-47)

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 pore water concentrations of sulfide and iron, and 2) inform protection of wild rice from elevated sulfate. Please note any specific questions or concerns.

The field data set is an important and very extended dataset and therefore very useful in the analysis of the wild rice sulfate standard study. Figure 17 is considered an important figure summarizing sediment pore water sulfide concentrations in field sites. Effects on wild rice are visible at sulfide concentrations above 75 µg/L. This preliminary threshold of 75 µg/L is an important result to be considered in the MPCA analysis.

I have some concerns related to choices taken in the process of field data collection and analysis. In the field survey the general aquatic macrophyte habitat is used as a proxy for the wild rice habitat. More specifically, in the analysis of the field data, Water Lily habitats have been used as a proxy for wild rice habitats. However, there might exist some clear differences among different macrophyte habitats. E.g., wild rice can excrete O_2 by their roots (a feature that is also included and addressed in the analysis performed by MPCA), which is not a characteristic of each aquatic macrophyte. Moreover, Water Lily can grow in anoxic organic sediments. Therefore and in my view, the wild rice habitat is most probably a subset of the full dataset as collected in the field survey. The analysis of the data also clearly shows this. Therefore, my concern is that potential wild rice habitats as selected from the field data collection, might not be representative for real wild rice habitats.

As the analysis of the field data survey is based on correlations, those correlations can be used for hypothesis generation. Subsequently, causal relationships need to be tested experimentally. The mesocosm experiment performed and included in this set of studies, is at least partly applicable to test the generated hypotheses.

To me it seems that the field dataset has not been fully explored. Already very early in the process and analysis by MPCA, it is concluded that some variables are not relevant or important, thereby focusing on the most important variables: sulfate, sulfide and iron. However, as there might be interactions between the sulfate/sulfide/iron geochemical cycle and other geochemical cycles like that of phosphorus, ammonium and nitrate (see further in this review), these geochemical cycles and interactions should be more carefully considered in the analysis.

Mesocosm Experiment (see Analysis, pp. 26-32)

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.

I have evaluated the mesocosm study and in my view, the mesocosm study includes a number of drawbacks in its experiment design:

- Sulfide concentrations have been monthly measured in 2012 and 2013. However, more frequent measurements of sulfide in time are lacking.
- I suggest to replace nominal sulfate concentrations by actual time-weighted average sulfate concentrations in order to approach the real exposure concentrations as closely as possible.
- I miss the interactions with nitrate and phosphate geochemical cycles (see also the next pages in my review).
- The mesocosm study did not include a real control (value of 0). The treatment including the lowest treatment of sulfate at the level of the sulfate standard, was considered as the control.
- I suggest to deduce a NOEC (No Observed Effect Concentration) from the mesocosm study by means of an ANOVA analysis e.g., the Williams test (ANOVA; Williams, 1972).

- The mesocosm study mimics sulfate pollution via surface water and not via groundwater. Is this a correct approach ? How does sulfate pollution in groundwater influences the sulfate and sulfide concentrations in surface water, e.g., in the field survey ?
- What was the percentage organic material and organic carbon in the sediment ?
- How deep has the pore water been sampled (see Fig. 12) ?
- Please evaluate the mesocosm study by calculating NOECs and EC_{50} values and EC_{10} values for endpoints (the latter ones only when assuming the controls are the 7 mg/L sulfate treatments). If such an eco-toxicological evaluation is applied, these values can be compared with those from the hydroponic experiments and maybe with the results from the field survey in a risk assessment approach.

We received additional information that the mesocosms included aerobic ànd anaerobic sediment layers: 1 mm aerobic top layer and deeper anaerobic layers. It is expected that the roots of wild rice – excreting O_2 – have contributed to the pattern of aerobic and anaerobic patches in the sediment. Therefore it might be concluded that oxidation and reduction processes have occurred in the sediment of the mesocosms.

Wild Rice in Relation to Sulfate, Sulfide, and Iron (see Analysis, pp. 35-39)

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 pore water above 300 µg/L can be toxic to wild rice? Why or why not?

I do not support the threshold value of 300 µg/L. If the EC_{20} is considered as a proxy for the NOEC, then effects can already be expected above values of 200 µg/L (EC_{20} in the range of 210 – 322 µg/L, see pages 36 and 37 of the MPCA report). Values can be compared to values published by Lamers et al. (2013) for a range of macrophytes. In the MPCA report this paper by Lamers et al. (2013) has been referred to. The values for wild rice as referred to above, are in the lower range of values for *Oryza sativa* in Lamers et al (3013).

The value of 300 µg/L for effects of sulphide to wild rice is too high. As stated before, if a Time Weighted Average approach will be applied to the data, this will end up in lower effect concentrations than currently included in the MPCA analysis. Also the field survey shows a threshold value of 75 µg/L (see figure 17 in the MPCA analysis report), which is a value I support more compared to the value of 300 µg/L.

Control of Pore water Sulfide by the Availability of Sulfate and Iron (see Analysis, pp. 21-25, 45-47)

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

First of all, I suggest that in any modelling, and I have stated before, the sulfate / sulfide supplied by groundwater should be included in this analysis as well. This requires addressing the full hydrological system (supply by surface water and groundwater).

The use of Structural Equation Modeling (SEM) for pore water sulfide seems to be a promising approach. This type of modeling has many advantages over the multiple quantile regression as applied in the MPCA analysis report. I strongly support the use of Structural Equation Modeling.

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

If I understand correctly, the acid-extractable iron represents the available fraction and not the fraction that is actually present in the pore water. I support this approach to predict pore water sulfide concentrations. If I understand correctly, the available fraction includes the fraction that can potentially become available e.g., by a change in equilibrium between pore water and soil particles (e.g., by formation of iron sulfide).

Synthesis: How Sulfate, Sulfide, and Iron Interact to Affect Wild Rice (see Analysis, pp. 51-52)

Charge Question 9: The MPCA Analysis focuses on sulfide in the pore water as the sulfur parameter impacting wild rice, and the role of sulfate and iron as key variables controlling sulfide concentrations in pore water. 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?

Sulfur dynamics and geochemistry are very much related with and interact with other geochemical cycles, such as that of iron, phosphorous and nitrate. The iron cycle has in depth been considered in the MPCA analysis. However, I miss the interaction with the phosphorous and nitrate cycles. Therefore, and in my view, also these variables need to be taken into account in order to make a full analysis of the sulfate / sulfide issues:

a. The phosphor binding and phosphor cyclus need to be considered in my view. Iron oxides and iron hydroxide compounds may be bound to phosphate. Accumulation of sulfide lowers phosphate binding to iron oxides and iron hydroxide, thereby increasing phosphate availability in anaerobic sediments. (Lamers et al., 2013). E.g., in Paddy's more Iron oxides and iron hydroxide might be bound to phosphate in these probably nutrient rich systems (see Fig. 1, Smolders et al., 2003; see page 42 if the MPCA report). See Figure 1 as an example of interactions. An important feature of the processes shown in Figure 1 is that they interact via a loop back: the internal loading of phosphorus and ammonium has the ability to further amplify the sulfate-sulfide response. This type of interactions and loop back can be modeled by Structural Equation Modeling (SEM).

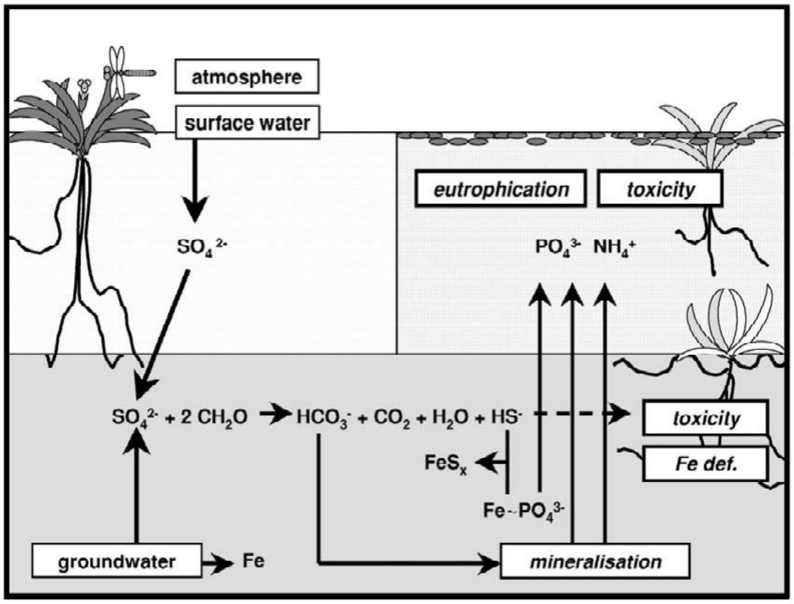

Figure 1: Mechanisms involved in the decline of Water soldier (*Stratiotes aloides*) in The Netherlands.

b. Interaction with nitrate: water bodies receiving high nitrate loads through the discharge of groundwater originating from fertilized agricultural lands, show low iron and low phosphate reduction rates (Lamers et al., 2013). See Figure 2 as an example of interactions.

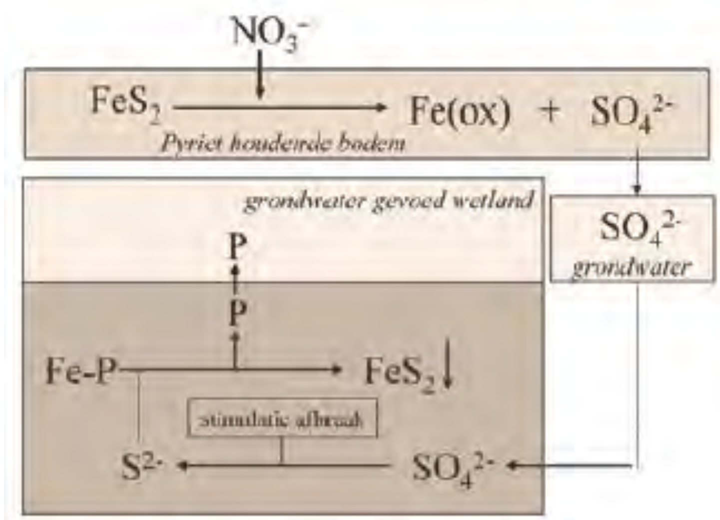

Figure 2: Interaction with nitrate cycle (Smolders et al., 2006).

c. The oxygen leaking out of the roots of Wild rice, will result in an aerobe layer around the roots, oxidizing sulfide to sulfate. This effect of Wild Rice is important and has been addressed quite late in the MPCA report.

Besides pore water, groundwater quality needs to be considered as part of the full analysis, especially the sulfate/sulfide, iron and nitrate concentrations in ground water.

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.

In my view, the question "what controls the accumulation of sulfide in pore water needs to considered more extensively, i.e., more variables need to be included in the analysis (phosphate, nitrate). Also the input via ground water into the aquatic systems under consideration (lakes, streams) is not considered in the analysis. In the analysis it is assumed that sulfate penetrates from surface water into the sediment. The full hydro-geochemical system needs to be included, incl. groundwater quality and groundwater discharge into surface water.

I advise to distinguish more precisely between rivers and lakes, especially in the conclusions made in the MPCA report. E.g., in Figure 20, streams show the lowest Spearman correlation (- 0.35, Appendix F). I wonder if it is correct to draw a straight line through these individual values. The same holds for lakes. The distribution of the values seems not to following any line, but the values seem to represent a cloud of values. I am not fully convinced if a straight line is the appropriate statistical regression here.

I suggest to consider paddies as a separate category, as they might be more heavily loaded with nutrients. Moreover, they are managed (dried and harvested), which might contribute to oxidative processes and iron bound to phosphate.

General Questions

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

In general, I support the synthesis performed by MPCA. Appropriate study components have been chosen. However, as stated before, I suggest to use the field study for hypothesis generation. These hypotheses can be tested in an experimental setting, e.g., in mesocosms. This has not been done that clearly. It seems the different study components were related to each other afterwards, making the overall synthesis correlative.

I also strongly recommend to use all studies in the analysis, i.e., also the incubation study and rooting zone geochemistry study.

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

- I suggest to make as much direct relationships as possible, e.g., in Figure 10 replace sulfate by sulfide.
- I suggest to distinguish more clearly between lakes and streams. See e.g., the difference as depicted in Table 9, page 39. Lakes represent a more worse-case scenario and maybe therefore the wild rice sulfate standard should be differentiated between these different habitats.

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.

- Please also address sulfate in ground water and include other variables in the analysis (nitrate, phosphorus).
- Several phosphate and nitrogen parameters have been measured in the sediment during the field survey (e.g., sediment exchangeable P; nitrate; ammonium). I suggest to include them in the analysis.

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Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Responses to Charge Questions by Dr. Donald M. Axelrad

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.

I do not believe that the hydroponics study is the best of the three 'sulfide effects on wild rice' studies from which to derive a no-effects concentration for sulfide to wild rice (the other two being the mesocosm and field studies). The hydroponics study was however useful in demonstrating that sulfate is not toxic to wild rice.

I do not accept the argument that the hydroponics study EC20 is a no effects concentration for sulfide to wild rice; consider using an EC5 or EC10.

The decision re which EC to use should be based on science and should be a number that allows for sustainability of wild rice cover – if that is MPCA's desired outcome for protection of wild rice. Such a number would be better derived from a long-term study, not the 10 or 11 day hydroponics study.

As well for the hydroponics study there is the experimental issue of the shoot being immersed in anaerobic, high sulfide water; a condition that might not take place in the natural environment.

The hydroponics study is also limited in that it only deals with two life stages for wild rice; germination, and seedling growth, and we have no basis to say that these are the most sensitive life stages for wild rice as regards sulfide toxicity. In contrast, the mesocosm and field studies obtain data on wild rice over several annual cycles.

My present feeling is that the field study is best suited to derive a safe sulfide exposure number for wild rice. Further statistical analyses of these field data are warranted.

It could however be informative to repeat the wild rice hydroponics bioassay, conducting it for a longer time period, and maintaining the plant roots in an anaerobic environment and the shoots in an aerobic environment.

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?

For reasons as stated in my answer to question number one, I do not believe that the hydroponics study is the best of the three 'sulfide effects on wild rice' studies from which to derive a no-effects concentration for sulfide to wild rice.

Answering question number two as posed, I do not believe it is appropriate to use the initial sulfide exposure concentration as the operative exposure concentration for the hydroponics test. There is consensus on the Panel for this position.

In the absence of further information on specific mechanisms of toxicity, I believe the best sulfide concentration data for use in statistical analysis of the hydroponics study is time weighted average.

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?

For reasons as stated in my answer to question number one, I do not believe that the hydroponics study is the best of the three 'sulfide effects on wild rice' studies from which to derive a no-effects concentration for sulfide to wild rice.

Answering question number three as posed, regression analysis is appropriate for the hydroponics data. However I question if an EC20 value should be used as a no-effects concentration; instead consider EC5 or EC10.

There is also the issue of use of initial, that is highest sulfide concentration, to develop ECs. This is inappropriate, and in the absence of further information on specific mechanisms of toxicity, I believe the best concentration for use in statistical analysis of these data is the time weighted average.

Importantly, the field study suggests lower no-effects sulfide concentrations than those derived from the hydroponics study. The field study suggests that sulfide above 75 µg/L in porewater is problematic, and one panelists further analysis of the field data, indicated that levels above 20-50 µg/L could result in toxic effects. It appears that the field study may be better suited from which to derive a no-effects concentration for sulfide for protection of wild rice.

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.

After the panel discussions, I felt that Question 4 was incomplete; in particular it assumed that the field study would not be used to derive a porewater sulfide number protective of wild rice, and I rewrote the question as follows.

1. Was the field study sampling such that it collected data representative of the range of surface water and pore water variables hypothesized to affect wild rice, and to examine the relationships between these variables?

2. Was field study data collection and statistical analysis appropriate for these purposes?

3. Can field data lead to proof of causation and establishment of pore water sulfide levels that are protective of an appropriate wild rice beneficial use endpoint?

The following comments I wrote with Dr. Fennessy and they are included in the ‘2014 08 20 Panel Conclusions of Analysis’.

The field survey study was comprehensive and provides a rich data set that can be used to address the question of how, and at what level, sulfate affects wild rice. The field survey encompassed a wide range of sites that varied in their concentrations of sediment water and porewater sulfate, sulfide, and iron. The panel agrees that the field survey provides some of the best data that the MPCA has available to investigate wild rice response to surface water sulfate levels. These data also offer a means of determining sulfide levels that are protective of wild rice. Much more analysis should be done to mine this data set, for instance, additional statistical analysis of the field data, such as defining breakpoints regarding wild rice cover or the probability of wild rice occurrence versus porewater sulfide levels would be instructive. Further, while trends in the data are important, the variability in the data can be equally instructive and should be considered more carefully in the analysis.

The analysis would have been improved if more wild rice sites in the two high sulfate areas of Minnesota were sampled in the Analysis; further discussion of the field locations where wild rice was absent, but were determined to be suitable for wild rice growth, is warranted.

Trends may be clarified if the data for lakes, rivers and wetlands were more fully analyzed independently. The hydrology, chemistry and biota of these ecosystems can vary dramatically; classifying these sites for analysis may reduce variability in the data and reveal a differential response to sulfate.

The initial modeling of the field data using a limited subset of variables only accounted for about 20% of the variance in wild rice cover. Further analysis with a more expanded set of variables needs to be conducted. For instance, N and P are important factors in wild rice growth and are affected by sulfate concentrations. Expanding the complexity of the models should help elucidate the interactions of the environmental variables that determine wild rice distribution.

Combining the field data with the sulfide dose-response results from the hydroponic and mesocosm experiments, and the finding of no-effect of sulfate in the hydroponics study provides weight of evidence that sulfide is the cause of toxicity. If possible, analyses looking at the decline of wild rice cover over time with associated water quality variables would provide support to link field results to the cause of declines.

The field study data (Figure 17 in the Analysis) support a working hypothesis of 75 µg sulfide/L as a threshold for toxic effects. Preliminary statistical analysis by the panel shows that this level may be as low as 20 to 50 µg/L. The panel concurred that the MPCA data support the preliminary finding of a threshold of 75 µg/L more than the proposed level of 300 µg/L.

Percent wild rice cover was a semi-quantitative measure used in the field survey. Stems per m^2, biomass per m^2, or the number of flowering stems should be considered instead as they are more quantitative biological endpoints that may more fully document the vigor of wild rice populations.

The integration of a biogeochemical model (e.g., structural equation modeling [SEM]) with data on the wild rice life cycle (e.g., a demographic model) is needed to better link the role of sulfate as a driver of wild rice population declines.

The lack of data on wild rice (presence/absence) in the field sites hinders use of an otherwise rich data set. Better definition is needed by MPCA of a wild rice beneficial use endpoint that could be used in future field surveys.

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 performance of wild rice over its various life stages in the mesocosm controls should be compared to wild rice performance in the natural environment at similarly low surface water sulfate levels to validate the mesocosm approach.

For analysis of mesocosm data, mixed-level (hierarchical) modeling would improve statistical power.

Actual rather than nominal sulfate concentrations should be used in statistical analyses.

Demographic data from the mesocosms could be used to develop a population model to calculate extinction probabilities for various sulfate concentrations (and time horizons).

Plant responses in mesocosms are presently plotted against nominal surface water sulfate concentrations and should instead be related to measured porewater sulfide concentrations.

The mesocosm experiment, like the hydroponics experiment, could be used to calculate acceptable porewater sulfide concentrations for protection of wild rice; this for different wild rice fitness endpoints and life stages. Among the data resulting from the mesocosm experiment were, number of seedlings produced and seedling survival, versus surface water sulfate concentration. By relating surface water sulfate concentration to porewater sulfide concentrations, and then with use of wild rice population dynamics information, and assuming a desired endpoint such as a number of seedlings needed to support a desired wild rice density, a target porewater sulfide concentration "standard" could be calculated. This approach is in fact more 'real world' than hydroponics data EC20 and EC50 numbers.

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?

I disagree in that the question implies that the hydroponics study should be central in deriving a wild rice sulfide no-effects concentration despite its major experimental limitations:

1. The hydroponics study only deals with two life stages for wild rice; germination, and seedling growth over only 10 or 11 days, and we have no basis to say that these are the most sensitive life stages for wild rice as regards sulfide toxicity; in contrast, the mesocosm and field studies obtain data on wild rice over several annual cycles;

2. The field study suggests lower no-effects sulfide concentrations than those derived from the hydroponics study. The field study suggests that sulfide above 75 µg/L in porewater is problematic, and one panelists further analysis of the field data, indicated that levels above 20-50 µg/L could result in toxic effects.

My present feeling is that the field study is best suited to derive a safe sulfide exposure number for wild rice. Further statistical analyses of these field data are warranted.

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?

Multiple quantile regression is not the best tool for predicting porewater sulfide concentrations as indicated in Panelists pre-meeting comments and in the '2014 08 20 Panel Conclusions of Analysis'. Structural equation modeling (SEM) is a better statistical tool for predicting porewater sulfide concentrations as a function of iron, sulfate, sediment total S, and sediment TOC.

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?

I disagree with this approach. This because the effect of sediment iron on porewater sulfide is mediated by its effect on porewater iron. The effect of sediment iron is therefore indirect rather than direct, and thus multiple quantile regression is not an appropriate statistical technique. SEM is a better statistical tool for predicting porewater sulfide concentrations.

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?

There is significant literature on sulfide in porewater impacting plants, on surface water sulfate being related to porewater sulfide, and on and iron controlling sulfide concentrations in porewater, and thus the focus of the MPCA Analysis is appropriate. SEM would determine if other variables are important in predicting porewater sulfide, e.g., sediment TOC, etc.

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.

The necessity to exclude alternative explanations for cause-effect linkages is a principle of causal analysis. In the Florida Everglades where there is also decline of cover of an important plant species, the emphasis re causation is on phosphorus concentrations rather than on surface water sulfate and porewater sulfide, though the latter have been demonstrated to be a possible factor. Phosphorus and nitrogen commonly affect plant abundance and succession. Sims et al. (2012) found that increased nitrogen increased numbers of wild rice stems, flowers seeds and seedlings. But it is possible that for some level of nutrients, other plants might out-compete wild rice. Have nutrients been ruled out as factors affecting wild rice cover in Minnesota?

The Synthesis leans on the hydroponics experiment to set a no-effects sulfide concentration for wild rice and multiple quantile regression to predict porewater sulfide based on sediment iron and surface water sulfate. Both of these approaches are suspect for reasons discussed previously and the Synthesis needs a rewrite.

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

The hydroponics, mesocosm and field studies are important and appropriate components to meet Study objectives; their findings support one another regarding toxicity of sulfide to wild rice, and provide support for the surface water sulfate, porewater sulfide and iron paradigm. I do not accept that the wild-rice-protective sulfide numbers derived from the hydroponics study, somewhere between EC20 and EC50, as the most appropriate to protect wild rice, nor do I accept that the hydroponics study is the best one from which to derive a wild rice sulfide no-effects concentration.

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

Structural equation modelling would be better at elucidating important factors affecting wild rice cover and this analysis should be extended beyond that already conducted.

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.

Wild rice cover endpoint needs definition: Natural Wild Rice In Minnesota, (2008, MDNR, p.2.) states *"Although stands of natural wild rice occur most commonly in central and north-central Minnesota, the historic range of wild rice included all of the state."* Is an appropriate restoration goal to improve water quality so as to promote wild rice cover statewide? If not, what is that goal? Certainly it is not to protect wild rice to some undefined extent by maintaining a porewater sulfide concentration somewhere between an EC20 and an EC50 as derived from a short term hydroponics study. The present lack of definition of a desired outcome for wild rice will hamper achievement of protection.

A principle of causal analysis is temporal precedence of the cause(s). Are there sites where there was once significant wild rice cover that now have little cover, where there are associated water quality data that can be used to determine sulfide no-effects concentrations? Are there 'dead zones' grading from no wild rice cover to significant cover, with associated surface water sulfate and pore-water sulfide and iron data?

Allam (1971) reported sulfide inhibitions of catalase, peroxidase, ascorbic acid oxidase, polyphenol oxidase and terminal cytochrome oxidase, which influence the oxidative capacity of rice roots.

Armstrong and Armstrong (2005) found evidence for sulfide-induced barrier to Fe^{2+} absorption in adventitious roots of rice, and wrote (p. 632), *"This is, to our knowledge, the first documentation of sulfide inducing barriers to root ROL (radial oxygen loss), blockages in the internal aeration and vascular systems and inhibiting lateral root emergence in rice…"* and, *"The influence of sulfide on anatomy and root growth can be correlated with a number of physiological effects"*

Examination of whether sulfide causes changes to wild rice anatomy related to physiological effects would add an important element to the examination of in particular, field data, and the calculation of a porewater sulfide concentration protective of wild rice.

Finally, field data should be more fully analyzed via SEM and other methods to better define the relationships between sulfate, iron, sediment TOC, sediment total sulfur, and sulfide and relate these to wild rice occurrence.

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Responses to Charge Questions by Dr. Patrick L. Brezonik

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.

This set of experiments represents a version of an acute toxicity test. The implicit assumptions seem to be that (a) seed germination and (b) growth of seedlings are sensitive (the most sensitive?) life stage of the plants. Although this appears to be reasonable, no evidence (e.g., literature citations) is provided.

I was surprised that there were no sulfate treatments lower than the current standard of 10 mg/L (e.g., 1-2 or 5 mg/L) nor any at small multiples above the standard (e.g., 20, 30, or 40 mg/L). The next lowest treatment level was 50 mg/L, which is rather high. The germination and growth experiments with sulfate were otherwise done properly and provide evidence that sulfate itself is not toxic or inhibitory to wild rice seed germination or seedling growth at sulfate concentrations much higher than the standard and even higher than the ambient concentrations in Minnesota surface waters shown in Figure 1 and Figure 7 (field survey data) of the *Analysis* document. The *Analysis* goes too far, however, in stating (line 775) that "hydroponic exposure of wild rice to sulfate showed that sulfate is not directly toxic to wild rice at the concentrations likely encountered in water bodies across Minnesota." It would be more appropriate to say that the hydroponic experiments did not provide evidence for sulfate toxicity to wild rice seedlings and sprouting of seeds. The experiments were not conducted through the entire life cycle of wild rice plants, and thus one cannot unequivocally say that sulfate is not toxic or inhibitory to wild rice plants.

The *Analysis* does not present data to support the above conclusions; interested parties must access the reports of Pastor for these data. It would have been easy to include a summary table or figure to support the "no-effect" statements. The conclusions about sulfate otherwise are not surprising insofar as the inhibitory effect of sulfate on wild rice long has been presumed to be mediated by sulfate reduction to sulfide and toxic effects of sulfide in the root zone. For the same reason, the sulfate results themselves cannot be used as evidence that the current sulfate standard is overly protective of wild rice. The MPCA appropriately does not imply that they can.

The range of sulfide concentrations used in the seed germination experiments was high compared to the range of typical sulfide values in porewater (e.g., as shown by field survey results in Figure 8A). High concentrations are common in short-term toxicity studies designed to determine a response; the paucity of low sulfide treatment levels is a bigger concern. Insofar as the experiment showed no negative effects on germination even at concentrations higher than found in all but a few sites shown in Figure 8A, the range used for the germination tests was satisfactory. The *Analysis* states that no sulfide effect on germination was found, but data are not provided to support the statement. Again, a summary table should have been included to support the "no-effect" statement.

I have three main concerns regarding the experiments to evaluate sulfide effects on seedling growth. First, the physical system used for the experiments was too simple to yield optimal results. Seedlings were completely immersed in the sulfide/growth solution instead of having the roots in the solution and upper (vegetative) part of the plant exposed to oxygenated water, as happens in nature. It should have been possible to design and build containers where a membrane with a small hole for the plant stem separates oxic and anoxic water layers. Such a system would have enabled the experimenters to continuously or periodically replace the anoxic growth/sulfide solution with fresh solution, thus maintaining more uniform sulfide levels. Assuming that oxygen entered the solution directly from the photosynthesizing leaves, such a system would have helped to control the build-up of oxygen in the water.

Second, it would have been highly desirable to have additional treatment levels at low concentrations: < 96 µg/L and between 96 and 320 µg/L. Although the measured values for a given treatment level actually produced a range of concentrations, as described in the text (line 375), the experimental design still resulted in a large gap (~ 1.5 log units) in measured initial sulfide levels, as shown in Figure 6.

Third, the MPCA used initial sulfide concentrations in their analysis, but the sulfide concentrations actually declined over time, in many cases by large fractions, over the exposure period; this is addressed further in charge question #2.

Aside from the concern about using initial rather than time-averaged values, the MPCA cannot conclude that the lowest level (134 µg/L) *was not toxic* based on the lack of a statistically significant effect using ANOVA. All they can say is there was *insufficient evidence to conclude that this concentration was toxic* under the conditions of the experiments. The ANCOVA test results make more sense from toxicological perspective. Finally, there is a large range in the EC20 results for the three tests; the results for D2 are substantially higher than those for R and D1. Similarly, the EC50 value for R is much bigger than those for D1 and D2. Although bioassay data are subject to greater uncertainty than one expects for strictly chemical analyses, the uncertainty in the EC20 and EC50 values at least partially reflects shortcomings in the physical set-up for the experiments and the small number of sulfide levels used in the experiments.

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?

The net effect of MPCA using initial concentrations was to produce EC20 and EC50 values that are higher than the actual EC50 and EC20 values for the seedlings. For a given initial concentration, the mean concentration over the total exposure period was lower, and there is little reason to think that the seedlings responded just to the initial (peak) concentrations, which apparently were of short duration. It is likely that toxic/inhibitory effects are related to time-averaged concentrations over the duration of exposure—or to the integrated value of $C_i t_i$, where C_i is the average concentration of sulfide for some exposure sub-period t_i, such that the actual range of sulfide concentrations in a given exposure sub-period t_i is relatively small (e.g., ± 5-10% of the mean for the sub-period). The *Ct* concept has long been used to model microbial die-off in disinfection and is the basis of Watson's law. The MPCA should re-analyze the data for the seedling growth experiments using time-averaged sulfide concentration values. Because the decrease in sulfide over time probably followed first-order kinetics, i.e., exponential decay, a good estimate of the time-averaged concentration would be the geometric mean of the initial and final concentrations.

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?

Regression analysis is an appropriate way to analyze the seedling growth data to identify effect levels, and fitting experimental data to a logistic equation is the standard way to calculate EC50 and other EC values. I wonder why EC20 was chosen as an effect level and not EC10, or even EC05. At EC20, 20% of the population is affected, which seems high in terms of protecting wild rice stands. Because of the nature of the short-term bioassays—that is, the number of treatment levels and replicates at a given treatment level, it is possible that inhibitory effects would not have been seen at the modeled EC20 value, but this should not be taken to imply that no effect would have occurred if more replicates and/or more treatment levels had been used. Consequently, it is not appropriate to link the EC20 to a no-effect concentration (i.e., a NOEC). If the MPCA re-analyzes the data using time-averaged concentrations of sulfide for the exposure experiments, they will obtain considerably lower EC20 and EC50 values than reported in the *Analysis*, but the EC20 still should not be used as a threshold level for deleterious effects of sulfide on wild rice. It is difficult to estimate what level of exposure to sulfide would be protective of wild rice populations based solely on the results of short-term bioassays like the hydroponic studies, but it seems to this reviewer that a level affecting 20% of the population (i.e., EC20) is not protective. EC05 would be a more reasonable target level, although again one still has the problem that growth of seedlings is not necessarily the most sensitive life stage of wild rice plants. A weight-of-evidence approach that includes results from all phases of the study—short-term bioassays, long-term, whole life-stage studies (the mesocosms), and field observations—should be used to derive such a value.

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.

The *Analysis* does a good job describing the data from the lake and stream field surveys and explaining differences in distributions of sulfate and other variables between the survey water bodies and Minnesota lakes and streams as a whole. I do not have concerns about the basic design of the surveys in terms of geographic coverage, which is broadly across the regions of the state where wild rice stands occur or about the distribution of surface-water sulfate, porewater sulfide and iron levels, and acid-extractable Fe and AVS in sediment in the survey results. I do not think that a probability-based design was needed or would have been appropriate. However, the survey should have sampled the few wild rice sites in SW Minnesota that occur in regions with high sulfate (Figure 1 of *Analysis* document). I also think that the MPCA used the field survey results appropriately to examine relationships between sulfate in surface water and porewater concentrations of sulfide and iron.

Why a Spearman (rank) correlation was done (line 463) and not a Pearson correlation should be explained. Spearman correlation does require fewer assumptions about data distribution, but these are easily tested assumptions, and Pearson correlations are generally considered to be stronger indications of quantitative relationships between two variables.

It is difficult to discern the negative relationship between Fe and sulfide from the two sets of maps in Figure 8 A and B. I would have been better to plot the concentration data one versus the other on a bivariate graph and include the correlation (line of best fit, r^2, etc.) on the graph. I note this sort of graph is included much later in the report.

Several possible explanations come to mind regarding the comment on line 563 that streams seem to have a higher sulfate frequency distribution than lakes. There is greater temporal variability in sulfate levels of streams versus lakes—streams generally are "flashier" and lakes more integrative of inputs and loss processes over time. The stream data thus may reflect "short-term" higher concentrations from anthropogenic sources such as wastewater effluent and farm fertilizer runoff more than lakes, as well as lower concentrations when rainfall-runoff constitutes much of the flow in some streams. Regarding the likelihood that wild rice can grow in streams with higher sulfate levels than they can tolerate in lakes, streambeds are likely to be less organic and thus less anoxic than lake sediments, leading to lower sulfide levels in the root zone in stream habitats than in lake habitats for wild rice.

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.

In my opinion, the *Analysis* document makes appropriate use of the mesocosm data. The MPCA evidently regards the experiments as providing useful information or they would not have continued to support the study into 2014. More detailed analysis of the sediment porewater profile data collected in 2013 in conjunction with analysis of growth and other condition data for wild rice plants should be useful in improving the MPCA's understanding the dynamics of sulfide formation and precipitation by iron.

The MPCA concluded on p. 37 (line 834) that the variability of sulfide in the mesocosms could not be explained by the variability in available iron because the sediment in all mesocosms was from a single source. Although the total iron in the sediments should have been uniform, it does not necessarily follow that the iron available for sulfide precipitation would have been the same in all treatments. Variations in mixing and exposure to oxygen when the mesocosms were established could have caused variations in available iron.

The statement on p. 7 (line 200) of the *Analysis*: "*…that the containers may not have reached equilibrium for the sulfide, sulfate and iron reactions (meaning there may be excess iron available to "buffer" the elevated sulfate, but once the iron is used up a toxic effect may be seen at lower sulfate...*" is problematic. I do not recall seeing this issue addressed in later parts of the *Analysis*. Moreover, it seems to imply that there is a fixed amount of available iron in the sediment at a given location but a *continuing (renewable)* source of sulfate in the overlying water. However, new sediment is formed on at least a semi-continuous basis, at least in accreting lake sediment environments.

Finally, as a general recommendation I think the MPCA should pay more attention to outliers and the large variances associated with certain trends and not just focus on the trends. They did this nicely in the case of the multiple quantile regression analysis showing the dependence of the sulfide-sulfate relationship on iron levels. An example where this is not done so well is on p. 30 (line 669) in reference to Figure 13: "*Apparently sufficient iron was available to precipitate much of the sulfide (as AVS) as it was produced, yielding an increase in AVS with increasing sulfate treatments* (sic) *concentrations.*" The figure itself, however, shows that the range of AVS production at a sulfate treatment level of 300 mg/L encompasses nearly the entire response range, and the interquartile range for the response (~1000-2000 mg/kg) was almost half of the total response range. Although the text correctly states that there was a trend of increasing AVS production with increasing sulfate, the trend doesn't tell the whole story. The large range of responses at the highest treatment level begs for additional analysis.

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?

In general, I agree that the field survey and mesocosm data support the conclusion that high levels of sulfide are toxic to wild rice. I strongly disagree, however, with the way the question is worded, which implies that only levels > 300 µg/L can be toxic. The field survey results in Figure 17 of the *Analysis* indicate that negative effects may occur at levels as low as 75 µg/L, and the mesocosms results in Figures 10 and 11 show generally linear decreases in seed production and seedling survival over the treatment range. There does not seem to be an obvious threshold sulfate concentration below which there are no effects, but the small number of treatment levels limits the reliability of this conclusion. The *Analysis* also noted (p. 37, line 832) that porewater sulfide levels are linearly related to surface-water sulfate concentrations, although variance in the relationship increases with increasing sulfate levels. Together, these findings from the field surveys and mesocosm experiments support the conclusion that elevated sulfide levels are toxic to wild rice plants. The concentration of sulfide above which effects may be deemed significant for survival of wild rice stands is uncertain, but the results do not support an interpretation of the above statement that *only* sulfide levels > 300 µg/L can be 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?

The use of multiple quantile regression is an innovative solution to a difficult problem and seems to be an appropriate tool to use in this situation. The technique is widely used in economics and ecology, where relationships between two variables are complicated by the existence of additional factors, as is the case with surface water sulfate concentrations and porewater sulfide concentrations. Conventional multiple regression analysis could have been used to explore the relative importance of surface water sulfate and various measures of iron in the sediment (porewater dissolved Fe and AVS in the solid sediment) in explaining sulfide concentrations in porewaters. Conventional multiple regression evaluates best-fit by minimizing the difference in sum-of-squares between the *mean* response and observed responses for a set of values of predictor variables, but quantile regression estimates the *median* response or *any selected quantile* response given a set of predictor variables. Because the MPCA was interested in the "near-maximum" response of porewater sulfide to surface-water sulfate, use of quantile regression makes sense. A level of pragmatism was involved in selecting the 75^{th} percentile for the predicted relationship; higher percentiles (e.g., 95%) would more closely approach the maximum sulfide expected for a given sulfate level, but the paucity of data as one approaches the maximum increases the noise and uncertainty in the relationship. Overall, I conclude the MPCA used reasonable judgment in selecting the 75^{th} percentile.

Regarding Figure 22, the first of several figures related to the multiple quantile regress analyses, the text is not clear concerning how the red line was empirically fit and why it isn't a 1:1 line with sulfate. If the line in Figure 22 is the same as the red line in Figure 23, this should be stated; if not, how it differs should be described. If the lines in Figures 22 and 23 are the same, then the statement in the legend for Figure 22 is not exactly correct because the red line in Figure 23 represents the 95^{th} percentile fit, not the true maximum sulfide for a given sulfate concentration.

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?

Arguments can be made on both sides depending on the purpose of the analysis. For example, if one is interested in determining what factor controls porewater sulfide concentrations at a given site and time, it would be appropriate to use the dissolved Fe concentration in the porewater. The results in Figure 19 convincingly show that dissolved sulfide and dissolved Fe concentrations are negatively related, implying that formation of FeS solid phases may control the concentrations of both sulfide and Fe in anoxic porewaters. It would be useful for the MPCA to re-plot the data in terms of molar concentrations of sulfide and Fe and determine whether the slope of the best-fit line is – 1, as it should be if the data fit the reaction: $FeS_{(s)} + H^+ \rightarrow HS^- + Fe^{2+}$. Equilibrium constants ($^*K_{s0}$) for this generalized solubility equation are known for various FeS solid phases, including amorphous ferrous sulfide and mackinawite (e.g., Davison, *Aquat. Sci.* 53: 309-29, 1991). This is discussed in the report by Johnson (2013) but not in the *Analysis*. Lines representing equilibrium conditions for a given pH (i.e., where the ion activity product (IAP) = $\{HS^-\}\{Fe^{2+}\}/\{H^+\} = {}^*K_{s0}$) could be drawn on the graph, and the graph could be interpreted in terms of which mineral phase controls the process and whether (on average) the porewaters are under-, super-, or just saturated with respect to the mineral phase. Davison (1991) found amorphous FeS was the controlling phase in anoxic freshwater environments he studied, but mackinawite or greigite was the controlling phase under seawater conditions. Johnson (2013) concluded that porewaters of the control and low-sulfate mesocosms were near saturation with respect to amorphous FeS, but high-sulfate treatments were significantly over-saturated, which suggests that sulfide was being produced in these systems at rates faster than FeS precipitation was occurring. It is pertinent to note that precipitation kinetics are complicated and frequently slow in natural environments such that supersaturated conditions can prevail for extended periods of time (e.g., Brezonik and Arnold, *Water Chemistry: An Introduction to the Chemistry of Natural and Engineered Aquatic Systems*, Oxford Univ. Press, York, 2011, pp. 393-400).

One issue of concern regarding Johnson's analysis is that it is not certain whether concentrations of the ions Fe^{2+} and HS^- were corrected to activities using activity coefficients computed from the ionic strength of the porewater. In addition, it appears that no effort was made to account for complexation (or binding) of Fe^{2+} by dissolved organic matter in the porewater, the net effect of which would be to decrease the concentrations of free Fe^{2+} in the water. Stability constants for Fe^{2+} with natural organic matter are somewhat uncertain, and this is a complicated subject, but the MPCA may find the article by Rose and Waite (*Marine Chemistry* **84**: 85 [2003]) to be informative.

The main question of interest to the MPCA, however, was more complicated (p. 46, line 1086): *What would the porewater sulfide concentration be (and what would the effect on wild rice be) if the sulfate concentration in the surface water were increased by a given amount?* A physically-based analytical model to address this question would consist of several diffusion-reaction equations and a set of complicated partial differential equations; such models are referred to as "sediment diagenesis models." The numerous coefficients and boundary conditions in such a model would be very difficult to evaluate, and development and use of such a model would not be practical.

The MPCA used an alternative empirical approach using multiple quantile regression analysis (MQRA) in which sulfate is effectively the driver variable for sulfide production but the process is "mitigated" by the presence of variable amounts of acid-extractable iron (AEI) in the sediment. The implicit conceptual model behind the MQRA assumes porewater sulfide concentrations are the result of (i) sulfate loading via diffusion from surface water across the sediment-water interface and into the sediment porewater, (ii) reduction of sulfate by anaerobic sulfate-respiring bacteria once it diffuses into the anoxic sediment, and (iii) sequestration of some of the sulfide by reaction with the reservoir of iron in the sediment. Dissolved Fe^{2+} in the porewater at any given time is only a small fraction of the total iron available in the sediment to sequester the H_2S/HS^- produced by the sulfate diffusion-microbial reaction (respiratory reduction) processes. It thus made sense to use the more integrative measure, AEI. In a mechanistic sense, porewater iron is directly controlling; i.e., it reacts with hydrosulfide ions to form various mineral phases of FeS, but just as sulfate in porewater is continually renewed by diffusion from above, dissolved iron in porewater can be renewed by dissolution of the reservoir of solid-phase iron. It is important to recognize that AEI may not be the only sediment characteristic affecting the sulfate-sulfide relationship. It is highly likely that sediment total organic matter (TOC) also plays an important role insofar as it is the main driver for microbial sulfate reduction. It thus would be useful for the MPCA to redo its MQRA using both sediment characteristics (AEI and TOC) rather than only the one they used (AEI).

Important questions left unaddressed by the MQRA modeling are about the dynamics of important diffusion and reaction processes. Examples include: the relative rates of sulfate diffusion into the sediment, sulfate reduction in the sediment, dissolution-diffusion of Fe from solid phases of the sediment into the porewater, and precipitation of FeS. For example, if surface-water sulfate concentrations suddenly increased, causing rates of sulfate diffusion into the sediment and formation rates of sulfide also to increase, would the porewater concentration of Fe^{2+} be sufficient to sequester all the "new" sulfide?" If not, would dissolution of iron solid phases and precipitation of FeS be sufficiently rapid to prevent a build-up of sulfide to toxic levels? It is pertinent that Johnson (2013) found substantial supersaturation with respect to amorphous FeS in high-sulfate mesocosms, implying that downward sulfate diffusion and sulfide formation by sulfate-reducing bacteria can exceed the rates of solid-phase FeS formation.

Questions related to the dynamics of various processes cannot be answered directly with empirical models based on MQRA, but a more comprehensive analysis of the large amount of field data collected as part of the study, including the porewater profiles collected in 2013 from the mesocosms, should be useful.

Finally, it is important to note that there are intermediate modeling possibilities between the two "end-members—empirical regressions and analytical sediment diagenesis models discussed above. Structural equation modeling (SEM) is one such intermediate approach that combines the use of qualitative conceptual (box and arrow diagrams) with statistical analysis. The SEM developed by another panel member for the sulfate-sulfide-iron-TOC system shows considerable promise in developing a more quantitative understanding of the factors controlling sulfide levels in sediment porewaters, and I encourage the MPCA to use this approach in its continuing studies.

In summary, the MPCA's approach to use acid-extractable iron rather than the porewater iron to predict porewater sulfide concentrations based on surface-water sulfate concentrations is reasonable, but the MQRA approach they used has important limitations. Uncertainties remain about the dynamics of the various processes affecting porewater sulfide concentrations under field conditions. The MPCA should conduct further data analyses and modeling that include other sediment variables and other modeling approaches.

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?

For the most part, my answer is yes. Sulfate at concentrations higher than found in Minnesota surface waters (and higher than sulfate concentrations in waters where wild rice grows) was not found to be toxic or inhibitory to seed germination or seedling growth. Although this does not completely address the possibility that sulfate could cause direct problems to wild rice growth and reproduction, the likelihood of direct effects is small. Sulfate is one of the major ions in natural waters, is ubiquitous in surface waters, and is generally recognized as not toxic at low to moderate concentrations.

It is well known that sulfate produces sulfide in anoxic environments, and sulfide is known to be much more toxic to biota than is sulfate. It thus was reasonable to focus on the role of sulfide in controlling wild rice occurrence and abundance. The role of iron in controlling free sulfide concentrations in sediment porewater is known from studies on sulfur biogeochemistry during the focus on acid deposition and its effects on aquatic ecosystems in the 1970s to 1990s and additional studies on sulfur biogeochemistry related to mercury pollution and cycling processes that have been a research focus of aquatic scientists since the early 1990s.

I do not think there is a need to explore other variables that could control concentrations of porewater sulfide, but further studies should be undertaken to evaluate other factors (aside from sulfide) that may affect wild rice growth and survival at various sites. As noted elsewhere in my responses, Figures 15 and 16 could be interpreted as evidence for iron limitation of wild rice growth, and this bears further analysis of the data and literature on wild rice and possibly additional studies. In addition, the important linkage between elevated sulfate levels and phosphorus internal cycling processes in lakes needs further investigation. Elevated sulfate levels could lead to less control of phosphorus levels in sediments by iron, which could exacerbate eutrophication problems—leading to more frequent and more intense algal blooms and a decline in water clarity. Such changes in water quality characteristics could have detrimental impacts on wild rice growth and abundance.

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.

Overall, the text of the Synthesis has many problems and needs to be rethought and rewritten. It does not provide a true synthesis of all the study components. The following paragraphs describe specific technical concerns I have about the text.

The statement on line 1174 about toxic effects above EC20 determined in the hydroponic experiments will need to be modified once the time-varying nature of sulfide exposure concentrations in those experiments is taken into account. In addition, the statement should not imply that there are no effects at concentrations below the EC20. The statement on line 1176 that responses of wild rice estimated from the field survey and mesocosms experiments are consistent with the seedling bioassay (hydroponic) results is incorrect or at least misleading. The field survey results show effects above 75 µg/L (much lower than the range of EC20 estimates), and the mesocosm data show linear effects with increasing sulfate levels and no indication of a threshold effect.

The statement on line 1206 that sulfide immediately reacts with iron and precipitates is inaccurate. Similarly, use of "immediately" on line 1237 is inappropriate. Data from the root zone profiles (Johnson 2013) apparently show substantial supersaturation with respect to amorphous FeS in mesocosms pore water at high treatment levels. The scientific literature on mineral precipitation kinetics, which indicates that precipitation of solid phases from ions in solution is often very slow under environmental conditions, also does not support this statement.

The statement (line 1222) that some oxygen leaks from the roots into the surrounding water may be true in theory, but data from the root zone profile study do not support this as a significant mechanism for aerating the root zone. This would seem to be a much needed subject for further field measurements and experimentation.

The statement on line 1231 is speculative and based on observations on a single plant. No evidence was provided in the analysis for the widespread existence of ferric hydroxide crusts on wild rice roots.

The statement on line 1239 that ferric oxy-hydroxides are potentially available for bacteria to reduce to dissolved Fe^{2+} has some problems. If sulfate reduction is occurring, the ferric oxy-hydroxides presumably should have been depleted, given that Fe(III) is an energetically preferred electron acceptor compared with sulfate. To the extent that they actually are not depleted in sediments where sulfate reduction is occurring, the "disequilibrium" indicates that the sediments are heterogeneous environments with pockets or "microzones" that are not in equilibrium with the porewater. This raises questions about the timeframe required for iron in such zones to undergo dissolution and for the resulting Fe^{2+} to diffuse into the bulk porewater, where it could react with accumulating HS^-. On the other hand, it may not be necessary for ferric oxy-hydroxides to undergo reductive dissolution to react with sulfide species. Heterogeneous redox reactions involving dissolved sulfide and ferric oxy-hydroxide surfaces are known to occur.

Because of slow and uncertain kinetics of FeS formation and related reactions of solid phase Fe, the statement on line 1248 should be modified with the word "potentially"; i.e., "…to potentially keep sulfide from accumulating…." The rest of the sentence on line 1249 would be stated more correctly as "…low values of Fe/AVS are indicative of systems where there is less potential for further removal of sulfide produced by sulfate reduction activities in the sediment." I also do not think lines 1256-1257 are chemically correct statements.

The concluding paragraph goes too far in implying that the MQRA model can accurately predict concentrations of sulfide in sediment porewaters. If for no other reason than the uncertainties in the kinetics of solid-phase FeS formation, the statement at the beginning of the paragraph is not realistic. The conceptual model in the Synthesis seems qualitatively correct, but a schematic figure would be useful. In my view, the section and especially its last paragraph presents an overly optimistic picture of our state of knowledge regarding: (a) the quantitative effects of sulfate and sulfide on wild rice and (b) our ability to predict accurately whether toxic sulfide levels will occur in a wild rice stand from knowledge of sulfate levels in the surface water and the acid-extractable iron content of the sediment. I think the Synthesis needs substantial rethinking and rewriting.

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

The study components can be grouped into two main categories, each having two components: (a) *bioassays*: short-term hydroponic assays of seeds and seedlings and longer-term mesocosms studies, and (b) *field observations*: field survey and rooting zone depth profiles. A fifth component, the sediment incubation laboratory experiment, represents a third category—controlled experiments on biogeochemical processes, but it played only a small role in the *Analysis*. Overall, these three categories of investigation are appropriate for assessments of complicated questions like that facing the MPCA in evaluating the scientific basis for the wild rice sulfate standard. To answer the above question more fully, two issues need to be addressed for each category: (i) was the design for each category appropriate and sufficiently comprehensive, and (ii) were the studies conducted competently and did they produce data of sufficient quality and quantity to support the *Analysis*?

Regarding the bioassay category, the overall design of short-term tests on sensitive life stages and long-term (life-cycle) tests on whole plants was appropriate. Specific designs for various experiments had some deficiencies, as described in responses to earlier questions. The following problems are noted for the short-term bioassays. (1) No evidence was provided that seed germination and seedling growth are the only or most sensitive life stages of wild rice plants, which raises the question of whether the short-term bioassays were sufficiently comprehensive. (2) The number of treatment levels for both sulfate and sulfide exposures was too small, particularly at low concentrations, resulting in larger uncertainties in calculated EC50 and EC20 values than would have been obtained with more exposure levels. (3) The physical design of the systems used in the experiments on sulfide effects on seedling growth made it difficult for the experimenters to control sulfide concentrations, which varied markedly during the experiments, making it difficult to interpret the results. Design deficiencies notwithstanding, the experiments themselves appear to have been carried out carefully and competently. Data from experiments on sulfate and sulfide effects on seed germination and sulfate effects on seedling growth appear to be sufficient to answer the questions they were designed to address. It is unclear whether sufficient data were obtained in the experiments on sulfide effects on seedling growth to overcome the problem of time-varying sulfide exposure levels.

My largest concern about the design of the long-term mesocosms bioassays is the lack of treatment levels near the current standard. The lowest treatment level of 50 mg/L was five times the current standard. The controls had ~ 7 mg/L of sulfate (the ambient level in groundwater used for the experiments), which is near the current standard and thus does not serve as a true control. I understand the desire for a broad range of sulfate levels (the highest was 300 mg/L) to increase the likelihood that some responses would be seen, but the lack of treatments in the range between 10 and 50 mg/L of sulfate decreases the reliability of extrapolations back to this critical range from responses at much higher exposure concentrations. The mesocosm experiments themselves appear to have been carried out carefully and competently.

The field survey design was appropriate and involved collection of data across the broad range of wild rice stands in Minnesota. A large number of water quality and sediment parameters were measured, and the resulting data appear to be of high quality. My main criticisms are that (i) it would have been useful to have sampled all wild rice sites in the two high-sulfate regions of SW Minnesota in Figure 1 of the *Analysis* (there are only eight such sites), and (ii) the lack of analysis and interpretation in the report. The MPCA has made good use of the field observation data at the aggregate level, but it could learn much more by examining in detail the data collected for individual lake sites where wild rice stands apparently occur in the presence of high surface-water sulfate levels.

The second component of the field observation category—the study on rooting zone depth profiles (Johnson 2013)—was well-designed, and the work appears to have been done carefully and competently. The data appear to be of high quality, and the report provides a clear written summary and analysis of the accumulated data. The author reported that no differences could be found in profiles for redox-sensitive species between sediments with wild rice roots and sediments in compartments isolated from roots. He concluded that wall or edge effects in the compartments without roots may have allowed downward transport of oxygen, creating conditions similar to the downward transport expected in compartments with roots. An alternative explanation may be that the roots are not very important in terms of oxygenating the sediment. It would be useful for the author to provide further evidence that the lack of difference in profiles for the two conditions was in fact an experimental artifact.

Finally, the lab experiment on the effect of temperature on sulfate diffusion into and out of sediments was appropriately designed and carried out, but it is unclear how this work fits into the overall study plan.

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

The *Analysis* document states (lines 204-207) that two of the five components of the study—the rooting zone depth profiles and Sediment Incubation Experiment—are only briefly reviewed in the document and that "further analysis and possibly additional study is needed before general findings can be drawn" from them. I encourage the MPCA to incorporate the findings of these two studies into their final report. The root zone porewater profiles, in particular, should be very useful in improving understanding results of the mesocosm studies and the sulfate-sulfide-iron paradigm.

Regarding the state database on sulfate (line 235 and Figure 1), I wonder whether there was an analysis to see whether data exist for the few lakes in SW MN where wild rice is found. The map shows modeled sulfate distributions, but values for individual lakes, even in an area with generally high sulfate levels could be low because of local hydrologic factors. The map of sites with sulfate data (left part of Figure 1 Myrbo 2013) shows that almost no sulfate data exist for extreme north-central, NW and SE Minnesota. It would be useful to show and discuss this map in the *Analysis* document. The number of individual sites with data also should be given. Many sites may have multiple measurements so that the 3,230 values represent fewer individual sites.

It would be useful to focus more detail on the few water bodies in Figure 1 of the *Analysis* in south-central and southwestern Minnesota where wild rice stands occur in the *apparent* presence of surface water sulfate concentrations substantially higher than the current standard (10 mg/L). I use the word "apparent" because the sulfate concentrations in the figure are *predicted* values based on measured sulfate values in MPCA and MDNR databases and not measured for specific sites with wild rice. Figure 1 shows four wild rice sites in a region with predicted surface water sulfate values in the range 34-96 mg/L, two sites at the boundary between that region and a region with sulfate concentrations of 94-260 mg/L, and two sites in the latter region. The database should be accessed to determine whether measured sulfate values exist for these eight water bodies. If not, measurements should be made. If sulfate levels are higher than the standard, it would be useful to evaluate porewater chemistry—e.g., determine whether sulfide levels are limited by high availability of Fe in the porewater and/or solid sediments.

Figure 1 also shows ~40-50 sites with wild rice across south-central, southeastern and west-central Minnesota in a region with predicted surface water sulfate concentrations in the range 12-34 mg/L. Studies should be undertaken to determine whether recent measurements of sulfate exist for the actual water bodies, and if appropriate, additional sampling should be undertaken to determine porewater sulfide and iron concentrations and acid-extractable Fe levels in the sediments. Some sites (~25) in the region were sampled in the field surveys of Myrbo and coworkers, and it appears (Figure 2, Myrbo 2013) that one site in the highest sulfate region was sampled in 2011 and 2012. Nonetheless, there seems not to have been a focused effort to understand what characteristics allow wild rice growth at these high sulfate sites and not at others. For example, Figure 6 (Myrbo 2013) shows seven sites with surface water sulfate concentrations of ~20-40 mg/L had wild rice coverage of ~ 30-80%, but no discussion of this finding is provided in that report or the *Analysis* document. A focused examination of field survey data from these sites could be useful in understanding what enables wild rice stands to occur at these sites.

Figure 15 is difficult to interpret. Is the lack of wild rice plants at low Fe and high sulfate causally related to hypothesized high sulfide levels, or is it just that the sampling scheme didn't find stands with this combination? How does one explain the one site with high wild rice cover (>20%) at a sulfate concentration of ~20 mg/L and Fe < 0.1 mg/L? Also, wild rice seems to be abundant across the range of sulfate—even up to concentrations > 100 mg/L when Fe is > 1 mg/L. An alternative explanation could be that wild rice cover is controlled by Fe as a limiting nutrient.

Figure 16 is somewhat better evidence for a toxicity effect of high sulfide levels as mediated by Fe concentrations, but the alternative explanation of Fe as a limiting nutrient should not be ruled out. It would be useful to see plots of actual percent abundance of wild rice versus (a) sulfide and (b) Fe concentrations rather than (or in addition to) the plot in Figure 16, which simply groups the wild rice responses into three broad ranges. The bar graph of percent cover versus porewater sulfide concentration in Figure 17 is a step in the right direction, but the bars do not provide an indication of the variability at a given sulfide concentration. This comment notwithstanding, Figure 17 is a nice summary of the information on percent wild rice coverage versus porewater sulfide. The text associated with the figure notes that it shows a sulfide effect at concentrations > ~ 75 µg/L; it would be appropriate to note that this is much lower than the EC20 value estimated from the laboratory toxicity studies.

Line 797 states that an abrupt water-level fluctuation in spring can virtually eliminate wild rice for that year. It would be useful to offer an explanation for this and indicate whether it is true whether the water-level fluctuation is up, down, or either way.

The discussion starting at line 851 provides further support for the idea that additional hydroponic experiments should be conducted with larger plants having roots, in which the upper part of the plant is in an oxic environment separated by a membrane from anoxic water containing the plant roots. Systems could be designed and built inexpensively that would allow continual replacement of the anoxic growth medium/sulfide solution at rates that would keep the sulfide levels constant. The opening of the membrane for the plant shoot could be sealed using a small amount of a nontoxic gel.

The discussion at lines 851-871 seems to be based in part on an implicit assumption that the leaves in the anoxic hydroponic experiments were the site of sulfide uptake and plant damage, but evidence is not provided that this was the case.

Regarding why DO levels were lower just above the sediment-water interface in lake than in stream environments, water flow rates in the streams almost certainly were higher than those in the lakes. The subsequent text notes that lake sediments likely were more organic because high stream flows would tend to suspend and wash away organic matter at the stream bed. Overall, the text provides a reasonable explanation and thus the initial statement (line 872): "*It is not clear why oxygen levels just above the sediment are much greater at the stream sites…*" is not correct; in my opinion, it is an expected phenomenon and thus is clear.

Lines 976 and 981 are inconsistent. The first line states that 1 N HCl was used in the field survey; the second line states that 0.5 N acid was used in the field survey (to be consistent with the 2011 pilot study). Which is correct?

The regression equations in the legend for Figure 20 and 21 are unclear; e.g., "lake=TRUE" should be defined. A better explanation should be provided for why are there not separate equations for lakes, streams and paddies.

Spearman nonparametric correlation rather than Pearson parametric correlation was used in three parts of the *Analysis*: page 18, line 463; page 30, line 666; and page 48, line 1134. The report should explain why the MPCA decided to use a nonparametric method rather than the more common practice of using a parametric method and transforming the data (e.g., as a log transform) if that is needed to normalize the distribution.

The lack of correlation between surface water sulfate concentrations and soil sulfate levels (p. 48, line 1137) is not surprising. Sulfate in surface waters most likely is derived from sources other than sulfate in surficial soils or even soil parent material. Sources include atmospheric deposition, oxidation of sulfide in organic matter in watersheds, groundwater, and anthropogenic inputs, including sulfate from fertilizer use. Sulfate salts are fairly soluble, and it is unlikely that the solubility of the least soluble (probably $CaSO_4$ and $CaSO_4 \cdot 2H_20$ – anhydrite and gypsum) is exceeded in Minnesota soils, except perhaps in a few areas of western MN.

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.

The data in Figure 6 of Myrbo (2013) show that stands of wild rice occur in streams with very high sulfate levels—much higher than found in lakes. A better understanding is needed of the factors affecting wild rice growth in streams with high sulfate concentrations, and I recommend that additional analyses be conducted separately on the lake, stream and paddy data sets.

The MPCA has a wealth of data from the 2011-2013 studies that have been only partially analyzed. Priority should be given to completing that analysis and using what is learned from this to decide whether additional field and/or laboratory studies are needed. What is learned from the additional analyses will be invaluable in designing the additional work to fill in the gaps that may still exist.

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Responses to Charge Questions by Dr. M. Siobhan Fennessy

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.

This experiment was designed to investigate a vulnerable life state for rice (seeds and seedlings) under controlled conditions using a range of sulfate and sulfide concentrations. The care that was taken to develop SOPs for the experimental design, and to control variables such as pH is commendable. However, due to limitations in the experimental design the sulfide seedling hydroponic test has limited ability to assess the toxicity of sulfide to rice.

Elevated sulfide concentrations were found to affect seedling growth (no effects were seen for seed germination). The primary limitations of the study are the short duration of the experiment (11 days), and serious issues with maintaining experimental conditions in the hydroponic treatments. Thus, any treatment effects (or lack thereof) cannot be linked to a known concentration of sulfide (see Charge Question 2), and no conclusions can be reached about the level of sulfide that induces toxicity effects with any certainty.

The fact that the seedlings experienced anaerobic conditions does not seem unrealistic (lines 889-891); wild rice plants are likely to face these conditions in the wild, and rice is known to germinate and begin growth under anaerobic conditions (Cronk and Fennessy 2001). However, the ability to extrapolate the results to the overall growth and survival of rice plants is limited by the short duration of this study. It is not clear whether the same effects might occur at other life stages (in lab studies or in natural populations). The response of wild rice at the seedling stage to sulfide cannot be generalized to subsequent life stages due to shifts in plant physiology that occur during growth. Treatment effects at one life stage may not hold over the life cycle of the rice plant.

The study would have been strengthened by changes in the experimental design (focusing on the sulfide-seedling experiment). For instance, taking more frequent water quality measurements in the sulfide treatments could have helped document the actual concentrations to which the seedlings were exposed. The authors state "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." This is certainly true, but the measurements needed to ensure that experimental conditions were maintained weren't made. The systematic reduction in sulfide levels seems fairly predictable from the literature, for example Li et al. (2009), in a hydroponic experiment using *Typha* and *Cladium*, changed solutions daily to address the changing water chemistry issue (this study is cited in the report). It is clear that changing the solutions every 2 to 3 days was not sufficient and led to large reductions in sulfide concentrations (ranging from a 30% to 96% reduction in concentration). Since the sulfide concentrations that the seedlings were exposed to are not known, but were clearly much lower than intended, an accurate assessment of the growth response cannot be made.

Other minor comments on the experimental design are about the number of replicates used. I appreciate the issue of managing an appropriate number of replicates given the relatively large number of treatments, however, using more than three replicates would make the results more robust. A power analysis to determine the appropriate number of replicates and treatment levels would be useful. Finally, it is not clear why the two 'definitive' sulfide – seedling trials used different concentrations of sulfate in the experimental treatments.

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?

Given the large reductions in sulfide concentrations over the 2 to 3-day intervals between changes in the water solutions, using the initial exposure solution concentrations is not appropriate. It cannot be assumed that the initial concentrations, even if present for a short time, were responsible for the impacts to growth. As mentioned above, other studies have had difficulty maintaining initial, experimental concentrations and so changed solutions each day (and this was without the problem of photosynthesizing seedlings in a closed container). The decline in sulfide in the experimental solutions was predictable, and should have been anticipated by the research team. Any effects on growth are likely an integrated response to the range of concentrations that the seedlings experienced during the experiment; if sulfide losses in the bottles were rapid, then the lower concentrations could be responsible. Using the initial concentrations is the least conservative approach in gauging effects since it represents the maximum possible concentration; it is unknown how much of the experiment took place under lower concentrations.

Although seedlings were exposed to the higher, initial sulfide concentrations at the time of water renewals, there is no information as to how rapidly the concentrations decreased, so no any way to assess the exposure the seedlings experienced. One approach might be to use a time-weighted average of concentrations for the calculations. At minimum, the high and low values should be clearly reported in the text of the report.

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 values presented here for EC20 and EC50 are based on the hydroponic experiments using seedlings over 11 days of growth. While the approach of using the four-parameter logistic equation is standard to create this type of dose-response curve, the effect levels presented here (EC20 and EC50) are not useful in evaluating the EC20 and EC50 concentrations. This is due to:

- The EC concentrations reported are likely higher than they should be since this analysis is based on the initial sulfide concentrations, while the effective sulfide concentrations were lower than intended over some (perhaps a substantial) part of the incubation.
- While the EC20 values (range of 210 – 322 µg/L) support that higher sediment porewater sulfide concentrations negatively affect the growth of wild rice seedlings, this threshold is not supported by other data presented in the report. For example, the field data presented on lines 820-823 indicate that the proportion of sites supporting at least 5% wild rice cover begins a steady decline

at concentrations above 75 µg/L (Figure 17, line 825). Relying on the field data (which also deals with plants over their entire life cycle) seems more appropriate.

- Using the EC20 for the no effect threshold is not conservative and may not sufficiently protect wild rice. Use of the EC05 is more standard, and will offer more protection against population declines. The possibility of a 20% decrease in growth across multiple years could compound, and cause loss of wild rice populations.

- Again, extrapolating the data beyond the seedling stage (i.e., beyond 10 days) cannot be done with any certainty with respect to the EC20 and EC50 values over, for example, the growing season. Would the relationships found here between sulfide concentration and growth continue over the full growing season? Would longer exposure to experimental conditions show effects at the lower sulfide concentrations? The data do not allow this type of assessment to be made.

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.

While it is difficult to judge definitely whether or not the sites used in this study are fully representative of Minnesota lakes and streams with respect to these questions, the report provides detail on the site selection process and a description of the range of environmental conditions expected in the lakes, streams, and rivers of Minnesota (Figure 5, lines 261-265). Care was taken to sample sites in all ecoregions and across the range of surface water sulfate concentrations mapped by the MPCA or contained in their database. Special care was taken to find and include high sulfate sites, complete with reconnaissance visits to verify conditions. The field survey documented a wide range of sulfate concentrations in the field sites, and this range was similar to the range of sulfate concentrations documented by an USEPA probability based survey (Figure 9, lines 514-517). All that said, the information presented could have been more complete, for instance on Table 6, showing the minimum (detect) and maximum concentrations for the different types of field sites would have been useful. Comparing the ranges of concentrations using the 10^{th} and 90^{th} percentile would provide more complete information on how well the field sites represent aquatic sites in Minnesota.

Having completed several large regional surveys of wetland sites, the approach used here was sound and yielded a range of site conditions needed to inform the analysis. A targeted site selection process was used in order to ensure that a broad range of site conditions were represented, from low sulfate/ low rice to high sulfate/high rice, to sites that appeared suitable as rice habitat, but in which no wild rice was found. As a preliminary field study, the success of a project such as this depends on selecting sites that span the range of conditions in the state; this is best done with targeted sampling to ensure that range is captured (Karr and Chu 1999). A probabilistic study design could, by chance, miss part of that range, for example by selecting sites that are all low in sulfate (as the report points out).

Subsequent surveys could use probability-based sampling designs to randomly sample the population and test relationships developed in this study. Since the intent of this project was not to characterize a population of sites, but rather to use the range of field conditions to investigate the relationship between the occurrence of wild rice and water chemistry, this approach was appropriate. If the intent of the study had been to describe the biological and chemical condition of Minnesota lakes, streams, and wetlands, so that the results could be extrapolated to the population of aquatic sites as a whole, then a probabilistic survey that provides a geospatially balanced, stratified random sample would be appropriate (Herlihy et al., 2000, Stevens and Olsen 1999).

In this case, targeted sampling revealed relationships between sulfate in surface water and porewater chemistry, and ultimately informs the efforts to protect wild rice. The results of the field survey are some of the most informative in this report.

The mediation of porewater sulfide concentrations by iron seems clear, and is well established in the literature (Lamers et al., 2013a, b). Sulfate will diffuse into sediments as it is consumed there in the production of sulfide, along its concentration gradient. The concentration of acid-volatile sulfide has been used to make predictions about the bioavailability or toxicity of metals. The study design allowed the investigators to examine the relationships between sulfate, acid extractable iron, AVS, and porewater sulfide and iron in the sediments of the sites. Sulfate concentrations in the surface water were linked to porewater sulfide (positive correlation) and porewater iron levels (negative correlation). However, the relationships found should be considered preliminary and the data collected should be the subject of more analysis. The chemistry of sediments is complex, and not easily simplified into reactions between (in this case) iron and sulfur compounds. Other compounds that may affect these relationships include organic carbon (which was analyzed here), but also phosphate, the cycles of which can have substantial interactions with the S cycle (see Charge Question 9).

It is difficult to draw simple relationships between iron and sulfate/sulfide that are predictable over the range of field conditions and operationalize this as part of either water quality standards or a wild rice protection program. Total sediment iron, which is relatively easy to measure, was not predictive of the iron metrics used in the analysis. Figure 22 (lines 1072-1074) shows an empirically-fit maximum potential concentration of sulfide for a given concentration of sulfate; variability in porewater iron influences sulfide concentrations, but the scatter in the data (variability), as well as the influences of other compounds not accounted for, make this relationship difficult to serve as the basis for a sulfate standard. Trends may be more clear if the data for lakes, rivers and wetlands were analyzed independently. The hydrology of these ecosystems varies dramatically, and classifying these sites for analysis may reduce variability in the data and reveal a differential response to sulfate.

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.

Mesocosms have become a standard tool for ecosystem level experiments, in part because they can be easily manipulated to establish experimental treatments while allowing for variable environmental conditions. Plants were grown from seed for three seasons and their response to differing sulfate treatments was monitored. Sulfate concentrations in the surface water were measured throughout the experiment. The experiment ran for three years with a focus on key life history traits that influence population dynamics.

The data are reported using the treatment concentrations and not the actual concentrations that were determined weekly throughout the experiment. Reporting the actual sulfate values would be more informative, for instance, mean sulfate levels in 2013 ranged from 7.1 to 262.3 mg/L for the 0 and 300 mg/L treatments. The desired experimental conditions were not established in May 2013, when the average concentration in the 300 mg/L tanks was only 71 mg/L. By June, sulfate levels were much closer to the target concentrations. Reporting the effective concentrations would have strengthened the study. The data from 2011 and 2012 are not provided which is also an omission. As in the field study, porewater sulfide and AVS levels were positively correlated with surface water sulfate while porewater iron showed a negatively correlation. There was no analysis linking sulfide levels directly to plant performance.

Overall, the analysis of data could have been taken further, particularly the porewater data from 2013. On page 3 of the study report by Pastor, the author states that the goal is to determine the response of rice to a range of sulfate concentrations in surface water, and associated sulfide in the rooting zone, however, very little was done with the sulfide data that would help link these results to the other study components. Lines 662-671 describe the patterns in porewater sulfide, iron and AVS, but more could be done to relate the porewater data with the rice data. The statistical treatment is also not clear – for example Table 7 could be modified to show where significant differences were observed across years and treatments. The current presentation does not provide any statistical information. That said, the study provides valuable information showing a steady decline in many plant traits as sulfate concentrations increase.

The role of nutrient availability in the growth of the wild rice was not part of this study and although P was measured in surface water, it was not discussed). Nutrients are obviously critical to aquatic plant growth, particularly nitrogen and phosphorus. As the report illustrates, sulfide is toxic, impairing root growth and nutrient uptake. However, high sulfate levels also increase the rates or organic matter decomposition, which liberates nutrients such as P that promote growth (Table 8 shows a positive correlation between surface water sulfate and total P). The mesocosm approach seems an ideal way to address these types of relationships; more could be done with the data to address this.

Finally, much is made of the analysis of iron plaque on the roots from one plant. This was done it seems, as an interesting aside to the study, and not part of the study design. A sample size of one does not tell us much, so while interesting, it is not enough to draw any meaningful conclusions.

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?

It is not entirely clear how the concentration of 300 µg/L was settled upon given the evidence provided in the report. The preamble to this charge question states "the data showed deleterious effects of sulfide on seedling plant growth when sulfide exceeded the range of 150 to 300 micrograms per liter (µg/L)." Given this statement, the Report doesn't make clear why 300 µg/L was selected (and not, for instance, 150 µg/L which might also be toxic, given the data). There is too much uncertainly in some of the results to use the value of 300 µg/L, for example, the lack of data about the real exposure levels of seedlings in the hydroponic experiment. Furthermore, the Field Study indicates a threshold of 75 µg/L for the onset of effects, much lower than 300 µg/L. Even a visual estimate of Figure 7 in the Field Survey report (by Myrbo) indicates that the cover of wild rice declines at porewater sulfide concentrations above about 0.1 mg/L (100 µg/L; Figure 7). The data do not show a consensus worthy of making a definite conclusion on this question. Establishing a level of 300 µg/L as a threshold for the onset of effects is not supported by the data in the report.

While porewater concentrations above 300 µg/L can be toxic to wild rice, toxic effects may also be seen at lower concentrations. More work is needed before it is possible to make a conclusive statement about a threshold sulfide concentration. In a review on the phytotoxicity of sulfide, Lamers et al. (2013a) found that sulfide concentrations of 10 µmol/L (~320 µg/L) can be toxic to freshwater aquatic plants, although wild rice was not included in this study. *Oryza sativa* (rice) was shown to have intermediate tolerance to sulfide, with decreases in aboveground and belowground productivity, reduced nutrient uptake, and decreased radial oxygen loss at concentrations from as low as 30 µg/L, ranging up to 310 µg/L. In turn, these effects can reduce plant fitness and lead to changes in ecosystem function. This also leaves the question of how surface water sulfate levels are linked to porewater sulfide.

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?

Quantile regression is a suitable statistical tool to predict porewater sulfide concentrations. It provides estimates of quantiles (such as the median) of the response variable instead of the mean, as does traditional least squares regression. In this case it allows the influence of differing levels of iron in mediating the accumulation of sulfide in sediments to be assessed. One issue is that the quantile regressions are not presented with any statistical information such as their regression coefficients (e.g., pseudo R^2). This makes interpretation more difficult.

Different approaches to threshold analysis might be considered. Threshold analysis has been successfully applied in other studies to determine the ‘critical load’ of a pollutant that an ecosystem can assimilate without causing a shift in ecosystem state or function (Groffman et al., 2006). As this Report acknowledges, determining a critical load is complex due to the spatial and temporal variability in lakes, streams and rice paddies, which complicates ecosystem response; often the critical loads are different for different ecosystem types. Reviews by Qian et al. (2003) and Brenden et al. (2008) may be useful.

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?

Determining acid-extractable metals is a relatively standard means to estimate the availability of metals such as iron, and may better represent metal availability than measures of dissolved forms. However, there is confusion in the text about what was actually done in the quantile regression analysis. Line 1110 states that information on the ‘available iron in the solid phase of the sediment’ was used, reporting the data as µg Fe/g soil. Is this the same as the “concentration of available iron in the sediment” as it says in the legend of Figure 24 (which refers to acid extractable iron?) (line 1120)?. It is not clear how this links with statements found on Line 1141-1142, that the USGS sediment iron concentrations did not correlate with any of the iron metrics used in the analysis (although in the field report, sediment Fe and porewater

Fe are correlated). It would be clearer to consistently refer to the same variable by the same name, in this case 'acid-extractable iron'.

Given the exploratory nature of this analysis in the Report, it would have been useful to conduct this analysis for the different types of iron and compare the results. A more thorough explanation of the approach taken, and a more complete analysis of the available data, would help clarify the choice.

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?

Understanding the effects of sulfate on aquatic ecosystems generally, and wild rice populations specifically, requires an understanding of sulfide and its role in causing plant physiological toxicity stress. Understanding the role of iron in protecting against that stress through the formation of FeS and FeS_2 is also important, so the focus of the report on these variables is certainly warranted. However, there are other compounds involved in the sulfur cycle that are not addressed here, and the focus on sulfide and iron to the exclusion of other sediment compounds oversimplifies the chemistry of these systems. For example, some critical plant nutrients as well as the fate of mercury in aquatic ecosystems are also tied to the biogeochemistry of sulfur and iron. It has been shown that calcium and magnesium deficiencies may develop in plants grown on acid sulfate soils due to competition for cation exchange sites; rice (*O. sativa*) has been shown to be susceptible to calcium and magnesium deficiency (Lamers et al., 2013a).

The role of sulfate in eutrophication may also be a factor in some systems. As sulfate levels increase, its availability as an electron acceptor increases, leading to faster rates of organic matter decomposition and the mineralization of nutrients such as nitrogen and phosphorus (Smolders and Roelofs 1993, Lamers et al., 1998, 2013b). Increased nutrient availability may alter the competitive ability of aquatic macrophytes within plant communities (including wild rice), leading to changes in species composition. Pester et al. (2012) reports that as much as 50% of the anaerobic decomposition of organic matter in freshwater wetlands is due to sulfate reduction to sulfide. Conversely, sulfide can impede plant nutrient uptake, leading to lower growth rates.

The cycling of iron is also linked to phosphorus in the soil. Phosphorus binds with iron (both Fe III and Fe II), the availability of which is controlled by the degree of anaerobiosis. For this reason, Lamers et al. (2001) conclude that both iron and phosphorus are important controls on the response of soils to sulfate enrichment.

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.

The Synthesis could be strengthened in several ways, including by:

- Providing an introductory paragraph with an overview of the study, its goals, components, and key findings.

- Providing a true synthesis that fully integrates the findings of the study. As it stands, the section doesn't integrate the findings well by pointing out, for example, the commonalities between study components, or where findings differ. The Hydroponic experiments are too weak to be relied upon for drawing any conclusions about thresholds for something as important as the question of a sulfate standard for surface waters. It also is not credible to say that the results of the Field study, which suggest a concentration of 75 µg/L for the onset of impacts to wild rice, are consistent with the hydroponic study results as stated on lines 11776-1177.

- Removing some of the overview of sulfate and sulfide dynamics in freshwaters. This isn't really necessary, and seems out of place for a final, synthesis section. For example, lines 1215-1223 that describes plant root adaptations to saturated soils could be removed. While this is important information it was covered earlier in the Report; it doesn't add much to the synthesis of the findings.

- The section would benefit from more discussion of the plant responses that were noted in the different study components, and the empirical evidence that links plant response to sulfate levels. I expected to see much more about the ecology of wild rice in this section.

- Removing the discussion of the plaque that can form on plant roots in anaerobic soils in the paragraph on lines 1224-1233. Here the authors present information on the possible role of plaques in protecting plants from sulfide toxicity. The authors go so far as to offer support for one of the hypotheses, namely that the plaque serves as a barrier that intercepts and precipitates sulfide before it can enter the roots. This is problematic because 1) it is based on an analysis of plaque from one plant in one of the mesocosms, and 2) the roots were sampled for plaque as an interesting 'aside' when the plaque was noticed, and was not part of the study design. It is surprising the topic is included in the Synthesis.

- Should line 1242 say "…the potential reservoir of ***available*** iron…?"

- Lines 1251-1254 are unclear; what is meant by "solid phase" iron? Is this the acid-extractable iron, or perhaps the sediment iron (line 990)? The text refers to Figure 24, which displays "available iron in the solid phase of the sediment (line 1110), however the legend of Figure 24 says it is the concentration of available iron (acid extractable?). As discussed in Charge Question 8, the lack of clarity in identifying which parameters are being used in the analysis is confusing and makes the findings unclear.

- The Synthesis ends with a discussion of Fe/AVS and its use to indicate whether the supply of iron can keep pace with the production of sulfide. How does this relate to the iron content of the sediment, as shown in Figure 24? What circumstance would "trigger concern that the available iron at a site has been consumed by the past production of sulfide"? Are potential inflows of iron with ground water taken into account?

- I suggest the Synthesis be revised to addresses the ultimate question of what conclusions can be reached about a sulfate surface water standard based on the component studies (if any).

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

An assessment of several of the component studies is presented in earlier Charge Questions. Overall, some of the studies proved more valuable than others. The two studies that received limited treatment in the Report are the analysis of rooting depth profiles and the sediment incubation laboratory experiments to assess diffusion rates. In these there is some preliminary analysis of the porewater chemistry, but more could be made of these data (more in-depth analysis of the data, inclusion of plant data from other studies) with a more complete discussion of its ecological implications. For example, the authors state that the diffusion rate of sulfate over a 3-5 cm distance in the sediment is approximately 13-36 days. This leaves the question of how this finding informs the assessment of surface water sulfate loads?

The study of temperature dependent diffusion rates of sulfate, while interesting, is difficult to apply to field situations where waters move, carrying sulfate, iron and other compounds that might affect the reactions. The final sentence of the study report reads: “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.” An explanation of how the data could be applied in this way would be helpful. Ultimately the Field and Mesocosm studies are the most useful in addressing the study objectives, however they do not provide a consensus in support of a specific sulfate standard that would prevent sulfide toxicity, given available iron concentrations. Additional studies to identify relatively rapid indicators of iron in the soil and its ability to sequester sulfide in lakes, rivers, and wetlands, would be valuable (e.g., total Fe, porewater Fe, AVS). Further data analysis to investigate whether different sulfate standards are needed for the three water body types would help meet the goals of the study.

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

Essentially all of my comments on the report are included in answers to the Charge Questions above. A final editorial comment is to ask that the authors be sure that references to different parameters are consistent and clear. As discussed above, the same forms of iron are referred to by different names in different places in the text; such inconsistency creates unnecessary confusion. It would also be helpful if units were displayed more consistently in the text, and that the units are clearly explained. For instance, reporting sulfate as mg/L could mean mg SO_4/L or mg SO_4-S/L, two very different measurements.

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.

Many of the issues or critical data gaps have been discussed as part of the other Charge Questions. To emphasize one point that was briefly mentioned above, future research and/or data analysis could treat the lake, stream and wetland field sites as independent groups. The hydrology, hydrodynamics, water chemistry and biota of these three ecosystem types varies, which could lead to different responses of wild rice to sulfate levels. Classification is important to reduce variability in samples; this is one reason why USEPA has developed three very different approaches to the surveys of water quality in lakes, streams, and wetlands. Differences in water chemistry are noted on page 22, but the differences are not used as part of the data analysis.

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Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Responses to Charge Questions by Dr. Susan Galatowitsch

Laboratory Hydroponic Experiments (see Analysis, pp. 13-16, 38-39)

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.

A short-term laboratory assay of seed/seedling responses to sulfides has the potential to be important to understanding the effects of elevated sulfides on wild rice. There are, however, a few problems with the execution of this experiment. First, belowground portions of the seedlings needed to be exposed to sulfides while leaves should not. Second, there should have been more sulfide treatment levels and more replicates. There needed to be more treatment levels in the low end of the range, in particular. The duration of the experiment (10 days) seems reasonable—longer time frames would be beyond the scope of seedling growth and better approached in another way (such as mesocosms).

Other points:

While it is reasonable to assume that the seedling stage is vulnerable to sulfides, the results should not be extrapolated more broadly to the overall effects on wild rice (i.e. it should not be used out of the context of a population assessment).

Creating relatively uniform sulfide treatments in sediment would have been exceedingly difficult and prone to artifacts from complex microbial transformations of other elements. Also, stagnant waters of wetlands typically have pre-dawn water column oxygen levels of zero (BOD exceeds PS) – their benthic invertebrate fauna are usually dominated by species that can tolerate low oxygen conditions, providing evidence that the anoxia is significant enough to cause biological responses in many wetlands. I assume wild rice in sites without flowing water potentially would be exposed to sulfides daily, at least for a few hours. Flowing water is less prone to becoming anaerobic because oxygen will be added by turbulent mixing with the atmosphere.

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?

Because sulfides impaired growth, rather than causing mortality, it seems more appropriate to assume the response was the result of prolonged exposure to sulfide and so the initial concentration should not be taken as the treatment dose. Instead, a time-weighted average (the norm for ecotoxicology) or geometric mean should be used.

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?

A regression analysis seems appropriate for identifying effect levels; however model fit needs to be considered to select the most appropriate statistical approach (e.g., non-linear vs linear). Also, wild rice response should be evaluated based on actual concentrations, not nominal concentrations.

Other point: EC20 and EC50 are not adequately protective of wild rice—20% or 50% mortality/impairment should be considered a significant adverse impact to a wild rice population. EC5 or EC10 is, therefore, more appropriate.

Utility of the Field Survey Data (see Analysis, pp. 21-25, 35-36, 41-47)

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 pore water concentrations of sulfide and iron, and 2) inform protection of wild rice from elevated sulfate. Please note any specific questions or concerns.

This study generated a large, potentially useful data set even though it is an observational study with inherent limitations—notably that there are a number of other factors, such as water level fluctuations and eutrophication, which can affect habitat suitability for wild rice. The lake and stream sites survey seemed representative of lakes and streams in Minnesota, but the data from these two types of ecosystems should be analyzed separated. A recently published study from Minnesota showed that riverine wild rice populations have lower seed mass than lake populations, suggesting that wild rice populations respond differently to conditions found in these two types of ecosystems. Also, I think it would be useful to use discriminant function analysis or logistical regression to see if sulfates emerged as a factor that correctly classified a site as occupied or unoccupied by wild rice.

Mesocosm Experiment (see Analysis, pp. 26-32)

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.

One of the key benefits of multi-year mesocosm studies is the demographic details that can be measured. In this study, there appears to be enough information on key parameters to do some life-table based population simulations. Wild rice is an annual plant so populations can be modeled with relatively few transition probabilities (seeds to plants, plant production of seeds, seed mortality). It would be useful to use these transition rates and simulate the fate of populations with different levels of sulfates—what are extinction probabilities (i.e., for 50, 100 years) under different sulfate levels? RAMAS is one tool that is widely used for this kind of analysis. The point is: will these sublethal effects cause population extinction. It's hard to know without projecting the fate of populations using the demographic data gathered.

A major limitation with using the experimental data is the range of treatment levels. The control had a sulfate level of 7 mg/L and the lowest elevated treatment was 50 mg/L—five times the current standard. Because this mesocosm study lacks a true control (close to 0 mg/L) and sulfate levels do not bracket the current standard, the results of this study are likely to be of little to no value.

Other notes:

Observed PW sulfide concentrations should be used to determine exposure responses, rather than nominal sulfate levels.

Wild Rice in Relation to Sulfate, Sulfide, and Iron (see Analysis, pp. 35-39)

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?

I agree with the assertion that the data indicate elevated sulfide in sediment can reduce wild rice growth. However, there is not a sound rationale for the threshold of *300 µg/L*—this appears to be higher than is supported by the evidence presented.

Control of Porewater Sulfide by the Availability of Sulfate and Iron (see Analysis, pp. 21-25, 45-47)

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?

The approach seems reasonable and logic sound but I do not have experience with other options.

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?

I do not have sufficient expertise on this topic to respond.

Synthesis: How Sulfate, Sulfide, and Iron Interact to Affect Wild Rice (see Analysis, pp. 51-52)

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?

I agree that MPCAs focus for sediment chemistry was appropriate. However, the level of sophistication in analyzing and interpreting the environmental chemistry of the situation was much greater than analysis of the ecological response of wild rice, which seemed to be lumped into "growth". Twenty percent decrease in growth of individuals doesn't mean that you'll have a population of somewhat smaller plants -- it probably means you'll have less carbon to produce seed, which in an annual can cause a population to trend downward and be more vulnerable to other stressors. A logical assessment endpoint is likely to be seed production since it likely regulates population persistence of this annual species and because it is related to beneficial use of the species.

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.

The synthesis should be framed on the ecological response of wild rice to sulfates (and sulfides) rather than on the environmental chemistry of the system. There is almost no discussion of the wild rice response, which is problematic given the aims of the study. Also, results of assays and experiments need to be integrated with results of the field survey to establish causation and determine defensible threshold levels of sulfate effects on wild rice.

General Questions

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

The set of studies provide a reasonably comprehensive “picture” of the response of wild rice to elevated sulfate. However, we don’t know why wild rice responds like this—or how other stressors in the system (i.e., Hydrologic alterations) could cause synergistic impacts. That’s may viewed as beyond the aims of the study—yet most of the application of a standard will be for systems that experience multiple human-caused stressors, which this set of studies didn’t address.

An additional study component of value would have been an investigation of conditions leading to wild rice loss. Wild rice has declined in distribution across the region—assessing which environmental factors are linked to loss would be valuable for understanding the potential for multiple stressor effects from sulfates and other human-caused stressors. Of course, it would be helpful to know if losses have occurred more or less evenly across the range of sulfate conditions tolerated by wild rice. As importantly, it would be important to know if other stressors (especially eutrophication) increase the vulnerability of wild rice to sulfates. See Pillsbury and McGuire (2009) for some findings related to wild rice habitat loss.

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

None.

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.

As previously mentioned: 1) there needs to be attention to population responses, not only individual responses to sulfates, 2) there needs to be attention to wild rice response to multiple stressors, especially those that could affect sulfate-sulfide conversion--including eutrophication.

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Responses to Charge Questions by Dr. Mark L. Hanson

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.

Most of my original comments stand, and should be considered by the MPCA when interpreting the data. They should look to the expectations of standardized test protocols in terms of assessing the validity of the results. A mainstay is consistency of control performance. I don't see that here for these tests. While they do provide reasonable evidence that sulfate is not very toxic, and that sulfide is the primary toxic agent for wild rice, extracting concentrations at which effects can be expected should be done with caution. As always the ecological relevance of the endpoint must be considered (here, as well as in the mesocosms and field surveys), especially for an annual plant that requires seed production to propagate, which is more sensitive to impairment than perennials, or annuals that rely on vegetative reproduction. As well, the relatively short duration of the tests needs to be addressed in any extrapolation to a field scenario where exposure is over the entire lifespan of the plant, and not just for a brief window. Finally, the fact that there are sub-species of the rice needs some consideration. How confident is MPCA in the field identifications and is there any reason to think that different sub-species would display significant differences in sensitivity?

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?

Again, the consensus is that initial measured concentrations lack the conservatism, and hence the protection required to ensure the long-term sustainability of wild rice in the regions where it is currently found. Some sort of time-weighted average or other value that better describes what the plants are seeing over the course of their exposure is needed.

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?

Non-linear regression analysis is the most appropriate way to derive the EC20 and EC50 estimates (plus their associated confidence intervals). As was noted throughout the meeting, EC20 is not likely to be protective for these plants with their life history and anticipated exposure duration. I'd recommend the EC05, but even then, uncertainty factors should considered if any laboratory value is to be used as the threshold in guideline setting for when effects in the field would be anticipated. Environment Canada has an extensive document that describes experimental design and statistical interpretation of results that might be of value to the MPCA is this effort. It can be found at: http://publications.gc.ca/site/eng/278313/publication.html

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.

This is an impressive data-set that is in the early days of being understood. I think it has a lot of potential to understand what water quality factors limit the range of wild rice in the region. As I mentioned at the meeting, I think water hardness needs to be more fully explored as an important parameter for wild rice. While a number of measures were taken related to wild rice directly in the field survey, the focus should be on those with most directly linked to reproductive success. Stalk number appears to be a viable candidate. As well, the percent cover values should be used more effectively, the significance of selecting certain levels (e.g., 5%) justified from the perspectives of the ecological, toxicological, and socio-cultural perspective. For example, is 5% cover a density at which traditional harvesting can occur?

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.

Due the relatively high levels measured in the controls (approx. 7 mg/L sulfate), any observed responses need to be taken with a grain of salt. It would be useful to link endpoints across all three study components (lab to cosm to field) and so those most linked to reproductive success of wild rice should be emphasized. As well, the biological data should be modeled in relation to both sulfate and sulfide. Finally, the performance of wild rice in the mesocosm systems must be contrasted with wild rice from the field. If the growth and development is significantly different, than any responses in the cosms are suspect. Best to be transparent about the strength and weaknesses of this work.

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?

As stated at the meeting, I believe the consensus is that 300 μg/lL can be toxic to wild rice, but that the MPCA's data-set as a whole support significant effects at lower concentrations, and that using this as a threshold is not warranted at this time. The data have simply not be analyzed to the degree needed at this stage to make any claims about where a toxic threshold for wild rice exposed to porewater sulfide might reside. The percent cover data, or stems per m2, as it relates to sulfide concentrations will likely provide the best indication of when sulfide passes a threshold (Figure 6 by C. Pollman highlights this quite nicely). Building a weight of evidence around the various biological parameters and sulfide in surface waters will help create a strong understanding of when sulfide matters. Of course, the degree of change that is or is not acceptable in terms of wild rice in Minnesota surface waters still needs to be defined (and I recommend it be directly inked to reproductive success).

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?

I think panel members made a number of viable recommendations around this charge question that the MPCA should consider.

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?

I think panel members made a number of viable recommendations around this charge question that the MPCA should consider.

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?

Yes, the focus was appropriate with the knowledge at the time. I still recommend understanding the role of water hardness more fully.

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.

Clearly, the synthesis will need to be reconsidered in light of the panel's review. Considering the data were still in a preliminary phase of analysis, this is not surprising. I think making the case for sulfide and any specific threshold of effect should employ a weight of evidence approach. Do not be afraid to state when data are compelling, or not so much. Some sort of conceptual model linking all the facets of the studies and how they the evidence/data from each lead to the final conclusions would be useful.

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

I think the MPCA choose appropriate study components. The execution of some parts, may be lacking, but overall, there is a diverse data-set that will help them to accomplish their goal of protecting wild rice. The next steps are to make full use of the data at hand in the most scientifically defensible manner possible.

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

I think most of these have been summed up in my comments above, or in the panel's closing recommendations.

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.

Aside from the panel's other recommendations, the one area I'd suggest be looked further into that hasn't been stressed to much at this point is for the MPCA to make use of the paddy data, as well as the reports of the dead zones (which might be a natural experiment in action at the field-level). If there are gradients around sulfate/sulfide that can be defined as it relates to wild rice in these scenarios, the observations may help in defining a threshold at which declines in productivity are observed. Lastly, getting a sense of the seed back for these plants (if any) will provide some sense of how (and if) recovery might occur, should there ever been an incident where you see failure of wild rice, whatever the cause might have been.

Peer Review Meeting for Minnesota Pollution Control Agency's (MPCA's) Draft "Analysis of the Wild Rice Sulfate Standard Study"

Responses to Charge Questions by Dr. Curtis D. Pollman

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.

The specifics of the experimental design for the seedling hydroponic study are beyond my immediate area of expertise.

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?

There are, of course, two different "extremes" which can be applied towards choosing an appropriate sulfide concentration as the operative exposure based on the experimental data, and a multitude of intermediate variations that lie between. One extreme is to use the initial exposure concentration; the other extreme is use the final exposure concentration. The assumption here is that initial refers to the concentration measured on each solution renewal day after the existing sulfide levels have been amended to approximate the nominal treatment level, and the final values are the values measured several days later immediately prior to the sulfide amendment.

Choosing the initial exposure concentrations of course leads to a bias towards higher exposures levels *vis-à-vis* the true exposure level, and thus using the initial exposure concentrations to evaluate and model response will lead to a bias towards *underestimating* sensitivity for the measured response metrics to sulfide exposure. As a result, using initial exposure concentrations accordingly defines the *upper* limit for the response threshold; using the same logic, the final exposure concentrations likewise define the *lower* limit for the response threshold. The net result is that the true threshold (for the given set of experimental conditions) can be confidently ascribed to a number that lies in between these two limits.

I would recommend analyzing the data using both the initial and final exposure data to define the upper and lower thresholds of response. If we assume that sulfide levels decline with an exponential rate of decay over the duration of the time period between experimental additions, then the geometric (log) mean defines the average exposure concentration over the interval. These values then could be used to fit a third, "best estimate", model to define the response threshold.

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?

As a general consideration, the implementation of regression modeling, and in particular, the non-linear regression model using a logistic transformation of the sulfide data seems appropriate and is also conceptually satisfying. A comparison of observed with fitted values shows that the logistic model captures the shape of the response curve well (using change in plant mass as an example; see Figure 1) for wild rice to sulfide exposure, using initial sulfide concentrations to fit the model. Also, from a conceptual perspective, implicit in the logistic model is the idea that sulfide toxicity becomes increasingly more important above an initial, critical threshold; in addition, the model also implies that, above a certain higher threshold, the toxicity effects are maximized.

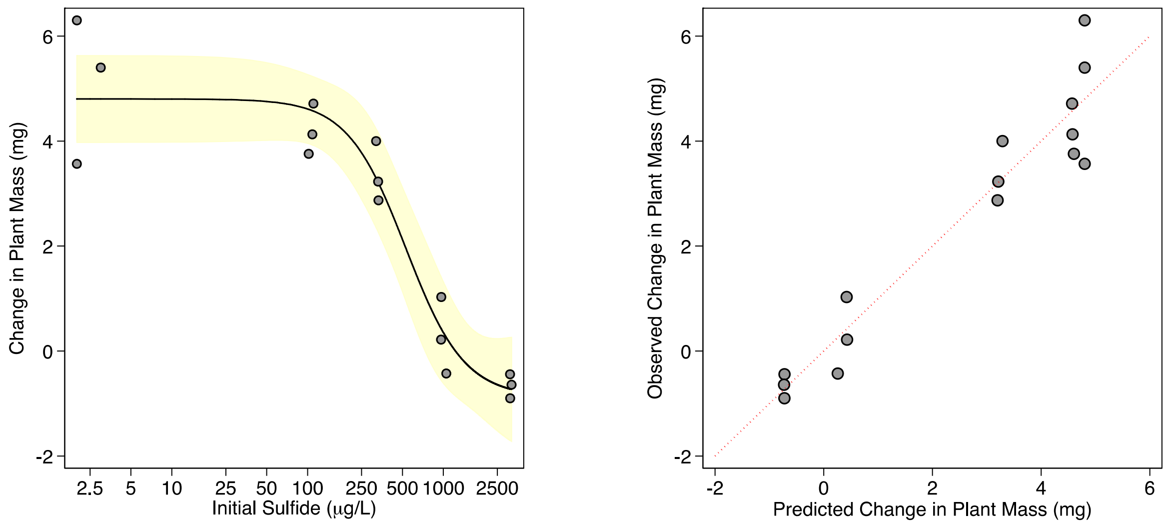


Figure 1. Logistic nonlinear model to predict change in wild rice plant mass as a function of initial sulfide exposure concentrations. Data are from the rangefinder hydroponic experiment (Pastor, 2013a). Left hand panel – fitted logistic function model response and observed values as a function of initial sulfide concentrations. The plot also shows the prediction confidence interval (90%; yellow shaded area). Right hand panel – observed vs. predicted values for change in plant mass.

Several questions nonetheless are important to consider. First is whether the use of initial sulfide concentrations leads to a systematic bias towards less sulfide sensitivity in the response curve. This of course relates to Charge Question No. 2. In other words, depending upon whether the initial or final sulfide concentrations are used, both the shape of the response curves (Figure 2), and the locus of maximal sensitivity (based on analyzing the fitted functions for where the maximal change in plant mass occurs for a unit shift in sulfide exposure levels; see Figure 3. The converse of this concern is that use of the final observed sulfide concentrations to construct the response model arguably imposes a bias towards an overestimate of sensitivity given that the final values are substantially lower than the exposure values the plants were responding to, particularly at intermediate concentrations where the greatest change in response is observed (Figure 4).

A second concern relates to model confidence. Inspection of the confidence intervals for the fitted parameters for three models developed by MPCA indicates that some of the fitted coefficients are not significant, and thus these estimates are unreliable. This is likely the result of using a comparatively small number of data points clustered within narrow concentration bands for each model. Restated, the experimental design for each individual experiment utilized only four treatment levels plus one control. This gives, in essence, results from five concentrations to fit a model with four adjustable parameters. Thus, even though there are technically 11 degrees of freedom used in fitting each of the individual models, the model approximates one that has been "overfit" with almost no degrees of freedom. In all likelihood, somewhat more stable, robust coefficient estimates would be obtained if the model were fitted with all the data combined across the three experiments and scaled appropriately (but not averaged). MPCA thus should consider refitting the nonlinear models with the data from all three experiments. It then might be useful to construct a derivative response plot similar to Figure 3 that shows where the model indicates the greatest sensitivity in response, and ensure that observations from that region in the model were available to support the model estimates. In addition, some form of model validation should be undertaken. Given the relatively small number of data points, I would recommend a jackknife cross-validation procedure, although the issue of small numbers of sulfide concentration levels used to construct the models may still be problematic, and needs to be considered.[1]

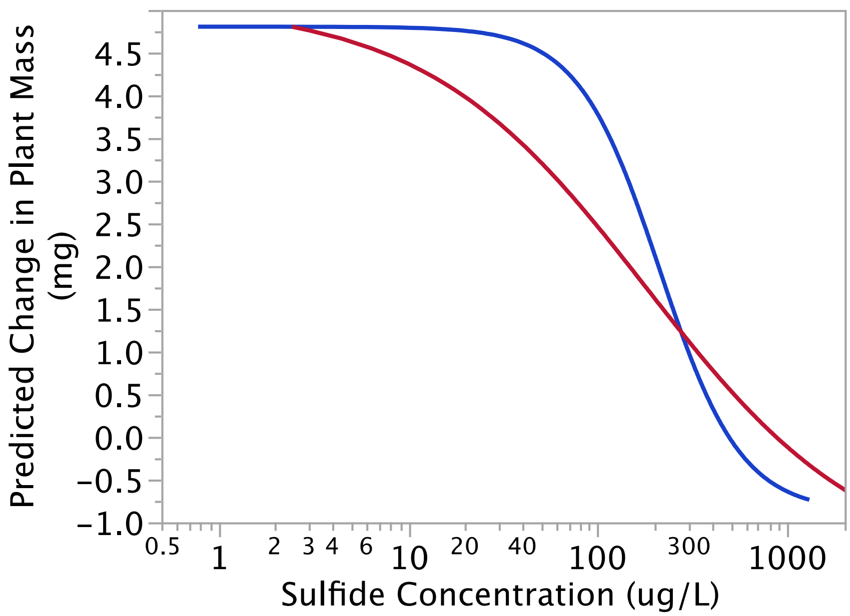


Figure 2. Comparison of logistic model response curves for changes in wild rice plant mass as a function of sulfide concentrations – rangefinder study results. Blue curve shows response based on initial exposure concentrations; red curve shows response based on final exposure concentrations.

[1]Assuming there are a total of N points to fit the model, this would involve refitting the model N times with each iteration involving removing one observation from the data set used to fit the model, and then using the fitted model to calculate the prediction error for the out-of-sample observation. The overall root mean square error (RMSE) for the full suite of N out-of-sample predictions would then be compared to the RMSE for the model constructed from the full data set.

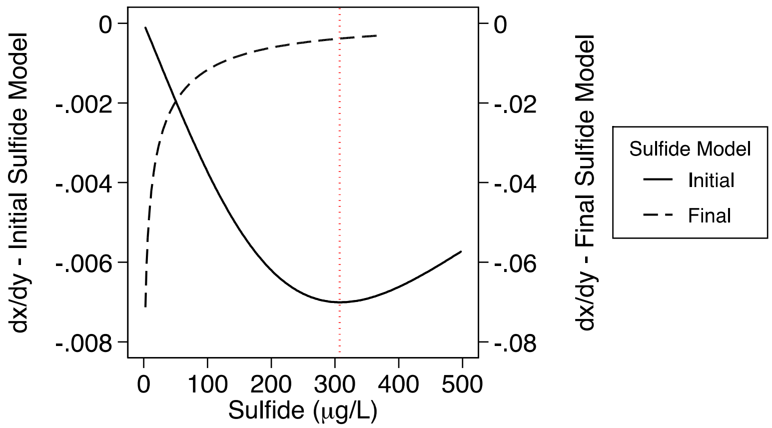


Figure 3. Predicted incremental (derivative dy/dx) changes in plant mass as a function of sulfide concentration. The change in the derivative is shown for the nonlinear models obtained using both initial and final sulfide concentrations.

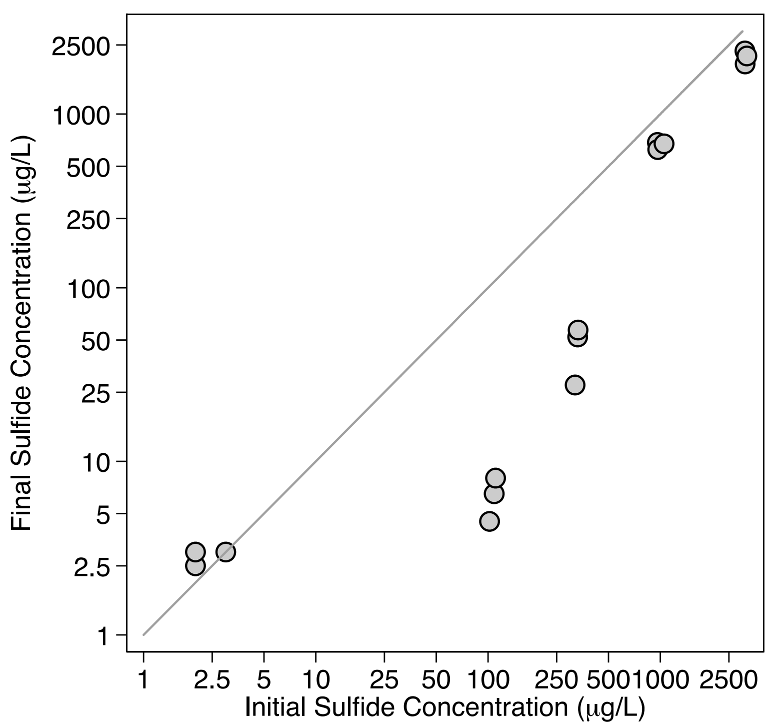


Figure 4. Comparison of final sulfide concentrations in the hydroponic experiments with initial concentrations.

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.

I agree with MPCA's emphasis to use the lake and stream field survey data "to explore the effect of elevated sulfate on the chemistry of the porewater of actual and potential wild rice habitat" and that the field survey sampling program thus did not require a probability-based sampling design (page 21). Given that, and as Question 4 states, a key question regarding the application of the survey data is whether is "sufficiently representative" of wild rice sites for the purposes of modeling sulfide relationships with other variables, and develop tools to adequately and appropriately protect wild rice from elevated sulfate. In order to demonstrate representativeness, MPCA compares interquartile ranges for the field survey data with results from both targeted sampling of wild rice sites (DNR survey – which included lake sites only) and from probability-based surveys conducted by USEPA. I have several comments.

1. I believe that the focus on comparing the interquartile ranges is somewhat limited, since by its very definition, the focus excludes the remaining 50% of the data that lie at the upper and lower ends of the distribution. If we assume that the data that lie within the lower 5% and the upper 95% quantiles are potentially all useful from both an effects and a modeling perspective, then the field survey data should encompass the upper and lower 5% quantiles observed for sites where wild rice is known to occur.

2. The comparison with the probability surveys is of limited value, since it is unclear how the probability-based distribution for the overall population compares to the weighted distribution that would be obtained for sites sampled during the survey that are restricted to the occurrence of wild rice. It may be possible given the design of the probability samples to use the survey data to subset and develop weighted distributional estimates for "ecoregions" known to support wild rice, compared with those that do not. This analysis would perhaps be very similar to the original analysis by Moyle (1956).

3. Figure 9, panel "B" of the MPCA report is a bit confusing as it includes a cumulative frequency distribution (*cfd*) *plot* for a PCA-DNR survey that the text indicates included lakes only, and also includes a curve for lakes labeled "DNR wild rice".

4. I cannot seem to reproduce the sulfate *cfd* for the 2012-2013 Field Survey for lakes shown in Figure 9, panel "A" of the MPCA report (see Figure 5). This may be related to how MPCA processed values below a given method detection or reporting limit. There also appear to be some differences between the *cfd* I generate for the 2012-2013 survey data for streams *vis-à-vis* the MPCA plot (comparison not shown). It would also be helpful if the abscissa of the Figure 9 plot in the MPCA report was scaled in a more accessible format.

5. Likewise, when I compute the interquartile ranges for the 2012-2013 survey data for both lakes and streams, the results do not match up with the values reported by MPCA. For example, I calculate the interquartile range for lakes only abstracted from the 2012-2013 survey to range from 3.3 to 29.3 mg/L; this is contrasted with a range from 2.5 to 14.5 mg/L reported by MPCA.

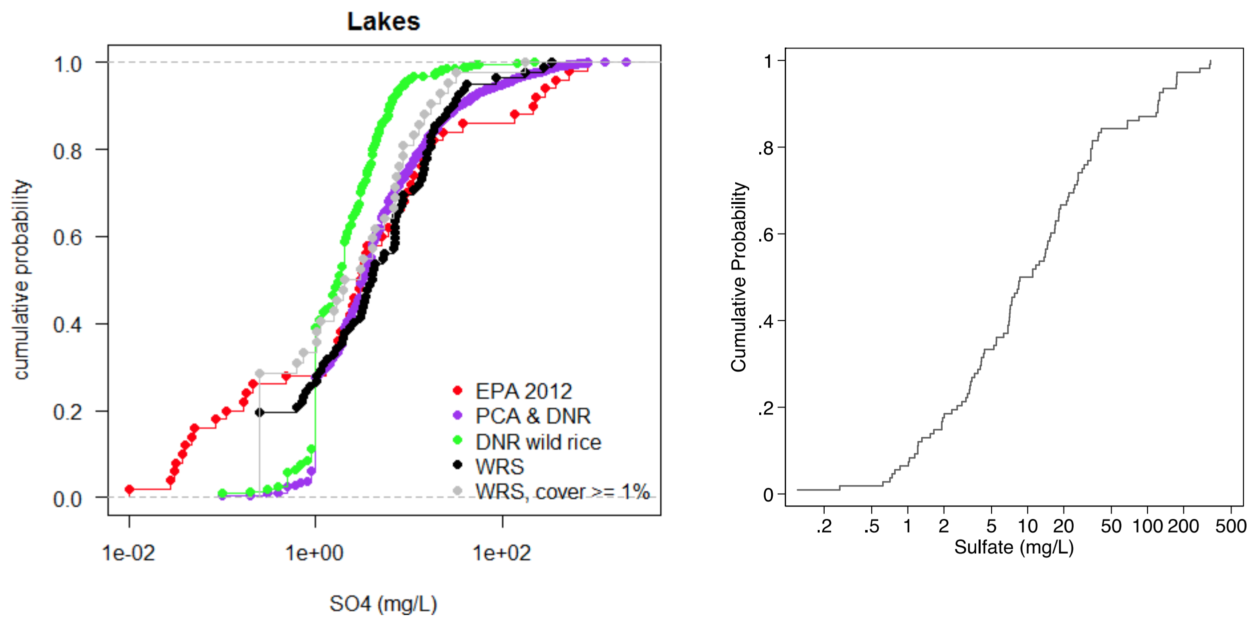


Figure 5. Comparison of *cfd* plots for the distribution of sulfate based on the 2012-2013 Field Survey data. Left-hand panel – *cfd* developed by MPCA (2014) based on several different surveys, including the 2012-2013 Field Survey (WRS) data. Right-hand panel – *cfd* plot generated from the 2012-2013 Field Survey data provided to the Peer Review Panel.

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.

Analysis of the mesocosm experiment results was conducted by using linear regression, apparently on the mean responses for six replicates at each of five treatment levels (including the control). This truncation of *N* leads to a loss of statistical power to detect significant effects. I would argue that mixed level linear regression would allow for a better assessment of the statistical strength of the *fixed* effect between response (*e.g.,* seed weight) and sulfate treatment levels, and evaluation of the magnitude of the *random* effects imposed by the different time periods. This presumes that the growing period for each treatment year was of the same duration. Of particular interest would be whether random slope effects are indicated by the experiments, rather than simply random intercepts. While the random effects are clearly important, it is the strength and nature of the fixed effect that helps lay the predicate for establishing a sulfate-related response that warrants establishing a protective criterion.

My own preliminary analyses (without the replicate data) of the Pastor (2013b) data using mixed level modeling indicates that the random effects of different years on the fixed relationship between sulfate and response are insignificant, and that the fixed effect significance is reinforced by using a mixed model approach (Table 1). Both of these results are important in establishing the utility of the mesocosm experiments for defining a toxic response. For viable seeds (normalized to area) and plant biomass, however, the high intraclass correlation (ICC) values for the models indicate that unmeasured effects associated with each given year contribute greatly to the response of the two metrics. As a result, the significance of the effect of sulfate on these two metrics is arguably weakened or masked by the unmeasured effects related to year. Nonetheless, the unmeasured effects do not significantly alter the underlying (fixed) relationship between these two metrics and sulfate.

Table 1. Summary of preliminary mixed level modeling results for selected plant and seed growth metrics as a function of nominal sulfate treatment level reported by Pastor (2013b). The analysis also includes the results for the intraclass correlation (ICC) for the Year groupings.

	Fixed Effects		Random Effects		
Response Metric	Slope	Significance	Intercept	Slope*Year	ICC
Viable seeds (per m2)	-0.977	0.029	Yes	No	0.886
Seed weight	-0.0275	0.011	No	No	0.197
Viable seeds (%)	-0.0608	0.001	No	No	0.075
Biomass	-0.0615	0.069	Yes	No	0.934

Other Comments

1. Statistical power analysis should be conducted to quantify the level of change the experimental design is capable of detecting.

2. The Pastor (2013b) report indicates that five plants were selected for analysis from each treatment tank (page 9). The spreadsheet of the 2013 data includes six replicates. Also, the 2011 and 2012 have an additional treatment level labeled "Row". It is unclear what the *Row* effect is, although it appears to be statistically significant.

3. The figures in the MPCA report that are derived from Pastor (2013b) should probably state that the values for r^2 that are reported (for example, Figure 5 in Pastor, 2013b) are *adjusted* values.

4. It appears that the regression modeling used the nominal rather than the actual sulfate concentrations. For example, the controls had a nominal sulfate treatment level of 0 mg/L, while the overlying water concentration had a concentration of 7 mg/L. It would seem more appropriate to use the actual levels if indeed the regression analysis was conducted using the nominal levels. Figure 2 in Pastor (2013b) indicates that the actual sulfate concentrations were almost always below the nominal level. The mean sulfate concentration that was measured after the initial stabilization period would seem to be more appropriate to use for the modeling.

5. The porewater geochemistry results presented in the rooting profile report (Johnson, 2013) may be useful, both in terms of defining toxicity in response to sulfide levels, and in terms of further informing the conceptual model for porewater sulfide dynamics. For example, it may be possible to use the porewater geochemistry to validate the structural equation model (SEM) developed in response to Charge Question 7.

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?

Mesocosm Modeling

The regression modeling results generally support the conclusion that elevated sulfide in the porewater can be toxic to wild rice (8 out of 12 analyses conducted related to declines in seed production), but the inconsistencies in the results need to be better explained. For example, why does the viable seed count normalized to area show no effect for two of the treatment years, while the percent viable seeds show significant effects across all three years? Likewise, the plant biomass results appear to support only a relatively weak effect or nonsignificant effect? The mixed level modeling suggested in the response to Charge Question 5 would establish more definitively the magnitude and significance of the underlying fixed effect between sulfate and seed production.

Given that (1) the hydroponic study results show that there was no direct sulfate effect on wild rice toxicity but that there is clearly a sulfide effect; and (2) the Field Survey results also show only a weak at best direct effect of sulfate on wild rice cover (see analysis below), and that porewater sulfide is a better predictor of wild rice cover; and (3) the mesocosm experiments included sulfide measurements in 2013 and 2014 – I would think that MPCA should consider modeling toxicity response using sulfide measurements conducted as part of the mesocosm experiments.

Field Survey Data

MPCA used histograms of the distribution of sites with a minimum of 5% wild rice cover plotted as a function of porewater sulfide to determine if there was concordance between the results of the hydroponic study showing sharp response (EC20) between 210 to 322 µg/L and the results from the Field Survey. The MPCA histogram analysis of the Field Survey data indicates a change in slope occurring around 60 to 75 µg/L. I believe the histogram assessment is a useful analysis for showing the underlying, overall response in the field survey data, although it is arguably more an assessment of the upper limit of response because it ignores declines in percent cover that may be occurring at lower sulfide levels, but not sufficient to drive percent cover below the 5% threshold. Given that concern, it might be useful to construct similar histogram distributions for higher thresholds (*e.g.,* 7.5 and 10%). One question I have regarding this analysis is whether the sulfide ranges used to construct the bins were based on the distribution characteristics of the survey data. Restated, do the delimiting concentrations of sulfide for each histogram bin correspond to a 12.5% quantile (8 bins), or were the concentration ranges selected based on some other criterion? If indeed the latter is the case, I think the analysis would be more robust if the bins were distributed according to quantiles.

An alternative, but related approach that is perhaps more sensitive to identifying threshold responses using the survey data involves constructing a curve that is based on the change in the cumulative percent cover of wild rice as a function of porewater sulfide levels. In this analysis, the data are sorted by sulfide levels and the cumulative percent cover is calculated for each concentration where cumulative percent cover is weighted by the number of observations for sulfide concentrations below a given value *x*, plus the number of sites with sulfide concentrations equal to *x.* In other words, for a given concentration *x*, the curve defines the overall mean for percent cover for all the observations that equate to sulfide concentrations ≤ *x*. Such curves are sensitive to the combination of large variations in the response variable coupled with a small number of observations used to calculate the cumulative mean distribution at the lower end of the range of concentrations. Conversely, as the concentration range approaches the upper limit of its distribution, the constructed curve is relatively insensitive to variations in the response variable. As a result, it is useful to construct the curve in both forward (cumulative response for concentrations ≤ *x*) and reverse (cumulative response for concentrations ≥ *x*) directions to help interpret the apparent break points that relate to a fundamental trend in the response rather than changes in the response driven by random noise. The results from this analysis, which are shown in Figure 6 for lakes and streams included in the Field Survey suggest that wild rice cover is quite sensitive to increases in exposure levels above ~ 40 to 50 μg/L, and may be sensitive to increasing sulfide levels at a threshold as low as 20 μg/L.

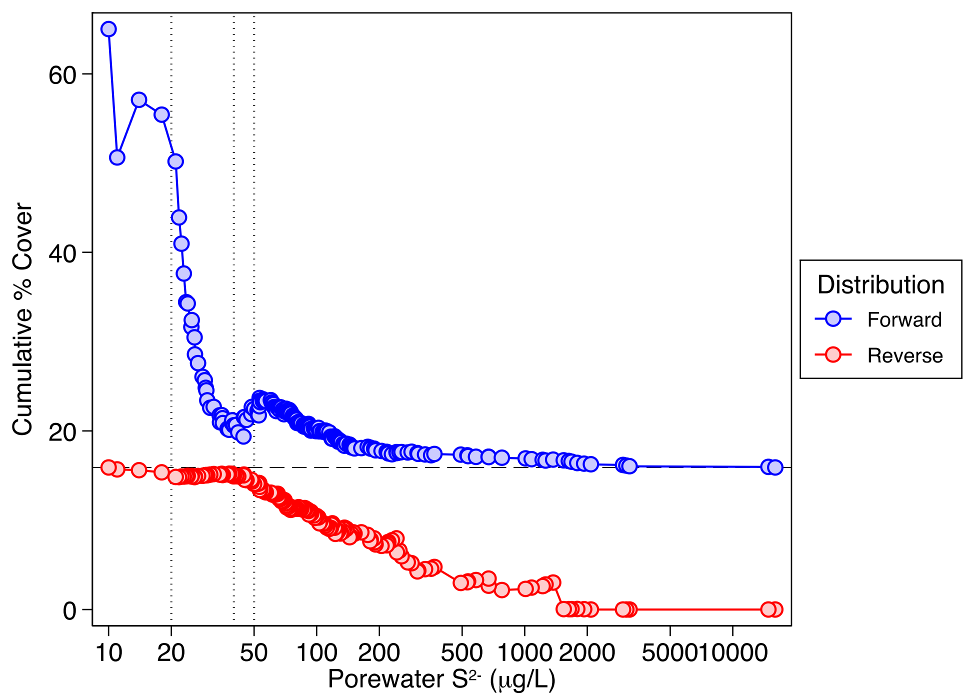


Figure 6. Cumulative percent cover of wild rice in Minnesota lakes and streams as a function of increasing porewater sulfide concentrations. Data from the MPCA Field Survey. The *forward* curve defines the overall mean response for all observations with observed porewater sulfide concentrations ≤ *x*; the *reverse* curve defines the overall mean response for all observations with observed porewater sulfide concentrations ≥ *x*.

Another approach towards the question of whether the Field Survey data support the hypothesis that porewater sulfide concentrations in excess of 300 μg/L involves logistic regression, with the occurrence or absence of wild rice cover used as the dummy response variable, and porewater sulfide as the independent variable. Confidence intervals can be constructed on the model, and inverse predictions can be conducted to predict the confidence interval (90%) for porewater sulfide concentrations that yield a probability of 0.50 that wild rice cover will be completely absent (Figure 7). This calculation yields a predicted porewater sulfide concentration of 495 μg/L, and a range of 272 to 1,740 μg/L. This prediction is biased towards defining a less sensitive threshold of response in that it considers the effect of sulfide precluding the occurrence of wild rice, and not just reducing the magnitude of its occurrence.

It should be noted that this analysis also indicates that there is no difference in response (presence or absence) between lakes and streams included in the survey (results not shown). This is not true if sulfate were used as the independent variable (results not shown), and this fact: (1) further supports the idea that the critical metric for quantifying toxicity effects is sulfide; and (2) suggests the difference between lakes and streams with respect to sulfate-related toxicity relates to differences between lakes and streams vis-à-vis the conversion of sulfate to sulfide, rather than the response of wild rice to sulfide. As later analyses will show, this difference likely reflects sediment geochemical differences, including organic carbon content in particular.

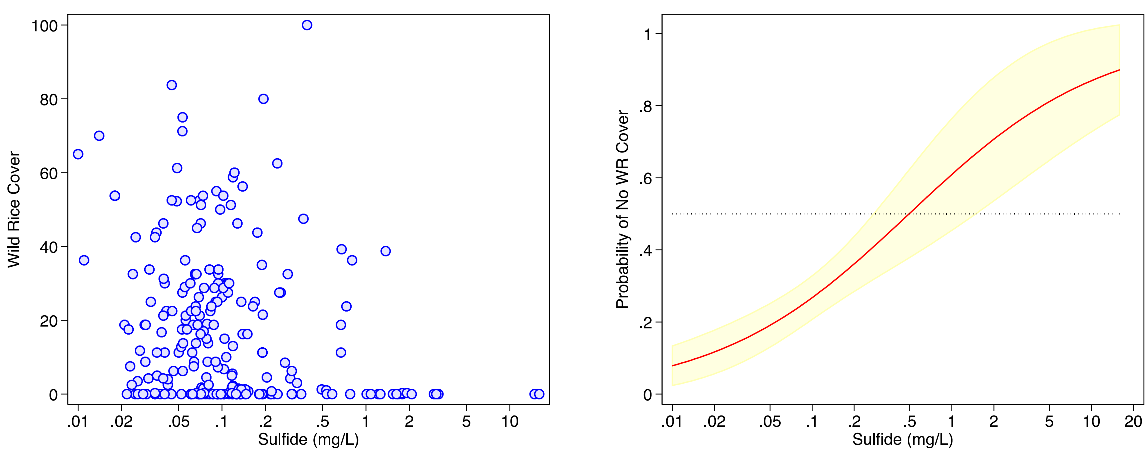


Figure 7. Sulfide – wild rice cover relationship derived from Field Survey data. Left-hand panel – percent wild rice cover as a function of porewater sulfide. Right-hand panel – logistic regression model predicted response (probability of the absence of wild rice) as a function of porewater sulfide concentrations. The logistic model also includes the 90% confidence interval (5% upper and lower tails) for the predicted response.

Structural equation modeling (SEM) further establishes that sulfide exerts a direct response on wild rice (using log-transformed wild rice percent cover as a continuous response variable), and that this effect is both highly significant ($p < 0.001$) and dominant compared to the effects of other variables (direct and indirect effects) included in the model (see Figure 8; see also discussion presented for Charge Question No. 7 which details the SEM approach towards modeling porewater sulfide). The SEM results, however, do not establish some sort of a threshold response, although the model could be used to predict the sulfide-dependent response of wild rice cover, and the results could be used in help evaluate the idea of a 300 μg/L “threshold”, assuming that a given relative decline in percent cover can be defined as a “threshold.”

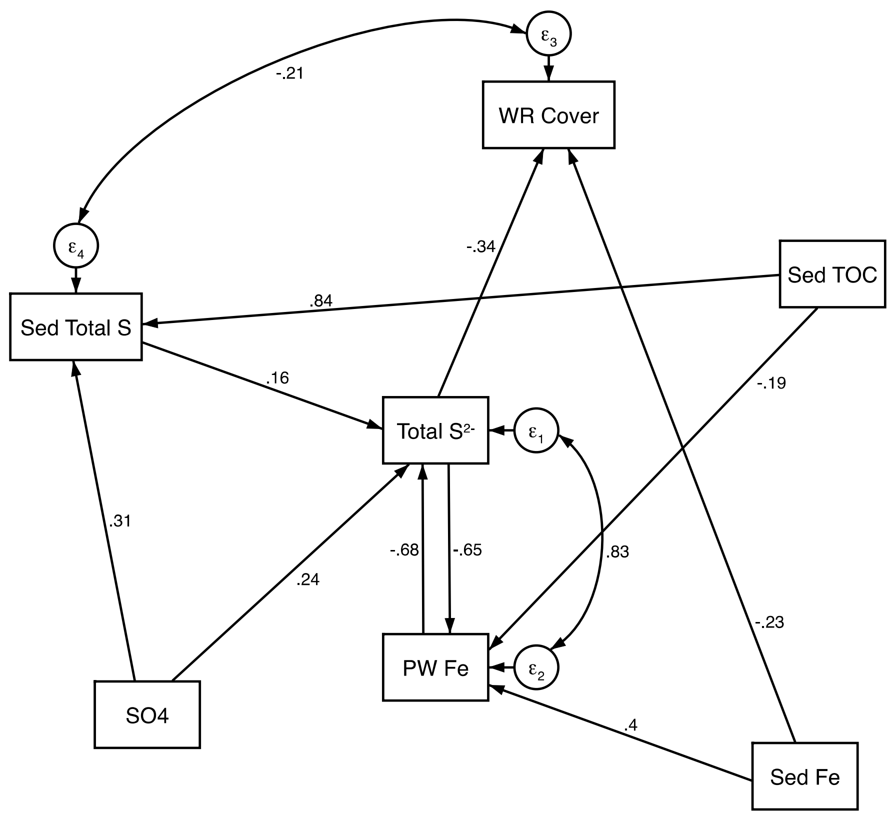


Figure 8. SEM model for porewater sulfide and wild rice cover response to sulfate and key sediment chemistry variables. Pathways shown are standardized (β) coefficients. This model is an extension of the SEM model developed for porewater chemistry alone (*i.e.,* excluding wild rice response) discussed in response to Charge Question No. 7 and presented in Figure 12).

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?

In evaluating this question, it is first useful to review the primary reasons when or why quantile regression is advantageous compared to ordinary least squares (OLS).

(1) Quantile regression is useful when the objective of the model is to characterize the distribution or the boundaries of the dependent variable. With respect to modeling sulfide, the supposition is that the upper quantile values are more meaningful from a toxicity perspective than characterizing variations in the mean distribution.

(2) Quantile regression also allows for essentially modeling separate effects of the independent variable on the distribution of the dependent value. Thus in the case of sulfide, the bivariate plot shown in the Figures 18 and 22 of the MPCA report indicates that increasing sulfate concentrations have little or no effect on *minimum* porewater sulfide concentrations, but it does govern the upper bounds of the distribution.

(3) Quantile regression is less sensitive to leverage imposed by influential *x* observations. Quantile regression is superior to OLS and robust regression methods in terms of producing unbiased estimators when leverage is important.

(4) Quantile regression is less subject to influence imposed by outliers (*y* values), resulting in improved model parameter estimates, although robust regression may be superior depending on the nature and severity of the outlier.

(5) Quantile regression makes no assumptions about the distributional characteristics of the error term in the model, and back-transformation to the original scale of a predicted variable does not impose a bias, unlike OLS.

With respect to the MPCA sulfate-sulfide data, leverage points and outliers are both problematic. This is illustrated by constructing a simple linear regression of porewater sulfide as a function of surface water sulfate (both log-transformed). An analysis of both leverage (Figure 9) and outlier influence (based on the calculated *Cook's D* statistic; Figure 10 shows that two sites (P-55 and FS-85) should be either excluded from a regression model, or their influence "down-weighted" through robust regression or quantile regression. The effects of both excluding these two outliers on OLS regression results and down-weighting through both robust regression and quantile regression (both median and 75th percentile) are summarized in Table 2.

Table 2. Summary of parameter coefficients for sulfide concentrations fitted by OLS, quantile (both median and 75th percentile), and robust regression methods. Models are constructed for (1) sulfate; (2) sulfate and sediment Fe; and (3) sulfate, sediment Fe and TOC concentrations as independent variables. The OLS results also compare the effects of excluding sites P-55 and FS-85 as outliers. All variables log_{10}-transformed.

Parameter	OLS		Quantile		Robust
	N = 215	*N* = 213	0.5	0.75	
SO4	0.213	0.181	0.127	0.349	0.143
SO4	0.242	0.209	0.157	0.308	0.181
Sediment Fe	-0.453	-0.402	-0.309	-0.517	-0.342
SO4	0.375	0.339	0.267	0.367	0.287
Sediment Fe	-0.682	-0.627	-0.536	-0.688	-0.538
Sediment TOC	0.464	0.444	0.409	0.426	0.397

The results for the sulfate-sulfide models show that the model coefficient estimates are sensitive to the choice of model estimation method. Moreover, eliminating the two most obvious outliers does not result very good agreement between the OLS estimates, and the robust methods (quantile and robust regression).

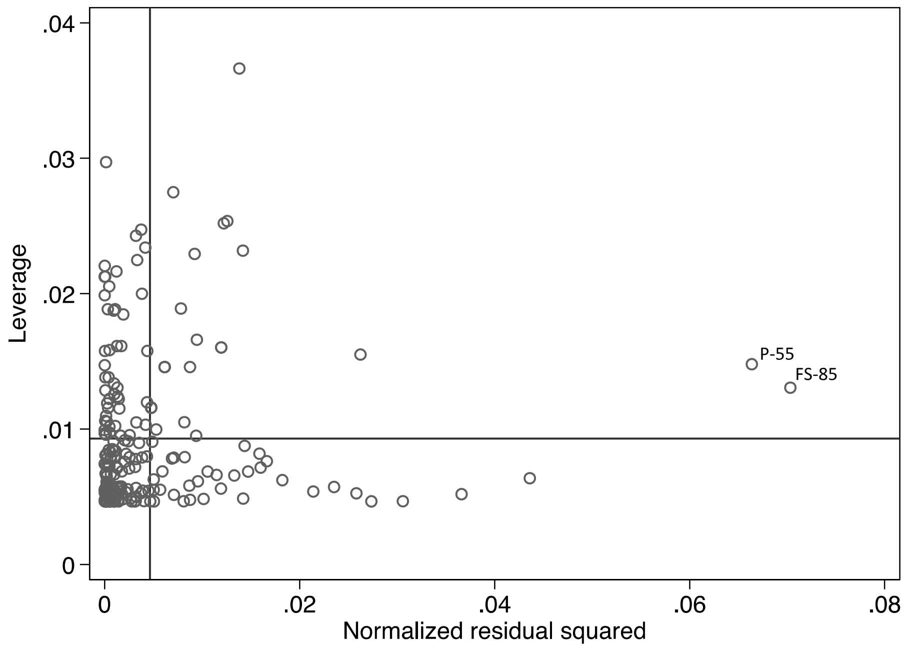


Figure 9. Leverage plot for bivariate sulfate-sulfide model.

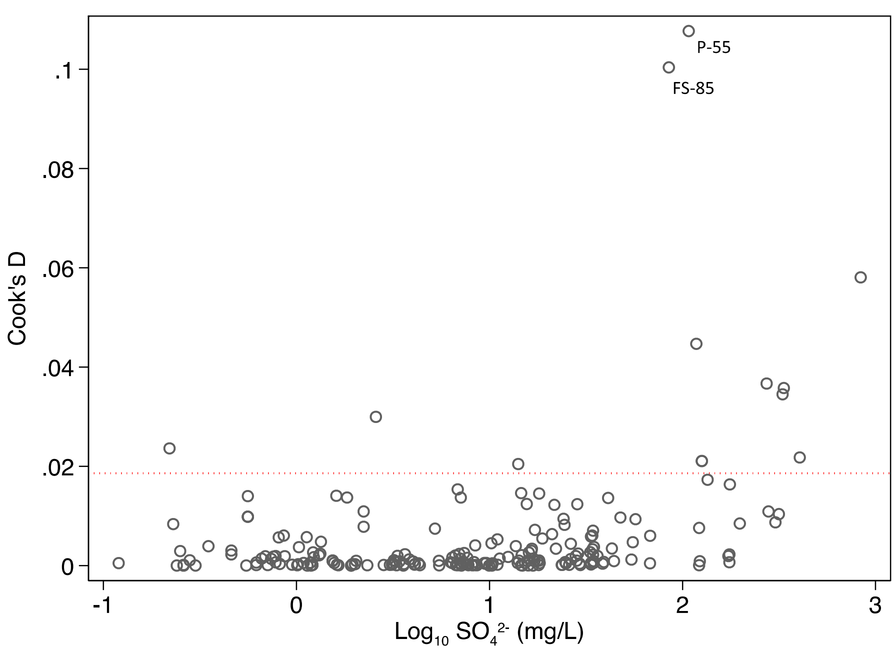


Figure 10. Plot of *Cook's D* statistic for the bivariate sulfate-sulfide model as a function of sulfate concentrations (log_{10}-transformed) showing the presence of two clear influential (outlier) sites – *FS-85* and *P-55*.

It should be noted that the fit statistics for the sulfate-sulfide model are poor for all three estimation methods (for example, r^2 = 0.10 for the OLS model with both P-55 and FS-85 included, and the pseudo r^2 = 0.07 for the quantile regression 75th percentile model). Including sediment Fe in the models as done by MPCA does substantially improve the model fits (r^2 = 0.21 and Bayesian Information Criterion or BIC = 316.6 for the single parameter sulfide model compared to 295.8 for the two parameter OLS model), and changes the slope of the sulfate relationship with sulfide (Table 2). These results would *initially* suggest that quantile or robust regression would be more appropriate for fitting a model for porewater sulfide.

Issues and potential problems with quantile regression for predicting porewater sulfide

I can think of a couple of reasons why quantile regression would be the method of choice for developing the sulfate-sulfide model relationship.

1. Quantile regression would be preferred if MPCA is concerned more about modeling the distribution characteristics of sulfide, rather than the central tendency. This would certainly be advantageous if, for example, the risk analysis of sulfide toxicity indicated that a certain quantile exposure better explained changes in toxicity than using models based on mean exposure. However, the question then devolves to which quantile to use. Choosing the 75th percent quantile may be more attractive because the model yields more certain parameter estimates (but poorer fits) compared to higher quantiles, but it needs to be demonstrated that sulfide estimates corresponding to this quantile better suits the risk needs of the wild rice exposure-response model. Moreover, the idea (page 46, lines 1098-1099) that "*i[I]t is more statistically robust to work with the 75th percentile, compared to higher percentiles, because as the number of data points progressively declines the uncertainty of the regressions increases significantly*" presumably relates to parameter coefficient uncertainty, and the ability to make conditional estimates of response. If so, then the same logic would indicate that a median response model would be superior to a 75% quantile model.

2. Quantile regression is useful for developing limits to the expected conditional response of sulfide to a covariate when other covariates that influence sulfide are unknown, not of interest, or not suitable for inclusion in the model (*e.g.,* porewater Fe – see response to Question 8). This is part of the overall question of model specificity, which considers (1) whether the form or the nature of the relationships between the dependent variable and the independent variables satisfies the underlying assumptions of the method used to fit the model, and (2) whether the model includes all relevant parameters. By acknowledging that, for example, sediment Fe influences the sulfate-sulfide relationship, and including it in the model, MPCA is clearly interested in improving model specificity, and better estimating how the response of sulfide to variations in sulfate is modified by other variables. I strongly concur with this approach. I would argue that, at a minimum, however, the model should also include sediment TOC. Inclusion of sediment TOC results in a quite substantial improvement in the sulfide model (for example, r^2 = 0.39 and BIC = 244.6 for the OLS model), and somewhat improved residual behavior (not shown), although the parameter coefficients across the three estimation methods still do not converge (Table 2). Inclusion of sediment TOC also results in shifts in the parameter slopes such that the slope for sulfate is increased substantially *vis-à-vis* the sulfate slope for the MPCA two-parameter model. This will of course lead to different estimates of the change in sulfide related to changes in sulfate when controlling for the other parameters in the two models. Determining whether these slope shifts

are significant, however, requires further analysis. Interestingly enough, if the focus towards modeling sulfide shifts more to the upper boundary, sediment TOC becomes a more robust determinant that sediment Fe.

I also have concerns about the material MPCA included in Appendix G to document the evaluation of model coefficient uncertainty. The model evaluated by MPCA (page 91) includes the ratio *logS04*:*logsedFe* along with *logSO4* and *logsedFe* as independent variables. The inclusion of the ratio along with its parent variables violates the assumption that the independent variables are indeed truly independent, and leads to parameter estimates that cannot be used to estimate conditional responses.

Another potential problem is heteroskedasticity of errors. Similar to ordinary least squares (OLS) regression, both quantile and robust regression assume that the residual errors are independent and identically distributed, and thus the standard errors and confidence intervals of parameter estimates will be unreliable if this assumption is violated (Hamilton, 2013). Inspection of the errors obtained from all three estimation methods are not constant, but vary with the magnitude of the predicted value for porewater sulfide (this is illustrated in Figure 11 for the errors obtained from the OLS regression model). Whatever method MPCA uses to develop their sulfide model, the method should use an estimator such as bootstrapping that is more robust to heteroskedasticity.

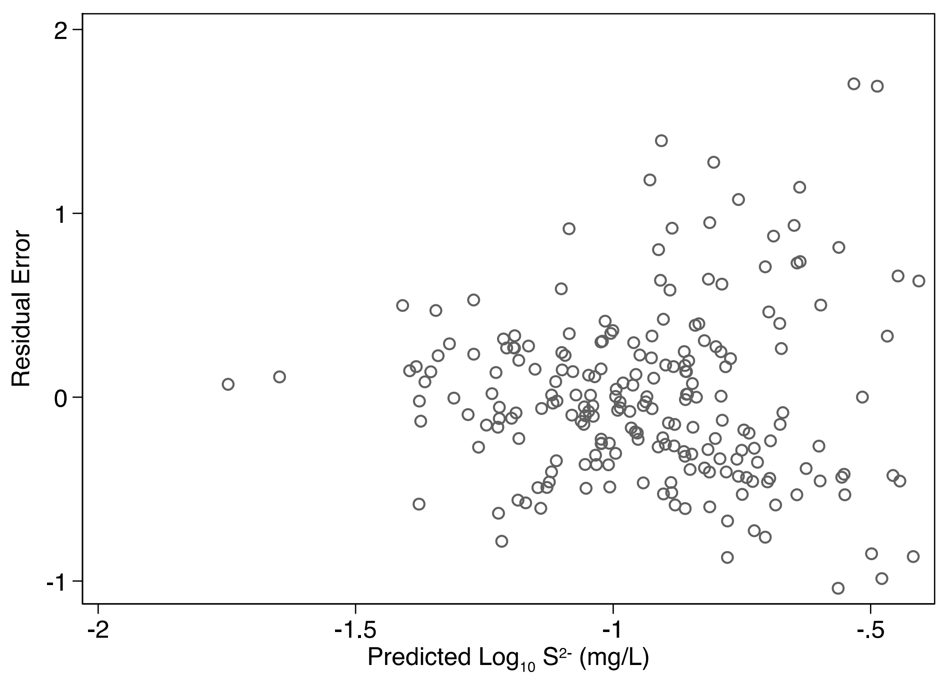


Figure 11. Plot of model residual errors *vs.* predicted porewater sulfide concentrations for the OLS model incorporating sulfate and sediment Fe as independent variables. All variables *log_{10}*-transformed.

A far more difficult problem with the MPCA quantile regression modeling again considers model specificity. Exclusion of relevant variables extends to porewater Fe. This is also an issue raised in Charge Question 8. This relationship is best characterized as a feedback loop that requires a set of simultaneous equations to solve and thus is not suitably modeled by conventional, single equation regression techniques, including simple multivariate linear regression, quantile regression, and robust regression. Moreover, the effect of sulfate on sulfide is not only direct, but is likely exerted indirectly through its effects on sediment sulfur. Several options are available for constructing a sulfide model that both includes the feedback loop effects of porewater Fe on sulfide, and integrates the direct and indirect effects of other variables as well. These options include structural equation modeling (SEM), and two- or three-stage least squares regression.

Structural Equation Model for Porewater Sulfide

I have constructed an SEM model to simulate both porewater sulfide and Fe concentrations using the MPCA survey data set to illustrate its application. The conceptual framework of the SEM is shown in Figure 12, and includes sulfate, sediment TOC, and sediment Fe as independent variables, and sediment total S, porewater sulfide, and porewater Fe as dependent variables. The number of observations that included measurements for the full suite of parameters used to construct the model was 184. The model is highly significant ($p > \chi^2 = 0.683$)[2], and fits the observed sulfide data better ($r^2 = 0.504$) than the previous three-parameter regression models.

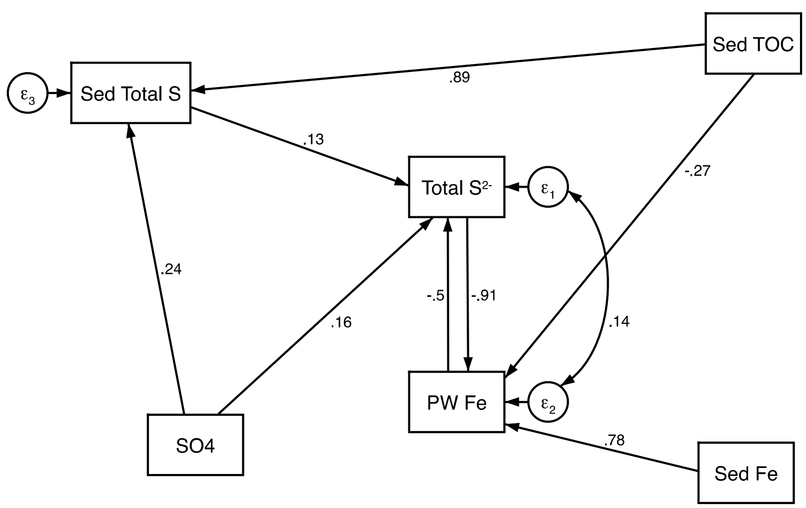


Figure 12. Path diagram showing the relationships between variables used to construct the porewater sulfide SEM. All coefficients are significant at $p \leq 0.001$.

[2] Note that the χ^2 statistic tests the null hypothesis that the model is equivalent to or reproduces the covariance matrix of the observed data. Thus χ^2 significance levels are used to reject the null hypothesis, and thus reject the model.

A critical advantage in the application of SEM to porewater sulfide modeling as a function of sulfate is that the SEM provides parameter estimates for both direct and indirect effects. By comparing the aggregated effects of each variable (using the standardized β coefficients to facilitate the comparison), we can assess the relative importance of each variable included in the model on controlling sulfide concentrations. This is illustrated in Figure 13. That analysis illustrates two key points. First, the model indicates that the feedback dynamics between porewater sulfide and Fe exert the greatest controls on sulfide levels, and thus underscores the importance of modeling that relationship. Second, the model indicates that the total effect of sulfate (which includes both direct and indirect effects) is essentially equivalent in importance to sediment Fe and TOC (both of which exert indirect effects) on sulfide concentrations. This in turn further underscores the necessity of including of including these variables in any efforts to relate changes in porewater sulfide to changes in sulfate concentrations.

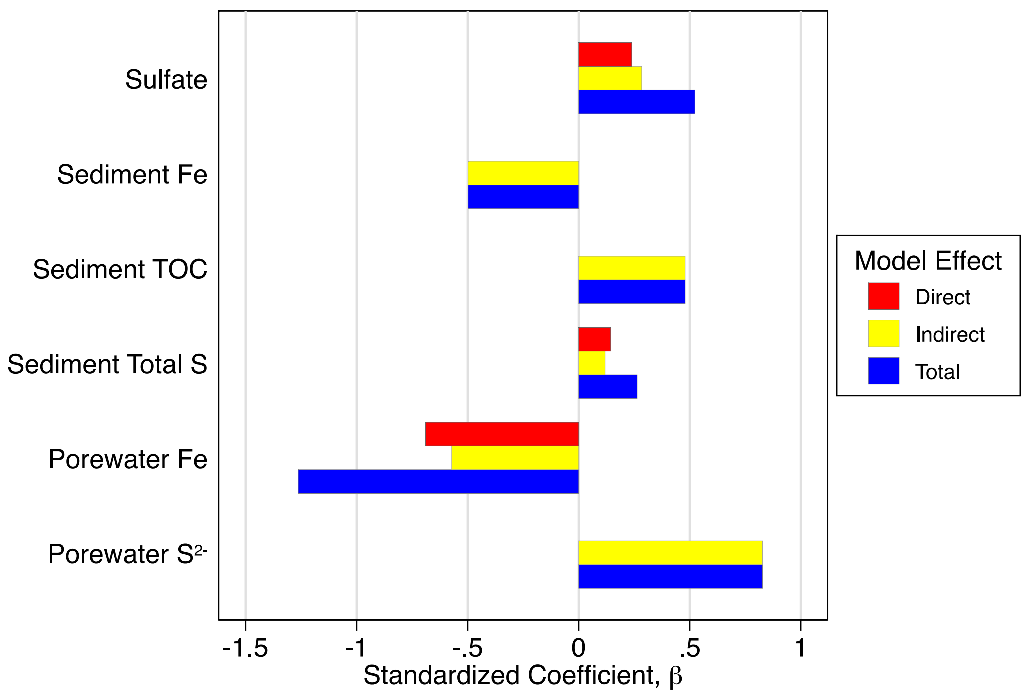


Figure 13. Direct, indirect, and total effects of SEM model variables on porewater sulfide concentrations. Effects are shown based on standardized (β) coefficients, which show the response of sulfide relative to its standard deviation based on a standard deviation change in the predictor variable.

The analysis of total effects afforded by SEM leads to a different assessment of the quantitative relationship between sulfate and porewater sulfide concentrations *vis-à-vis* that obtained from MLR. For example, the parameter estimate obtained from SEM for the total effect of sulfate on sulfide is 0.359, compared to 0.231 if MLR is used to fit the sulfide model using all the variables in the SEM that have direct influences on sulfide. <u>In other words, by not accounting for indirect effects, conventional regression modeling (as well as robust and quantile regression) can lead to a large underestimate of the effects of</u>

sulfate. Figure 14 compares the conditional response of sulfide to variations in sulfate for the SEM model (controlling for the other two independent variables in the model – sediment TOC and Fe) with that obtained from the quantile regression model (both median and 75th percentile) controlling for sediment Fe.

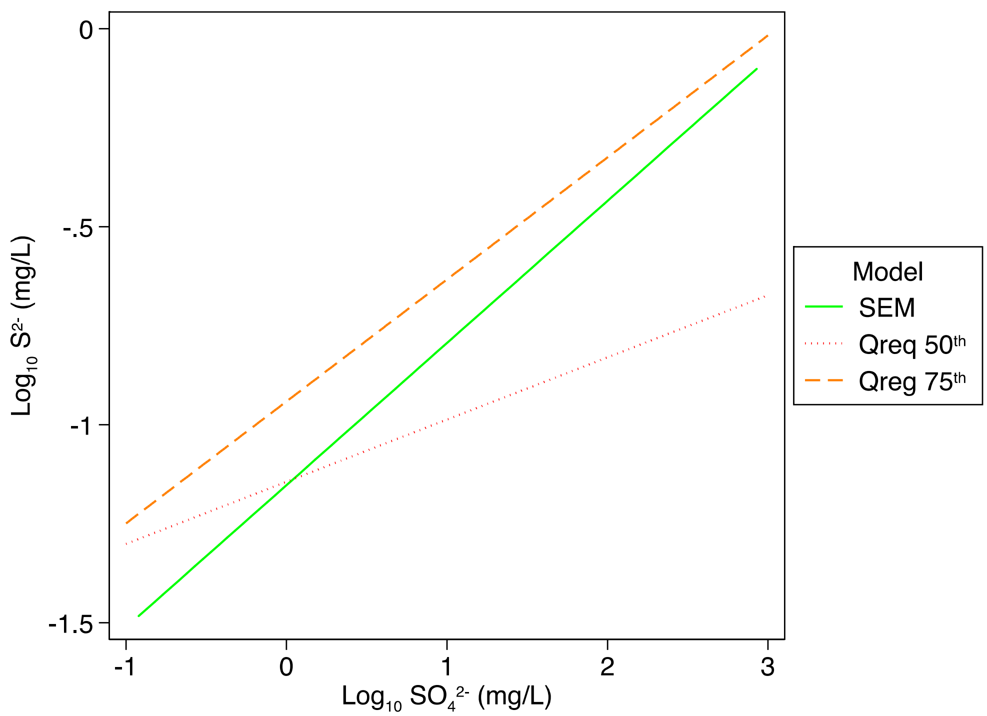


Figure 14. Predicted porewater sulfide as a function of sulfate concentrations: comparison of MPCA quantile regression (Qreg) model approach with SEM results. SEM model controls for sediment TOC and sediment Fe (fixed at median values), while the MPCA Qreg model controls for sediment Fe only (also fixed at the median value).

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?

This question is difficult to answer categorically. *Ceteris paribus* the dynamics of porewater iron Fe and sulfide concentrations are inversely related based on thermodynamics. Ideally, a statistical model that seeks to predict porewater sulfide concentrations would thus seek to include porewater Fe concentrations as well. The dilemma for MPCA is that quantile regression and other single equation regression methods will lead to biased parameter estimates if an independent variable included in the model is also causally influenced by the model dependent variable. Such would be the case if porewater Fe were included in the porewater sulfide model. This, however, leads to the proverbial *Catch-22* because, if MPCA wants to ensure that quantile regression is implemented properly, porewater Fe should *not* be included in the model. This overall problem is not trivial. If we examine the *total* effect of each of the variables that influence sulfide based on the SEM (Figure 13), we see that the two variables that exert the greatest effect of porewater sulfide are porewater Fe (which imposes a drag on porewater sulfide both directly, and indirectly through the feedback loop), and to a somewhat lesser extent porewater sulfide itself (which promotes higher concentrations through its effect on diminishing porewater Fe levels). Thus, if the goal of the modeling is to maximize our ability to predict the central tendency the conclusion is that porewater Fe needs to be included as a model variable, and that the appropriate model framework needs to be implemented to enable its inclusion.

However, if the goal of the modeling is to account for the upper limits of the conditional response of sulfide as a function of sulfate, then including acid-extractable iron, as well as other variables such as sediment TOC to help narrow the limits of the conditional response envelope is preferred. The distinction between the two approaches is both philosophical (e.g., the quantile regression approach should lead to lower estimates of acceptable levels of sulfate for a given target sulfide concentration because it is being used to predict the upper or 75% quantile boundary of the response envelope) and practical (SEM is not yet a widely used and accessible approach in statistical water quality modeling).

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?

I think that the focus by the MPCA on porewater sulfide as the parameter impacting wild rice is well supported by both the hydroponics study and the Field Survey results. The hydroponics experiment results are compelling in demonstrating a direct toxic effect related to sulfide exposure, while showing that direct exposure to sulfate (at concentrations up to 1,600 mg/L compared to a maximum of 838 mg/L measured in the lakes and streams included in the Field Survey) without the concomitant development of elevated sulfide concentrations had no demonstrable effect. Several different modeling approaches using the Field Survey data also indicate that porewater sulfide is the preferred variable for elucidating sulfate/sulfide-related toxic response. These approaches include the logistic regression and SEM modeling presented in the response to Charge Question 6.

The report is generally silent on whether other variables measured as part of the Field Survey have utility in characterizing or modeling the variability in wild rice cover. For example, MPCA report cites Moyle (1956) and the idea that SO4 toxicity may actually reflect Mg toxicity, Mg being correlated with SO4, but I did not see any analysis that explored that hypothesis. Some preliminary analyses on my part suggest the opposite – *viz.,* higher levels of wild rice cover correlate positively with Mg, while correlating negatively with sulfate. It thus would be useful to see a discussion on efforts to evaluate the effects of other variables such as magnesium and their significance. The SEM model could potentially be extended to include other variables, and serves as a useful framework for understanding and placing in better context the complex dynamics of indirect and direct relationships between wild rice response and key biogeochemical variables.

As stated in the response to Charge Question 7, the modeling of sulfide should include other sediment variables, including sediment S and sediment TOC concentrations, as well as the feedback loop dynamics of porewater Fe and sulfide.

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.

The Synthesis section identifies the following two key findings from the experimental studies and Field Survey:

1. The hydroponics study indicates sulfide toxicity related to wild rice growth in the range of 210-322 μg/L; and
2. The mesocosm experiment and the Field Survey are both consistent with these results.

These two basic findings are a critical outcome of the study, and I would like to see more discussion or integration of the results from these three separate studies. In other words, I believe that the data from all three studies support the fact that the effects of higher sulfate concentrations on wild rice relate to sulfide toxicity, and that the sensitivity of wild rice in the natural environment to sulfide appears more sensitive than indicated by the hydroponics study alone.

The Synthesis section then develops a conceptual model that "that describes how and under what circumstances sulfate negatively affects wild rice growth in Minnesota." At this juncture, it might be beneficial if MPCA included a schematic diagram, or a set of linked diagrams that showed the key biogeochemical processes that, based on the MPCA data and the literature, govern sulfide levels in porewater.

I have other several points for MPCA to consider:

1. The statement on lines 1213-1214 about gaseous H_2S being "carried along with other gases" seems to suggest that gaseous H_2S losses require an active transport mechanism such as convective movement of water upward or macrophyte pumping. I would indicate that ebullition and thus losses of H_2S can occur based on the partial pressure of H_2S in the porewater and that ebullition can occur without requiring co-transport of other dissolved gases.
2. I would agree that the results from this study tend to favor Ernst's hypothesis (lines 1231 to 1233). However, it might be useful to explain that the conceptual model essentially precludes high porewater Fe and high sulfide occurring simultaneously. The caveat to this of course is that somehow iron concentrations become overwhelmed by high sulfate loadings, but I see no evidence to support this as a reasonable likelihood event.
3. I am not convinced that the MPCA analyses shown in Figures 20 and 21 of the MPCA report (page 43) are particularly useful. As stated in the MPCA report (lines 1029 to 1033):

   > "There is a progressive decrease in the Fe/AVS ratio as sulfate concentrations increase (Figure 21). The parallel nature of the regression lines through paddy, lake, and stream sites indicates that the biological and chemical reactions are fundamentally similar, but that there are differences in oxidation of AVS, or in the supply of iron to the systems. <u>Lower values of Fe/AVS are indicative of a smaller reservoir of available iron to precipitate sulfide</u>." (emphasis added)

My concern in part is that ratios in regression models are notoriously difficult to interpret. In this case the MPCA model is:

$$\log_{10}\left(\frac{Fe_{sed}}{AVS_{sed}}\right) = b_0 + b_1 \times \log_{10}(SO4) \qquad (1)$$

Since there is no *a priori* reason why sediment Fe levels should be related to sulfate (nor are they based on a simple scatterplot analysis), a more useful approach would be to model AVS directly:

$$\log_{10}(AVS_{sed}) = b_0 + b_1 \times \log_{10}(SO4) + b_2 \times \log_{10}(Fe_{sed}) \qquad (2)$$

This leads to a greatly improved model based on the coefficient of determination (r^2 = 0.318 for the model in Equation 1 *vs.* r^2 = 0.510 for the model in equation 2). The model fit for the Equation 2 model is shown in Figure 15. The figure also includes augmented component plus residual (ACPR) plots for both independent variables to illustrate whether problems of curvilinearity are inherent in the model. No curvilinear problems are apparent, and this can be interpreted to also indicate no apparent issues with a smaller reservoir of iron that would be expected to show up in the lower end of the ACPR plot for sediment iron. It should also be noted the model slope for $\log_{10}$ sediment Fe is positive and less than 1 (using molar concentrations for both sediment Fe and AVS). As a result, the lower values of Fe/AVS do not appear to have any practical significance or consequence other than the obvious and to some extent, spurious: as Fe concentrations increase, so does AVS – *but not as fast* – and thus the ratio Fe/AVS increases as well. Restated, because AVS is correlated with Fe, the ratio of Fe to AVS reflects to some degree the ratio of Fe to Fe^b where *b* is the slope of correlation between AVS and Fe. Since *b* is less than 1, this means that the ratio Fe/AVS must increase as Fe concentrations increase. I think a better way to express the significance of the relationship between Fe and AVS is thus not in terms of the ratio, but that AVS formation is *jointly* limited by the availability of both iron and sulfate.

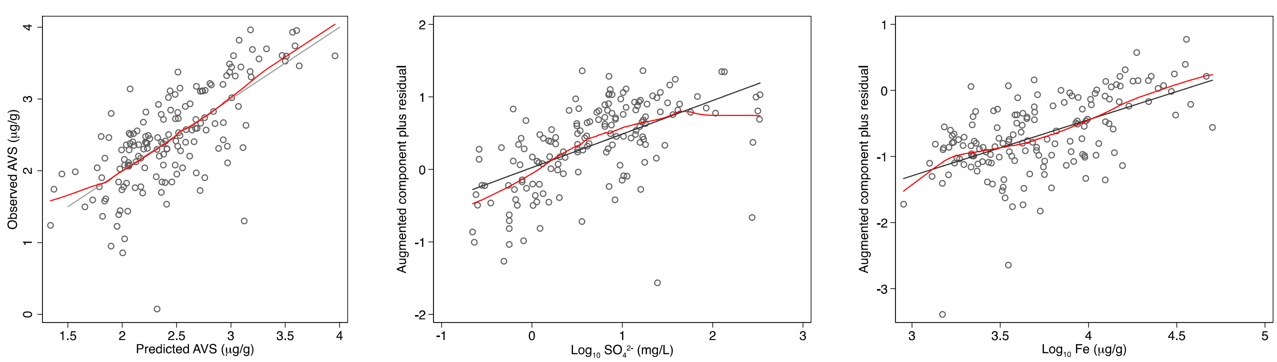


Figure 15. Sediment AVS model using sulfate and sediment iron as independent variables. All variables $\log_{10}$-transformed. Left hand panel – observed *vs.* predicted AVS concentrations (r^2 = 0.510; *N* = 159). Middle and right hand panels – augmented component plus residuals plots for sulfate (middle panel) and sediment Fe (right hand panel). Red lines show LOWESS smoothed plots of the bivariate relationships compared to the expected model relationship (solid black line).

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

My assessment of the study components really focused on the three components that the MPCA used to develop their analysis: the hydroponics study; the mesocosm study; and the Field Survey. While I have issues related to data analysis, and suggestions for how the analysis can be improved, I think the hydroponics and Field Survey are both are extremely useful in defining porewater sulfide as a causative factor in wild rice toxicity related to sulfate exposure. By isolating or removing the confounding effects of other environmental variables, the hydroponics experiments were able to clearly demonstrate that direct toxicity to wild rice was exerted by sulfide rather than sulfate. The mesocosm experiments in turn demonstrate that, although sulfate does not exert a direct effect on wild rice, wild rice effects are nonetheless observed as an increasing function in response to higher sulfate concentrations if sulfate reduction to sulfide can occur. Restated, the mesocosm results indicate that, in the natural environment, increasing surface water sulfate concentrations should lead to higher toxic responses by wild rice, all other factors held constant. As recognized by MPCA, however the strength of both the hydroponics and mesocosm experiments in removing or limiting variations in confounding variables is also its weakness in that the results are difficult to extrapolate to the natural environment.

The Field Survey thus provides the framework for both (1) extending and testing the conceptual model of sulfide toxicity and the indirect effects of sulfate on toxicity through sulfur cycling in the sediments; and (2) identifying and quantifying the effects of confounding variables. The Field Survey was well designed with respect to defining and measuring the sediment and water chemistry variables of interest, and appears to be well implemented from an analytical chemistry perspective[3]. The overall number of lakes and streams sampled (overall N including site replicates over time equals 255, plus 12 paddy sites) provides a well-sized data set for conducting multivariate analyses, including SEM. Further analysis needs to be conducted to establish that the range of concentrations for key biogeochemical variables for the lakes and streams sampled in the Field Survey suitably encompass the expected range for sites expected to support wild rice.

In brief, the three-pronged study design is a well-conceived approach that allows MPCA to not only establish convincingly a causal link between porewater sulfide concentrations and wild rice toxicity, but also how that link is manifested in the natural environment; the study also provides the fundamental data necessary to establish and model the relationship between sulfate and sulfide levels, and how that relationship changes from site to site based on the prevailing biogeochemistry.

[3] It would be useful, however, to have seen *cdf* plots of more of the critical variables as a means of both determining what sort of data transformations might be necessary as part of developing a particular statistical model, and whether analytical detection limits appear to limit the usefulness of the data without implementing an inferential technique such as multiple imputation to reconstruct censored values.

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

1. The caption of Figure 13 of the MPCA report (page 31) gives the results of a mixed model used to test whether seasonality was important. My understanding from reading the caption is that sample period was modeled as presumably a *fixed* effect, and that treatment was modeled as a *random* effect. I believe the effects ascribed to these two variables should be reversed.

2. MPCA should consider using the mesocosm data in conjunction with the final sulfate-sulfide model developed from the Field Survey data as a validation exercise.

3. I recommend that MPCA rethink its logic about separating the 2011 survey data from data collected in 2012 and 2013 (page 17). Using a dummy variable to separate the 2011 data from 2012-2013 and then incorporating that variable in sulfide regression models indicates that any effect attributable to different labs (or any other effect that might be associated with differences in the two groups of years) is insignificant.

4. Another way to examine the field data for significant relationships (page 18) is to conduct principal components analysis, or alternatively exploratory factor analysis (EFA). Extracted components or factors can then be regressed against wild rice cover to evaluate major determinants. In other words, the Field Survey should be “mined” to see if other measured variables can help explain the occurrence of wild rice in conjunction with sulfide.

5. The use by Johnson (2013) of calculated ion activity products (IAP) and comparing IAP values to Ksp to define whether under or supersaturation occurs in the mesocosm porewaters needs to be clarified. This would include stating the Ksp used to define equilibrium. Depending on the actual Fe-S solid phase modeled, K_{sp} values vary by over two orders of magnitude (Stumm and Morgan, 1996). Assuming that the plots of calculated IAP presented in Figure A5 of Johnson (2013) are indeed the actual IAP values and not the log ratio of IAP:Ksp, I would recommend include a vertical line on the profile plots to indicate the log K_{sp} and thus whether the IAP values indicate under- or supersaturated conditions. Also, the effect of DOC complexing Fe^{2+} is likely quite important in the porewater environment, and thus the activity of Fe^{2+} and the resultant IAP calculations are biased upwards. Thus, contrary to the text, the thermodynamics may not be indicative of supersaturation.

On page 38 of the MPCA report, this is a discussion on oxygen dynamics at the sediment-water interface, and the hypothesis that surficial groundwater could deliver more oxygen to stream sites than lake sites. I have the following comments. (1) Certainly streams are generally more turbulent and more shallow than lakes and thus oxygen exchange across the air-water interface should occur at higher rates and penetrate to the sediment-water interface more effectively. This could be stated more clearly in the paragraph. (2) In addition, I imagine that shallow groundwater that enters lakes and streams will have oxygen concentrations that are reduced well below saturation values. If monitoring data suggests otherwise, then those data should be cited. (3) Lastly, the sediment organic content in surficial stream sediments is generally much lower than the sediment organic content observed in lakes (Figure 16). This translates to generally lower rates of sediment oxygen demand in streams and, of course, higher concentrations of oxygen at the sediment-water interface.

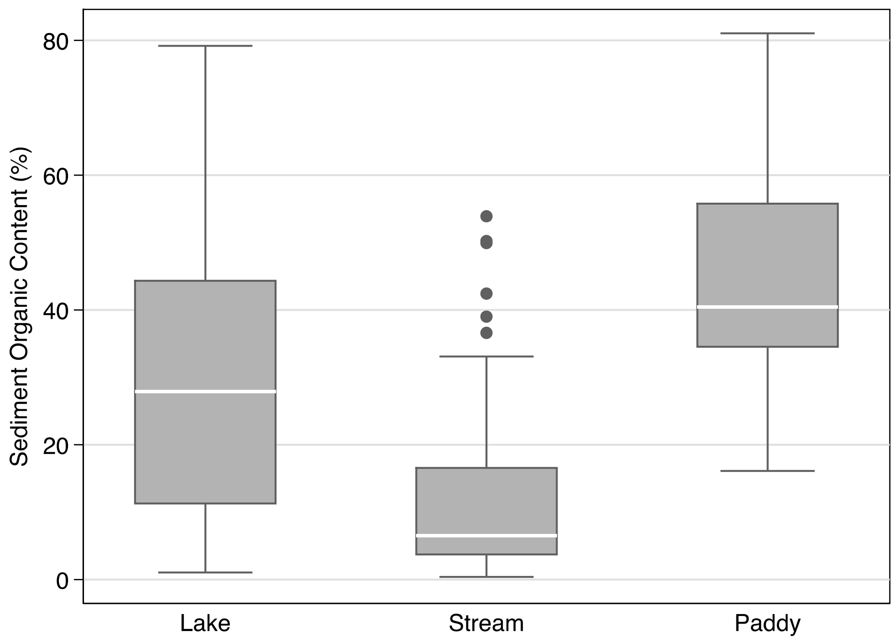


Figure 16. Box plot of the distribution of sediment organic content (%) as a function of hydrologic type (lake, stream or paddy) for sites included in the Field Survey.

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.

As a general comment, I would recommend that MPCA and its contractors exploit the utility of power analysis and initial statistical modeling of the data to ensure that the experimental design of subsequent definitive toxicity studies is optimized. For example, the initial rangefinder tests for the hydroponics study indicated a threshold response ~ 300 μg/L. Recognizing that resources are limited, it may be that a better experimental design would have included one or two more treatment levels within the critical region of most rapid response, and perhaps less replicates of individual treatment levels.

As stated elsewhere in my review comments, I believe that the mesocosm porewater chemistry from both the 2013 and ongoing (2014) studies may be useful with respect to both toxicity modeling and validation of the conceptual and statistical models for sulfide porewater chemistry.

The initial modeling of wild rice occurrence using the Field Survey data strongly supports the notion that sulfide exerts a deleterious effect. The initial modeling, however, only explains a small fraction of the variance in wild rice occurrence. From a management perspective, I would recommend that, at a minimum, the Field Survey data set be exploited more fully towards developing a more comprehensive model of wild rice occurrence. This in turn would confer the ability to better and more confidently model the site dependent effects of sulfate on wild rice occurrence. The statistical modeling ideally should be validated using methods such *k*-fold and jackknife cross-validation.

References Cited

Johnson, N.W. 2013. "Response of Rooting Zone Geochemistry to Experimental Manipulation of Sulfate Levels in Wild Rice Mesocosms" [submitted to] Minnesota Pollution Control Agency. Electronic file: Sulfate_Manipulation_Rooting_Zone_Geochemistry_final.pdf, found at: ftp://files.pca.state.mn.us/pub/tmp/wildRice/Collection_and_Analysis_of_Rooting%20Zone_Depth_Profiles/ MPCA

Moyle, John B. 1956. Relationships between the chemistry of Minnesota surface waters and wildlife management. Journal of Wildlife Management. 20(3):303-320.

Pastor, J. 2013a. "Effects of Enhanced Sulfate And Sulfide Concentrations on Wild Rice Germination and Growth: Results from a Hydroponics Experiment" [presented to] Minnesota Pollution Control Agency. Electronic file: Pastor_Hydroponics_Experiment_Report.pdf, found at: ftp://files.pca.state.mn.us/pub/tmp/wildRice/Laboratory_Hydroponics_Experiments/

Pastor, J. 2013b. "Effects of Enhanced Sulfate Concentrations on Wild Rice Populations: Results from a Mesocosm Experiment" [presented to] Minnesota Pollution Control Agency. Electronic file: Pastor_Mesocosm_report.pdf, found at: ftp://files.pca.state.mn.us/pub/tmp/wildRice/Outdoor_Container_Experiments/

Stumm, W. and J.J. Morgan. 1996. Aquatic chemistry, third edition. Wiley-Interscience, New York.

Data Sets Used

<u>Hydroponics</u>

Appendix8_ Rangefinder_Test_of_Effects_of_Sulfide_on_Juvenile_Growth2.xls

<u>Mesocosm</u>

2011_Sulfate_Field_Trial.xls

2012_Sulfate_Trial_Plant_Dta.xls

Appendix1_ Water_chemistry_of_mesocosms.xls

Appendix2_Mesocosm_Plant_Data.xls

<u>Field Survey</u>

LacCore_dataexport_updated_Jan_29_2014.xls

Appendix G

Slides Provided by Dr. Pollman

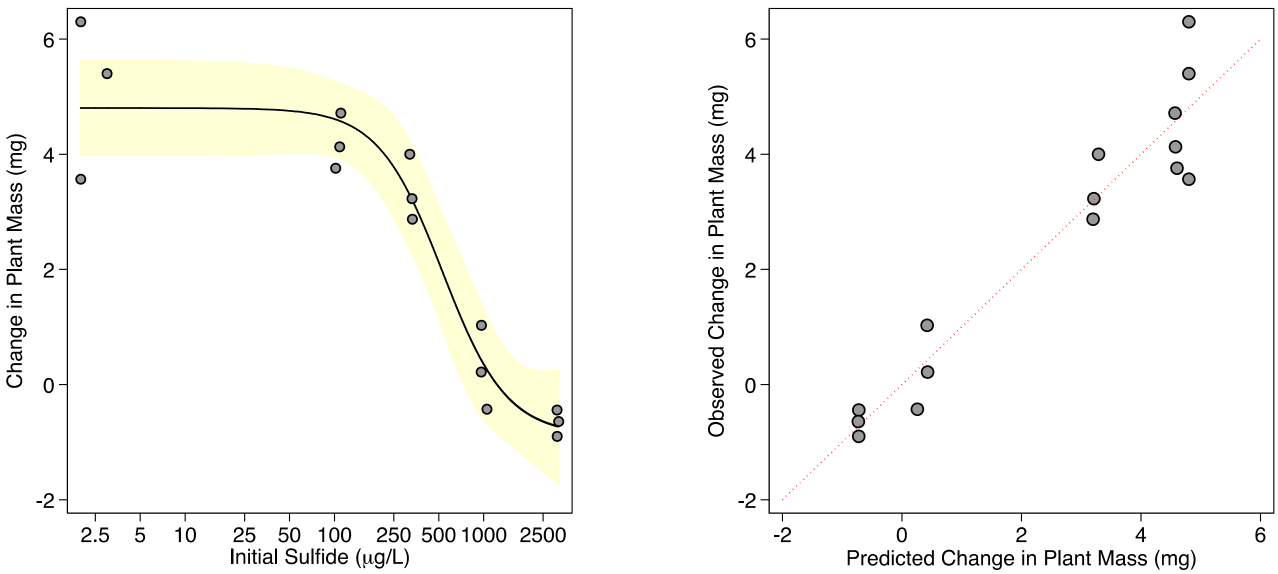


Figure 1. Logistic nonlinear model to predict change in wild rice plant mass as a function of initial sulfide exposure concentrations. Data are from the rangefinder hydroponic experiment (Pastor, 2013a). Left hand panel – fitted logistic function model response and observed values as a function of initial sulfide concentrations. The plot also shows the prediction confidence interval (90%; yellow shaded area). Right hand panel – observed *vs.* predicted values for change in plant mass.

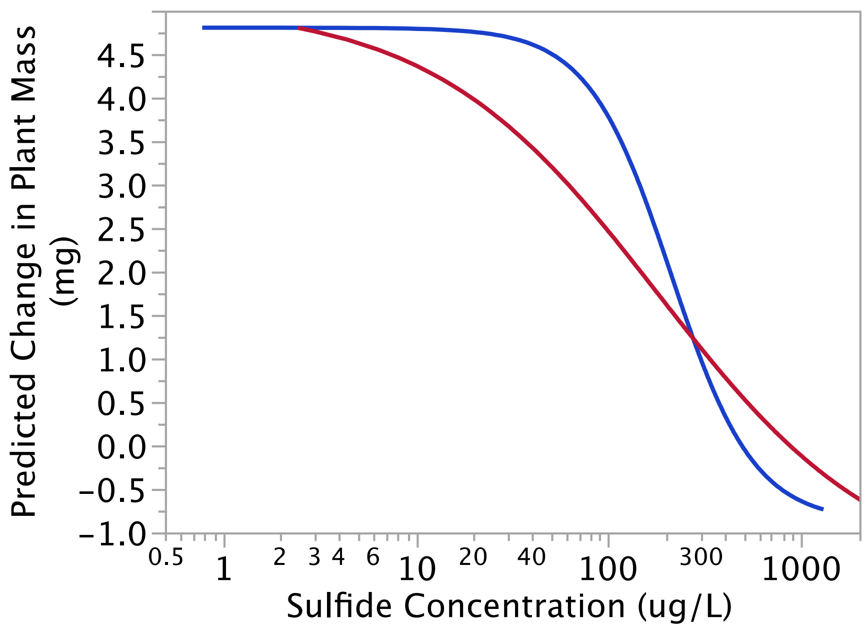


Figure 2. Comparison of logistic model response curves for changes in wild rice plant mass as a function of sulfide concentrations – rangefinder study results. Blue curve shows response based on initial exposure concentrations; red curve shows response based on final exposure concentrations.

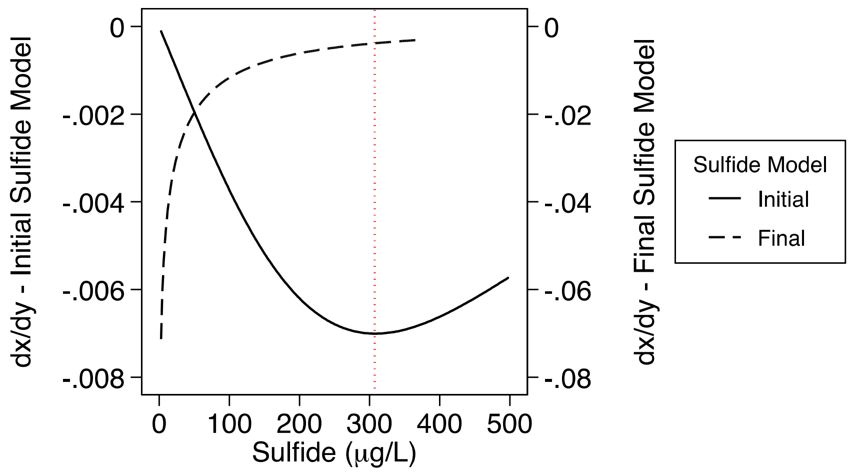


Figure 3. Predicted incremental (derivative dy/dx) changes in plant mass as a function of sulfide concentration. The change in the derivative is shown for the nonlinear models obtained using both initial and final sulfide concentrations.

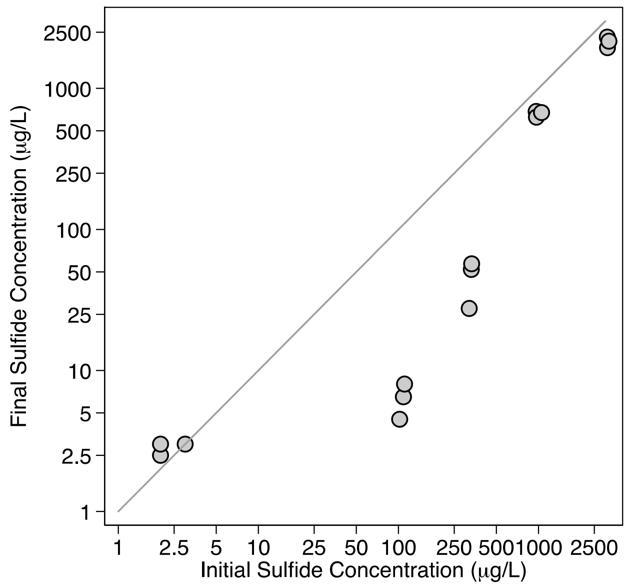


Figure 4. Comparison of final sulfide concentrations in the hydroponic experiments with initial concentrations.

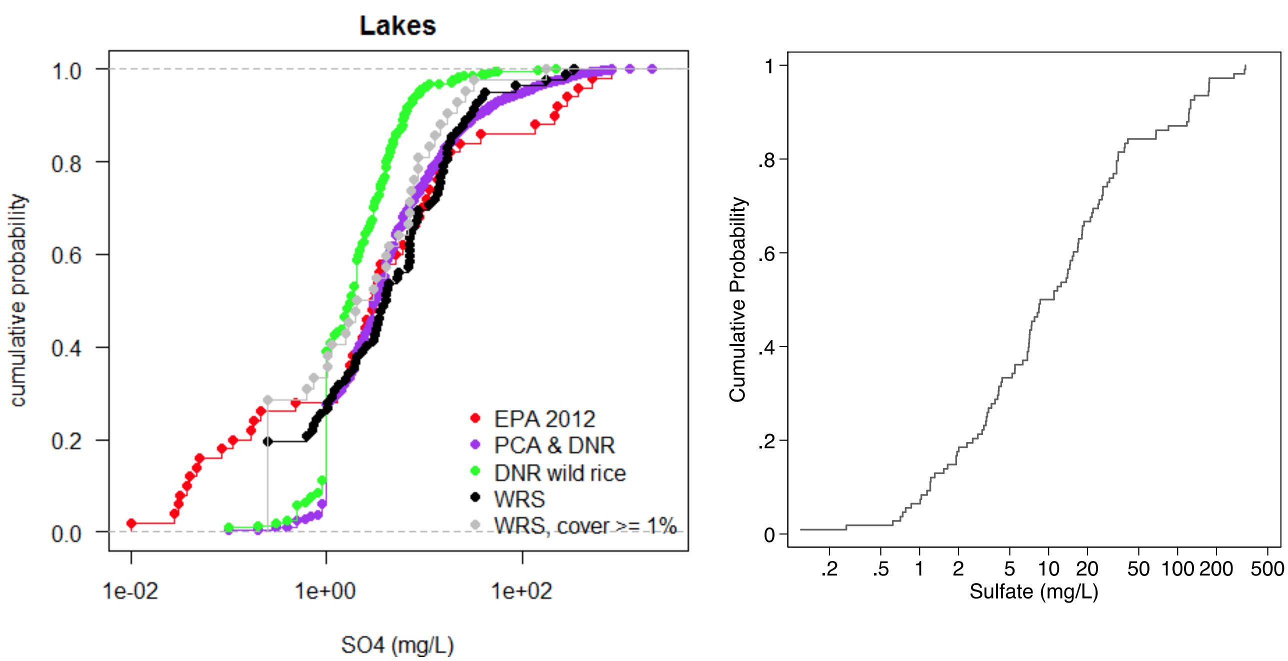


Figure 5. Comparison of cfd plots for the distribution of sulfate based on the 2012-2013 Field Survey data. Left-hand panel – cfd developed by MPCA (2014) based on several different surveys, including the 2012-2013 Field Survey (WRS) data. Right-hand panel – cfd plot generated from the 2012-2013 Field Survey data provided to the Peer Review Panel.

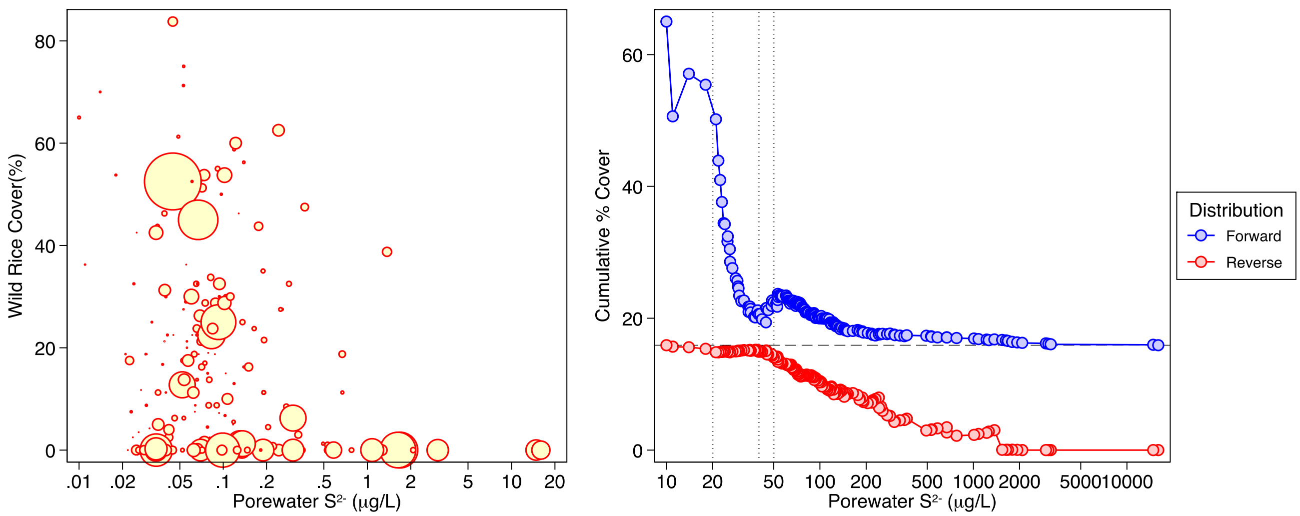


Figure 6. Percent cover of wild rice in Minnesota lakes and streams as a function of increasing porewater sulfide concentrations. Left-hand panel: bubble plot of percent wild rice cover with the size of the bubbles proportional to observed sulfate concentrations. Right-hand panel – cumulative percent wild rice cover. The *forward* curve in the right-hand panel defines the overall mean response for all observations with observed porewater sulfide concentrations ≤ *x*; the *reverse* curve defines the overall mean response for all observations with observed porewater sulfide concentrations ≥ *x*. Data from the MPCA Field Survey.

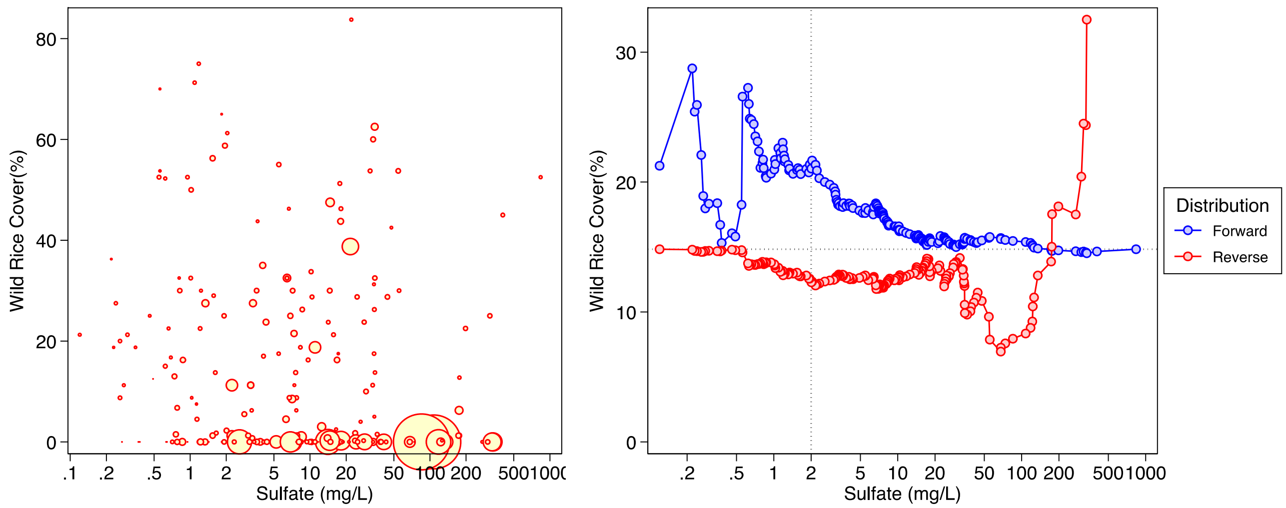
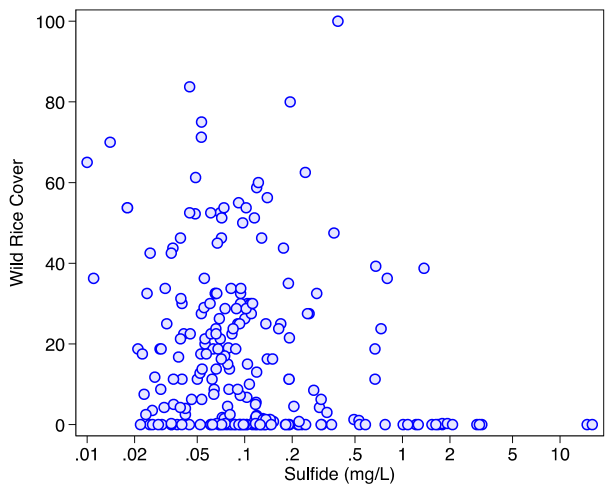


Figure 7. Percent cover of wild rice in Minnesota lakes and streams as a function of increasing sulfate concentrations. Left-hand panel: bubble plot of percent wild rice cover with the size of the bubbles proportional to observed porewater sulfide concentrations. Right-hand panel – cumulative percent wild rice cover. The *forward* curve in the right-hand panel defines the overall mean response for all observations with observed sulfate concentrations ≤ *x*; the *reverse* curve defines the overall mean response for all observations with observed sulfate concentrations ≥ *x*. Data from the MPCA Field Survey. Note that the two sites with the highest sulfate concentrations were not plotted for the reverse cumulative distribution curve (although the analysis did include these sites) so that details of changes in the response distribution curve are not obscured by the noise generated by the initial few data points used to construct the plot.+



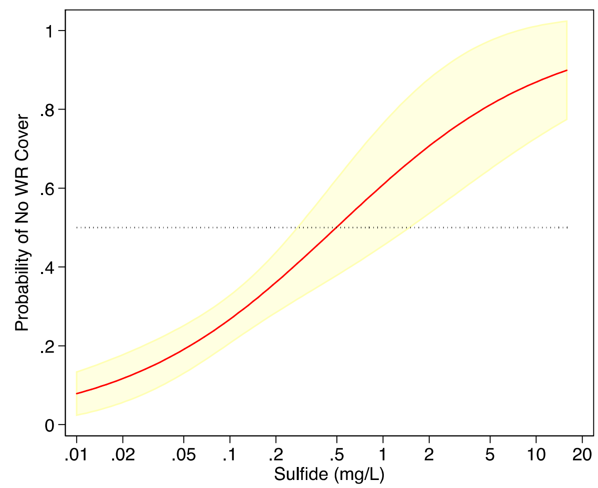


Figure 8. Sulfide – wild rice cover relationship derived from Field Survey data. Left-hand panel – percent wild rice cover as a function of porewater sulfide. Right-hand panel – logistic regression model predicted response (probability of the absence of wild rice) as a function of porewater sulfide concentrations. The logistic model also includes the 90% confidence interval (5% upper and lower tails) for the predicted response.

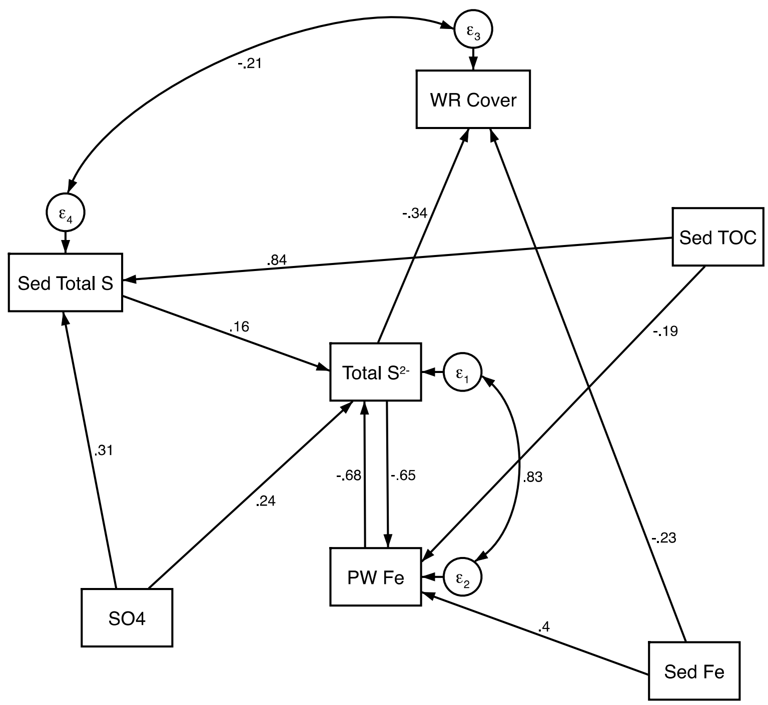


Figure 9. SEM model for porewater sulfide and wild rice cover response to sulfate and key sediment chemistry variables. Pathways shown are standardized (β) coefficients. This model is an extension of the SEM model developed for porewater chemistry alone (*i.e.,* excluding wild rice response) discussed in response to Charge Question No. 7 and presented in Figure 12.

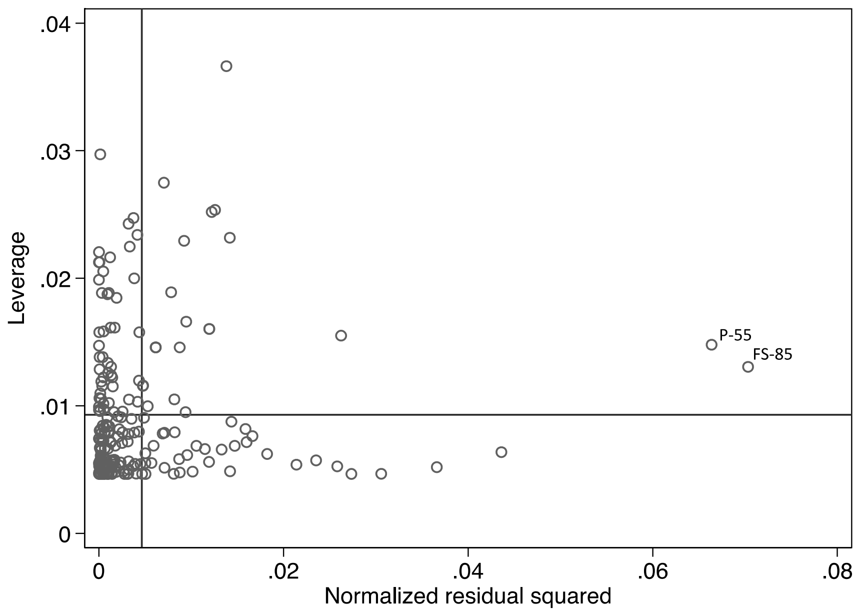


Figure 10. Leverage plot for bivariate sulfate-sulfide model.

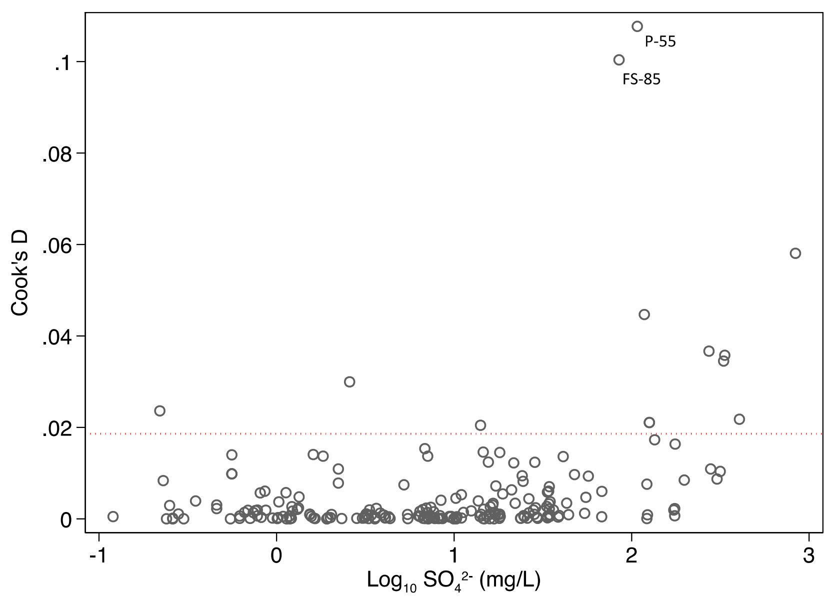


Figure 11. Plot of *Cook's D* statistic for the bivariate sulfate-sulfide model as a function of sulfate concentrations (log_{10}-transformed) showing the presence of two clear influential (outlier) sites – *FS-85* and *P-55*.

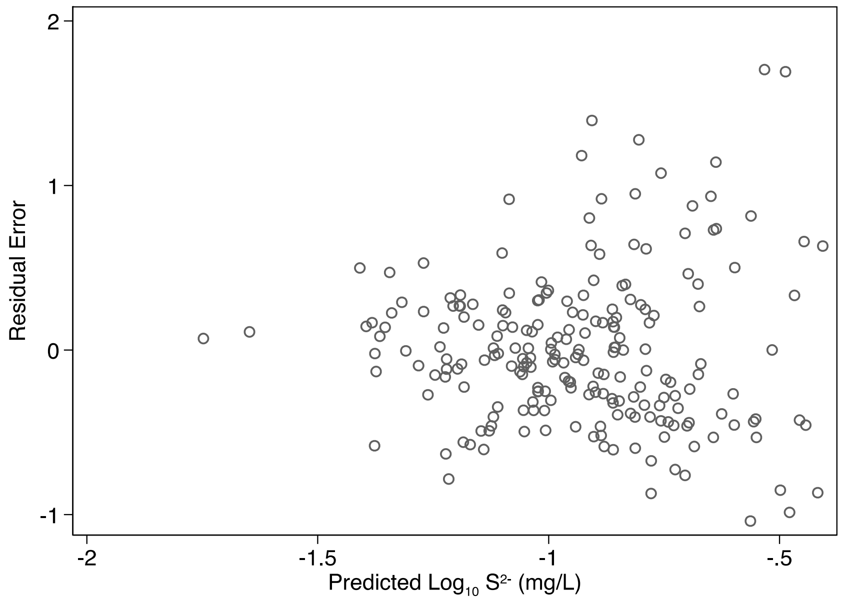


Figure 12. Plot of model residual errors *vs.* predicted porewater sulfide concentrations for the OLS model incorporating sulfate and sediment Fe as independent variables. All variables *log_{10}*-transformed.

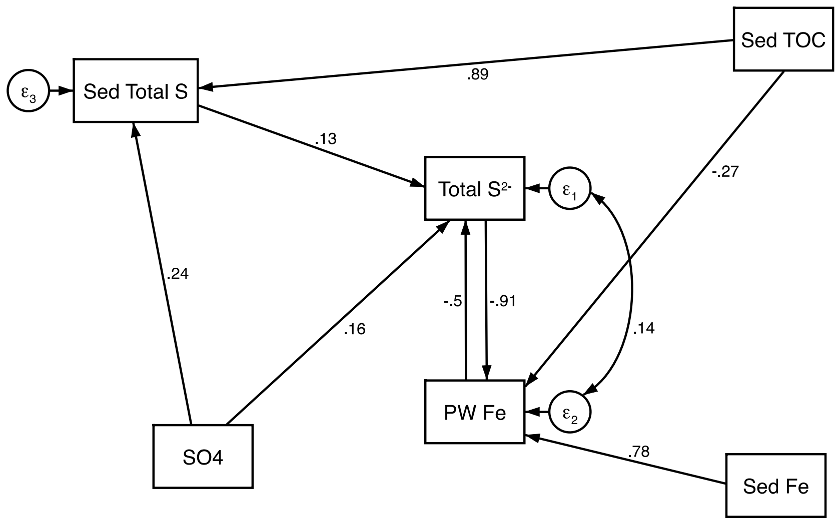


Figure 13. Path diagram showing the relationships between variables (log_{10}-transformed) used to construct the porewater sulfide SEM. All coefficients are significant at $p \leq 0.001$.

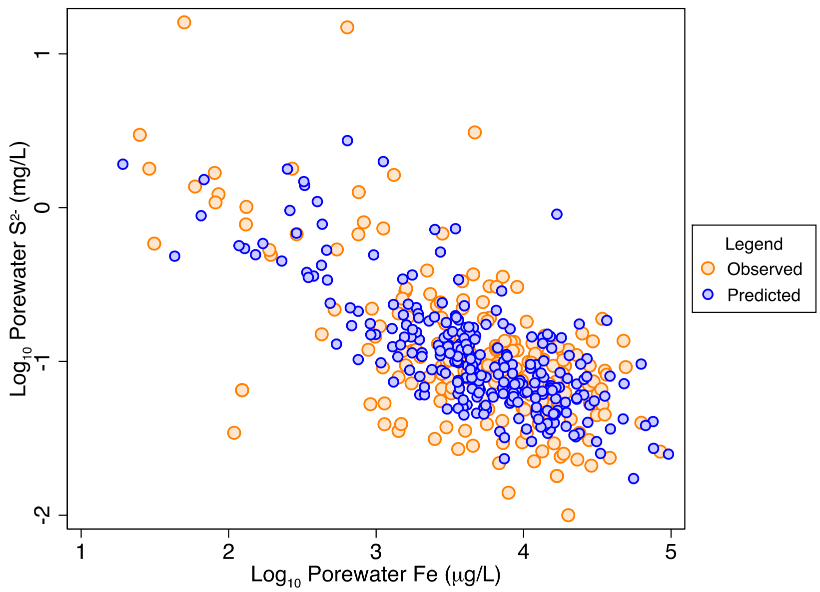


Figure 14. Comparison of observed S^{2-} vs. observed Fe with predicted S^{2-} *vs.* predicted Fe obtained from the SEM porewater S^{2-}/Fe model.

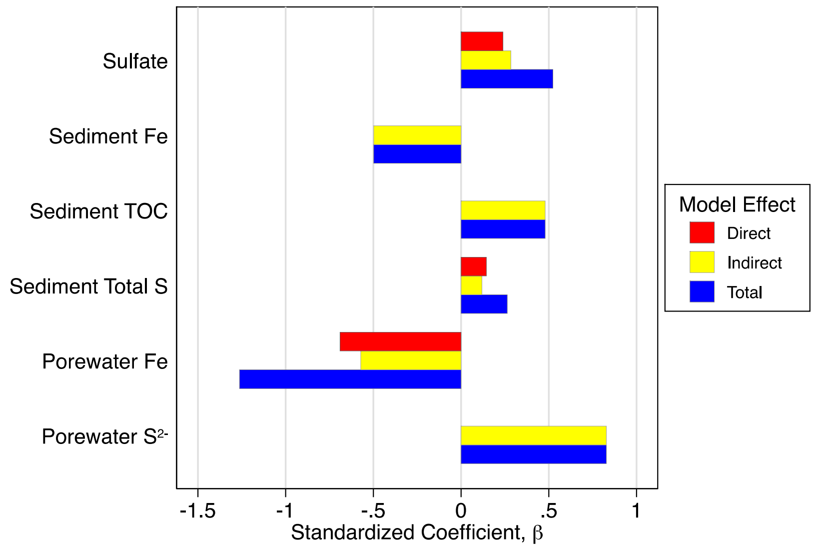


Figure 15. Direct, indirect, and total effects of SEM model variables on porewater sulfide concentrations. Effects are shown based on standardized (β) coefficients, which show the response of sulfide relative to its standard deviation based on a standard deviation change in the predictor variable.

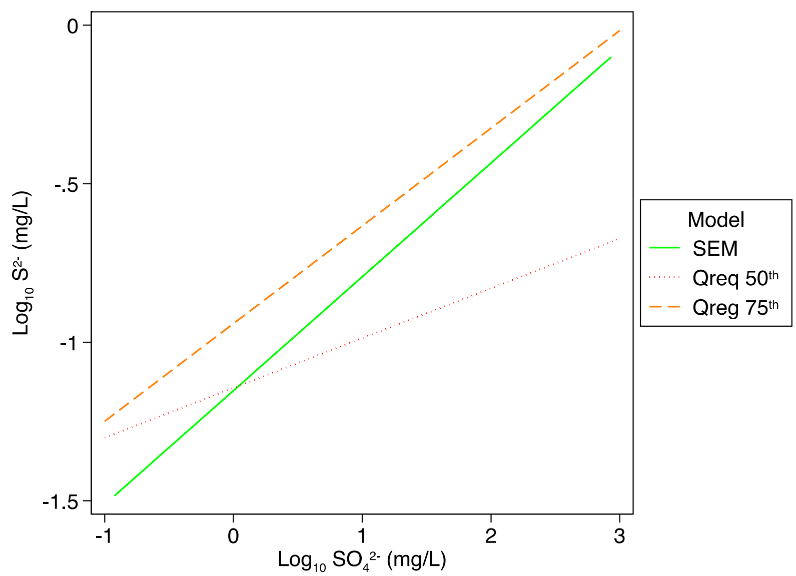


Figure 16. Predicted porewater sulfide as a function of sulfate concentrations: comparison of MPCA quantile regression (Qreg) model approach with SEM results. SEM model controls for sediment TOC and sediment Fe (fixed at median values), while the MPCA Qreg model controls for sediment Fe only (also fixed at the median value).

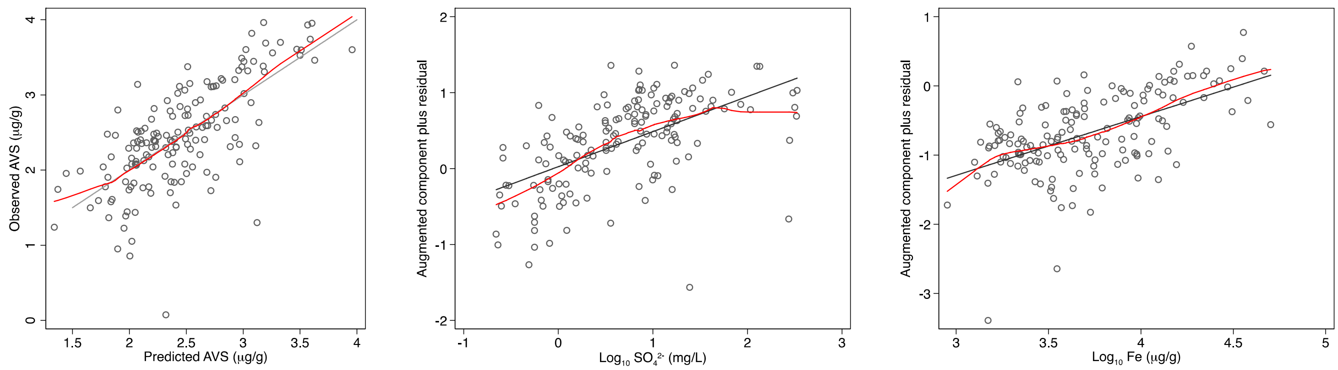


Figure 17. Sediment AVS model using sulfate and sediment iron as independent variables. All variables $\log_{10}$-transformed. Left hand panel – observed *vs.* predicted AVS concentrations (r^2 = 0.510; *N* = 159). Middle and right hand panels – augmented component plus residuals plots for sulfate (middle panel) and sediment Fe (right hand panel). Red lines show LOWESS smoothed plots of the bivariate relationships compared to the expected model relationship (solid black line).

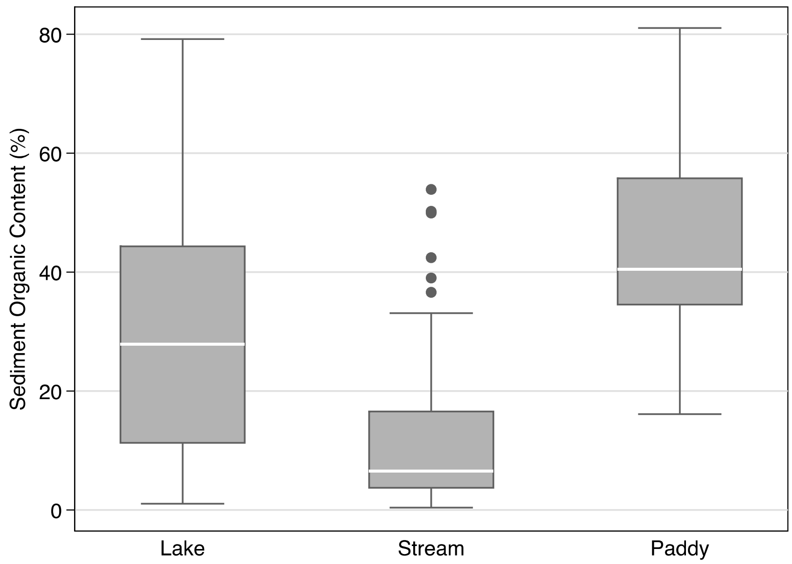


Figure 18. Box plot of the distribution of sediment organic content (%) as a function of hydrologic type (lake, stream or paddy) for sites included in the Field Survey.

Appendix H

Figures Referenced or Provided by MPCA

MPCA presented this figure, from the *Wild Rice Sulfate Standard Study Preliminary Analysis*, at the peer review meeting.

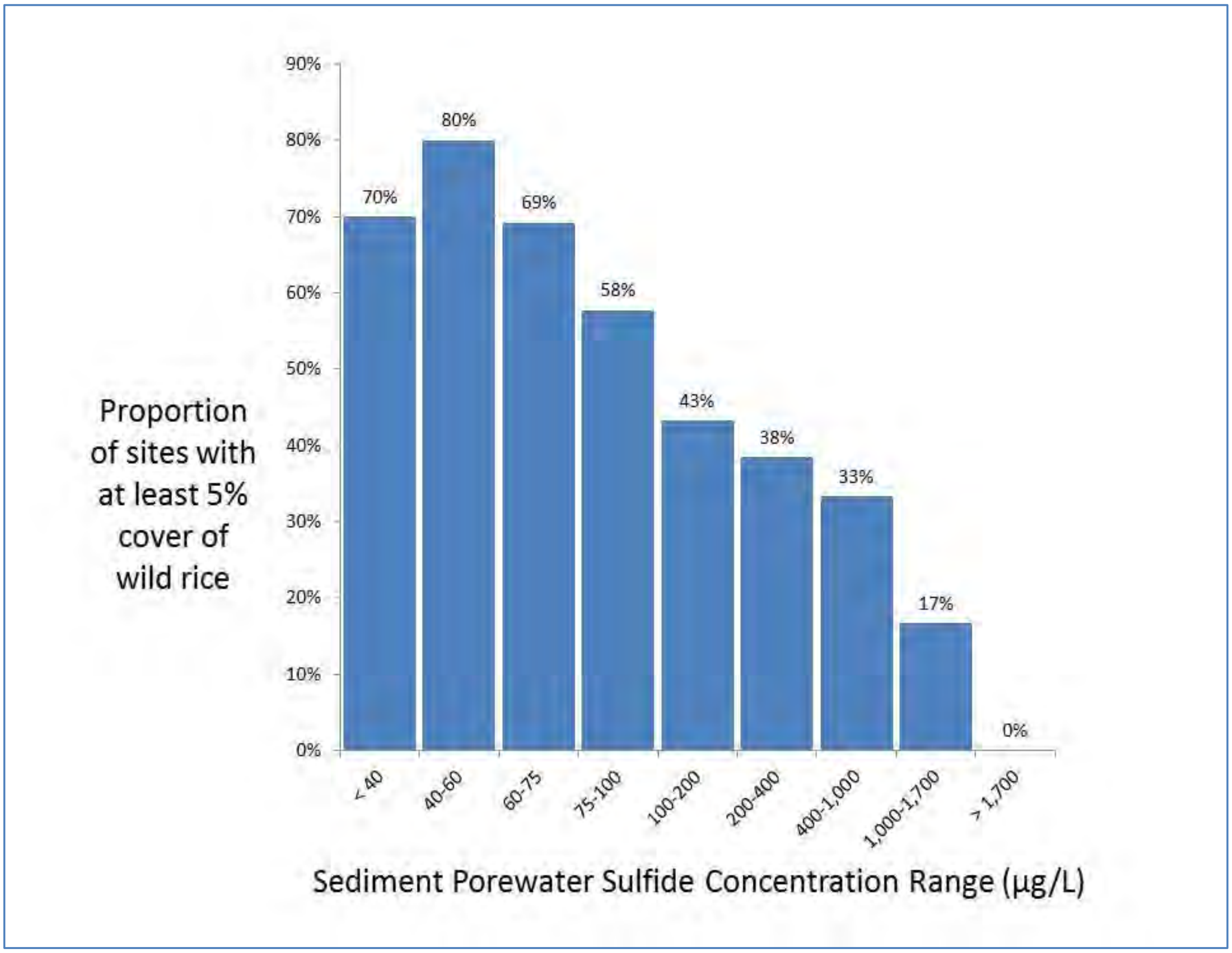
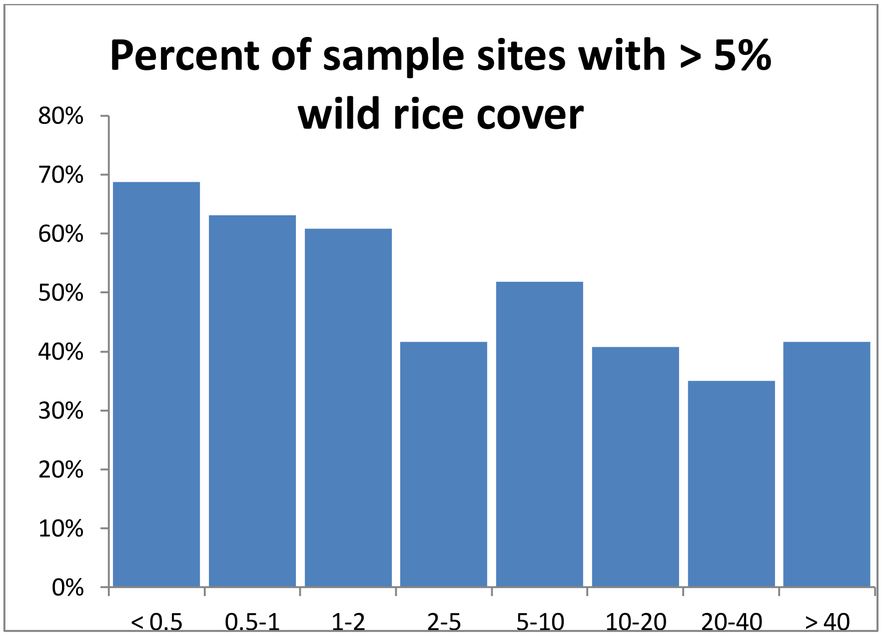


Figure 17. As sediment porewater sulfide concentrations increase at the Field Survey and Pilot Survey sites, there is a decreased proportion of sites where the wild rice exceeded 5 percent cover. (Based on 171 lake and stream samplings, where no site was sampled more than once a year, 2011-2013).

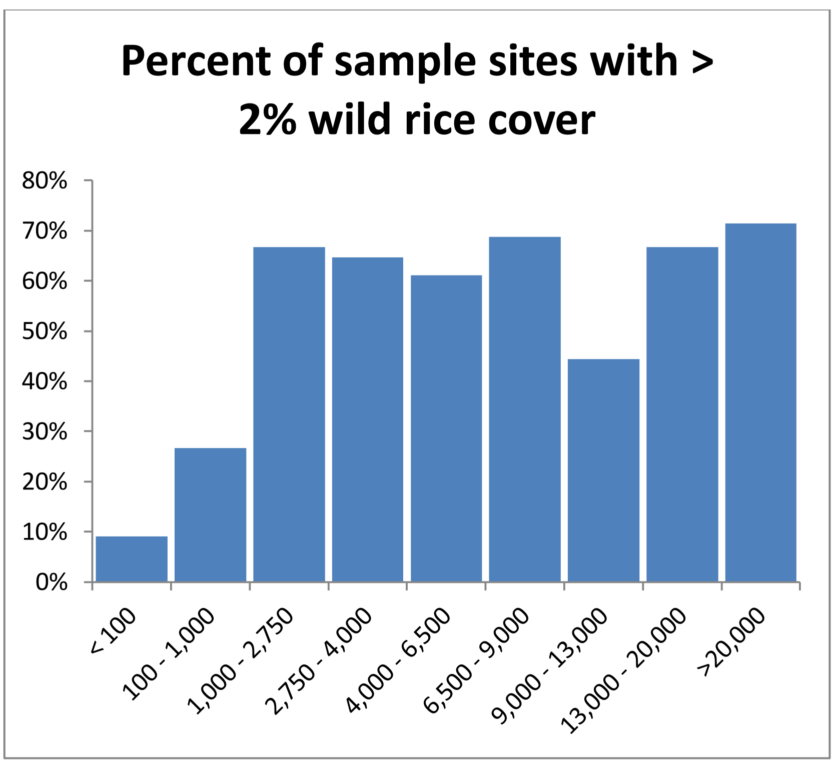
Source: Fig. 17, *Wild Rice Sulfate Standard Study Preliminary Analysis*, MPCA, March 2014

Hard copies of these three histograms were distributed to reviewers at the peer review meeting.

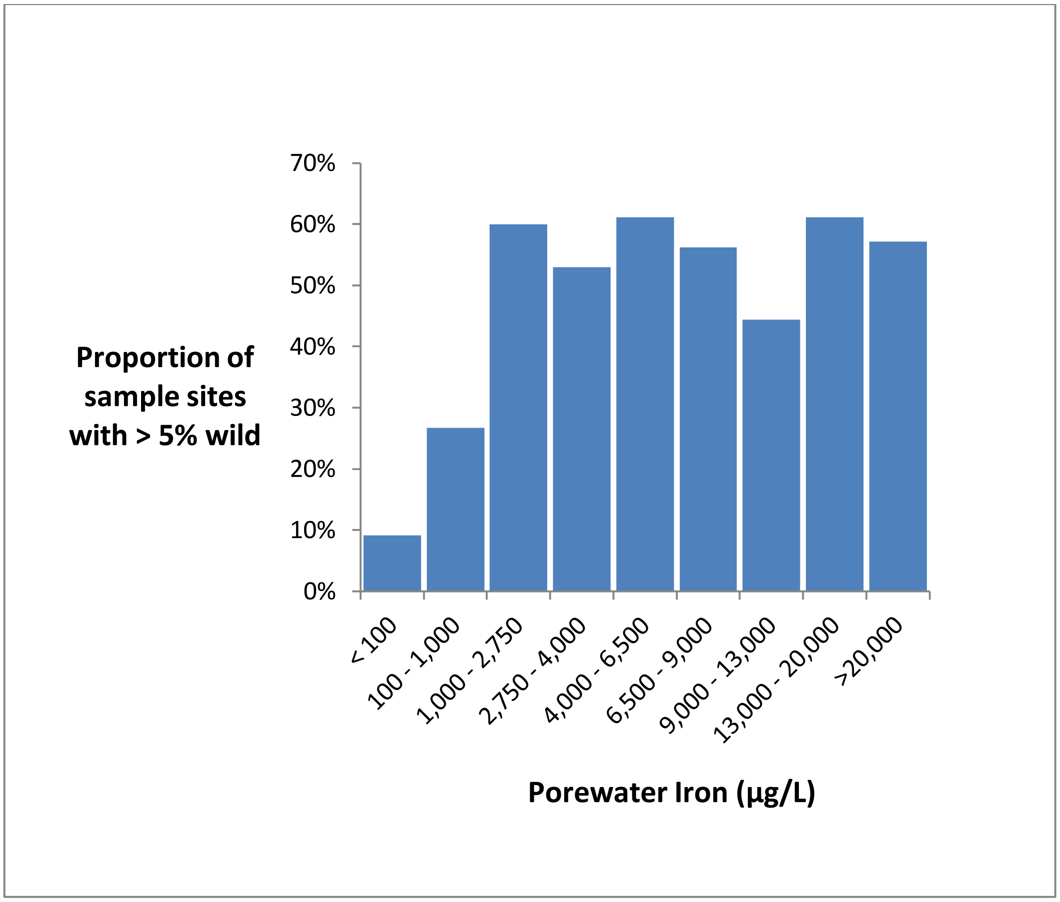
Source: From Fig. 10, *Wild Rice Sulfate Standard Study Preliminary Analysis*, MPCA, March 2014



Surface Water Sulfate Concentration Range (mg/L)



Porewater Iron (µg/L)


Proportion of sample sites with > 5% wild
70%
60%
50%
40%
30%
20%
10%
0%
< 100
100 - 1,000
1,000 - 2,750
2,750 - 4,000
4,000 - 6,500
6,500 - 9,000
9,000 - 13,000
13,000 - 20,000
>20,000
Porewater Iron (µg/L)

Appendix I

Figure Provided by Dr. Arts

Mechanisms involved in the decline of Water soldier (*Stratiotes aloides*) in The Netherlands (Smolders et al., 2003).