Comments on:
The proposed Amendment of the Sulfate Water Quality Standard Applicable to Wild Rice and Identification of Wild Rice Waters.
OAH Docket NO. 80-9003-34519
Revisor NO. RD4324A

November 21, 2017

Comments by: Robin L. Richards, REM on behalf of the Iron Mining Association of Minnesota

Comments addressing: Water Quality Criteria Development Process

Background
For the past 30 years, I have been providing consulting services focused on water quality protection. In this role I participated in work groups or committees tasked with commenting on or directly developing water quality criteria and their implementation into Clean Water Act programs. This participation has extended from the 1990 development of the USEPA "Technical Support Document for Water Quality-based Toxics Control" to serving on several state work groups dealing with state-specific water quality protection (e.g., the Illinois Sulfate Standard work group, 2002 to 2007). I was appointed and was an active member of the Minnesota Wild Rice Study Advisory Committee. My educational background is in biochemistry and plant physiology. I am a principal with Ramboll Environ US Corporation (Ramboll Environ) and serve as the Water Management and Planning Department Manager.

Executive Summary
MPCA did follow a few of the elements of the standard water quality criteria development process, however the elements that were either not followed or addressed and where MPCA did not incorporate the state of the art understanding/methodologies in criteria development have resulted in minimal confidence in the certainty of MPCA's proposed criterion in ultimately achieving protection of wild rice. Specific to my comments, MPCA has not demonstrated the reasonableness of the following:
- The porewater sulfide concentrations impacting wild rice health (SONAR 6.E.2)
- The MBLR sulfate equation (SONAR 6.E.4 and 6.E.5)
- The porewater sulfide analytical method (SONAR 6.E.7)

Introduction
<u>Why are water quality criteria developed?</u> *What has been left out in SONAR 4.A. pg 26 to 28?*
Water quality criteria are intended to prevent the occurrence of toxic pollutants in toxic amounts, as per Section 101(a)(3) of the Clean Water Act, and protect designated / beneficial uses of water (e.g., aquatic life, human health).

- Aquatic life criteria are intended to assure that toxic pollutants are not present in concentrations that would cause acute and or chronic adverse impacts on aquatic life.
- Human health criteria are intended to assure that toxic pollutants are not present in concentrations that would cause adverse impact to persons who eat fish or shellfish and/or drink the water. Similarly, wild rice criteria would be intended to assure that toxic pollutants not be present in concentrations that would cause acute or adverse impacts to wild rice.

In understanding the intention of water quality criteria, clear definitions of "toxic amount" and of "adverse impact" are needed. Hence, EPA, as per the Clean Water Act, develops water quality criteria using processes that result (with defined confidence), what dose of a chemical causes what type of response(s) to an organism[1]. Put simply, a dose-response curve is developed where the response or "adverse impact" is concrete and reproducible.

Furthermore, when the standard water quality criteria development process is followed resulting in a reliable dose response curve, there is an anticipation that a waterbody, all other physical and biological factors being equal, will have improvement in aquatic life and community health as the aquatic life criteria for toxic pollutants are achieved.

By the 1990's, EPA and the states were fully engaged in the control of the discharge of toxic pollutants in toxic amounts through the adoption of water quality criteria and implementation procedures. As anticipated, there are numerous examples where the levels of toxic pollutants were reduced in a water body, and related adverse impacts to aquatic life were no longer observed.

MPCA has not clearly defined the dose-response or toxic amount and the resulting specific adverse impact. These are the necessary underpinnings for a water quality criterion being confidentially developed and implemented to assure protection of the designated use.

Protective Level of Porewater Sulfide

MPCA discusses their process used to develop the protective level of sulfide (SONAR 6.E.2, TSD pg 31 – 39, 113-120) where MPCA attempted to develop dose-response curves between sulfide and a variety of "effects". However, there are errors in MPCA's development and presentation of their version of "dose-response" curves as discussed below.

<u>Pastor et al. 2017</u>

Pastor, et.al published their wild rice hydroponics sulfide toxicity testing data in Ecological Applications 27(1), 2017, "Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments". An EC50 of 245 µg/L was statistically determined and presented in the paper[2]. Very few details were provided in the published paper on the data used, definition of EC0, EC10, or definition of initial conditions. The peer-reviewed article does not contain an EC10 so it should be noted that any EC10 based on these data were not evaluated during the peer-review process for publication. In a meta-analysis performed for MPCA, Pastor calculated an EC10 of 299 µg/L.

MPCA conducted additional meta-analysis of the Pastor data to derive an EC10 using MBLR. MPCA did respond to questions on the data and statistical analyses, which were used to define various EC10s as presented in Attachment 1. However, as noted by the Minnesota Chamber of

[1] USEPA. 1991. Technical Support Document for Water Quality-based Toxics Control. Available on-line: https://www3.epa.gov/npdes/pubs/owm0264.pdf
and
Stephan, C.E., D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman and W.A. Brungs. 1985. Guidelines for Deriving Numeric National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses. EPA 822/R-85-100 or PB85-227049.National Technical Information Service, Springfield, VA.
and
USEPA. 1994 and subsequent online updates. Water Quality Standards Handbook. EPA-823-B-94-005a&b. Washington, DC: U.S. Environmental Protection Agency, Office of Water. Available online at: https://www.epa.gov/wqs-tech/water-quality-standards-handbook

[2] EC0, EC10, EC50 = Effect Concentration impacting 0% of (test) organisms, or 10% of organisms, or 50% of organisms.

Commerce[3] subsequent to Pastor's presentation of sulfide hydroponic work plans to the MPCA, interim results, and final results, the quality of the test design and execution are not considered of the quality typically used for determine chemical toxicity as per Good Laboratory Practices[4]. This is reflected by a variety of things:

- The scatter (huge variability) of the weight change
- The gap in sulfide concentrations between 100 µg/L and 1000 µg/L
- The high variability in the measured sulfide concentrations the implicit lack of control of aqueous sulfide
- The lack of daily sulfide measurements
- Treatment of what is really 3 range-finding tests as definitive tests.

Related to statistical analyses, some of the key decisions by MPCA used to calculate EC10s included the following:

- Considering normalizing the negative weight changes by assigning a negative growth as a zero value (identified as "growth" in Attachment 1 figures)
- Calculating the weight change by difference with the initials where the initials are an average of a random subset of the initial set of seedlings
- Defining the EC0 as control * 0.9 by inputting the MDL concentration for sulfide into the MBLR equation and using the equation output as control/EC0.

While the normalization certainly changes the shape of the sigmoidal curve and visually reduces the huge variability in growth or weight change, but it does not communicate the fact that the scatter in that data exists. It is not possible to generate the initial set of conditions (weights) as MPCA used a random subset. It is not clear whether MPCA has generated a different random subset and conducted a sensitivity analyses to determine that this is valid approach; nor is there an evident rationale for not using the entire initial weights. As presented in Attachment 1, an option of using the geomean of the minimally generated sulfide measurements was investigated by Ramboll Environ. It is not appropriate to use a geomean on this type of data, a time-weighted average is more applicable.

As presented in Attachment 1, when the influence of the sulfide test measurement issues are considered, the sulfide EC10s for the Pastor data vary by more than a factor of two, ranging from 103 µg/L to 255 µg/L. Given the variability in these EC10s and significant criticisms of the Peer Review Panel (see Section 3.2.2) these the sulfide EC10s, and any other ECs that may be based on the Pastor dataset, should be considered rough estimates and weighted less heavily in the determination of a porewater sulfide protective value then the other lines of evidence.

<u>Field Data</u>

My colleague, Dr. Michael Bock, has submitted in-depth comments on the MPCA errors in identifying the porewater sulfide threshold concentration. To reiterate, the MPCA presentation of probability of wild rice presence versus porewater sulfide is flawed as there is not a well-

[3] MCC. 2015. Technical Analysis of MPCA March 2015 Proposed Approach for Minnesota's Sulfate Standard to Protect Wild Rice.

[4] EPA Good Laboratory Practices (GLP) available on-line: https://www.epa.gov/compliance/good-laboratory-practices-standard-operating-procedures

And

OECD Good Laboratory Practices available on-line:

http://www.oecd.org/chemicalsafety/testing/goodlaboratorypracticeglp.htm defined no-effect level due to the high variability in porewater sulfide concentrations and the fact that sulfate is a necessary wild rice nutrient (TSD pg 53).

Summary
The proven and known approach of developing water quality criteria by developing a dose-response curve has not been reasonably demonstrated by MPCA. Their presentation contains errors and these errors undermine the confidence in understanding and defining the relationship between porewater sulfide and wild rice health.

State of the Art Science
State of the Art Examples of Water Quality Criteria: Direct Effect
USEPA has issued and continues to update guidance on criteria development including the type of data and statistical methods to define the dose-response. The current state of the science is recognition that the direct cause of an aquatic life (or human health) adverse impact (or effect) may not be due to water-column exposure[5]. This is similar to MPCA's finding that water column sulfate has no direct effect on wild rice.

Examples of EPA's state of the science for dose-response for water quality criteria include:

- Methylmercury where: If methylmercury in fish tissue > threshold value, chronic human health is adversely impacted (dose-response data from lab, some field, and model). And the EPA certainty in that statement and the threshold value is at a high confidence level; that is, minimal Type 1 or Type 2 errors.
- Selenium where: If selenium in the fish tissue > threshold value, aquatic life chronic health is adversely impacted (dose-response data from lab, some field, and model). And the EPA certainty in that statement and the threshold value is at a high confidence level; that is, minimal Type 1 or Type 2 errors.

The dose-response was demonstrated for fish tissue methylmercury and fish tissue selenium allowing EPA to confidentially define the "toxic amount" that caused risk to humans or impact on fish growth and reproduction. The development of water quality criteria for methylmercury, took 10 years of work (science and review) and for selenium, 19 years. Understanding of cause and effect takes time to allow thoughtful consideration given the importance of protecting humans and fish.

In addition, EPA's current state of the science, recognizes that laboratory techniques and methods, statistical computing, and ecological risk modeling have improved since the early 1990's; in effect, EPA is aware that one cannot "freeze" water quality criteria to the period of time it was first finalized. An example of this is the ammonia water quality criteria.

For example, ammonia-N aquatic life criteria, based on laboratory dose-response curves for a number of organisms, was established 1985. However, as knowledge increased about its behaviour in water and the sensitivity of invertebrate species, and as more data were validated,

[5] USEPA. 2001. Water Quality Criterion for the Protection of Human Health: Methylmercury. EPA-832-R-01-001. Available on-line: https://nepis.epa.gov/Exe/ZyPDF.cgi/20003UU4.PDF?Dockey=20003UU4.PDF
and
USEPA. 2016. Aquatic Life Ambient Water Quality Criterion for Selenium – Freshwater. Available on-line:https://www.epa.gov/sites/production/files/2016-07/documents/aquatic_life_awqc_for_selenium_-_freshwater_2016.pdf

revisions were issued in 2002 and again issued in 2013[6]. Now, if ammonia-N in water column is less than the determined threshold value, snail and mussel growth and reproduction will be protected (based on laboratory data). And the certainty in that statement is more than 95% confident. Besides laboratory data that demonstrate the impact of ammonia on mussel survival, there are in the field case examples where, all other factors being equal, reduction in water ammonia resulted in healthier mussel communities[7]. That is, there was documented data that a mussel community prior to ammonia reduction and post ammonia reduction showed improvement.

Defining the toxic amount of ammonia that would cause adverse impacts marched forward as science evolved. In addition to expanded knowledge, EPA removed older data that upon further review did not meet the current requirements for data validity. Clear understanding of the dose-response, the direct cause and effect, allowed science to continue support a valid and applicable criterion.

MPCA correctly states that water column sulfate does not have a direct effect on wild rice – there is no dose-response curve for sulfate vs. wild rice survival, growth, or reproduction. MPCA presents sulfate as having an indirect effect of wild rice. MPCA has defined porewater sulfide as a toxicant causing adverse impact to wild rice. However, as discussed previously, there is minimal confidence in the sulfide threshold developed by MPCA and MPCA's presentation of dose-response relationship is flawed.

Using state-of-the-art methods, EPA has shown more than once that a non-water column criteria can be developed from dose-response (aforementioned fish tissue based criteria) and that the confidence one typically has with laboratory water column data, can be achieved in defining a "toxic amount" in fish tissue. Without confidence in the dose-response for porewater sulfide, a "toxic amount" is difficult to define for use in assuring that protection of designated use is achieved. If MPCA followed the longstanding EPA approach to water quality criteria development, the wild rice water quality standard would be based on the chemical causing the direct effect, porewater sulfide.

<u>State of the Art: Water Column Translation of Direct Effect</u>

MPCA has created a challenge to translate the direct cause (porewater sulfide) of a wild rice effect to water column sulfate concentration. The water column concentration is used in water regulatory programs to assure toxic amounts are not discharged or that designated uses are attained (and maintained) based on a standard. EPA has not attempted to establish water quality criteria based on an indirect cause of the effect. EPA's water quality criteria are based on the direct cause. As discussed earlier, EPA has recommended criteria that are not water-column based i.e., selenium fish tissue, methylmercury fish tissue.

However, the implementation of these criteria to water column levels, or translation to a water column concentration, is considered a separate activity and is not part of the EPA's national recommended criteria. What this means is that while EPA criteria are suitable (and typically

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[6] USEPA. 2013. Aquatic Life Ambient Water Quality criteria for Ammonia – Freshwater. Available on-line: https://www.epa.gov/sites/production/files/2015-08/documents/aquatic-life-ambient-water-quality-criteria-for-ammonia-freshwater-2013.pdf

[7] U.S. Fish and Wildlife Service (USFWS) Midwest Region Freshwater Mussel Threats Geospatial Database.
DL Strayer. 2008. Freshwater Mussel Ecology: A Multifactor Approach to Distribution and Abundance.
J Farris and J VanHassel. 2007. Freshwater Bivalve Ecotoxicology.

encouraged) to be adopted into state water quality standards programs, translation of criterion to water column concentration is not encouraged by EPA to be part of state regulatory water quality standards. It is recognized that the models developed by EPA to go from fish tissue level to water column level are very site-specific and typically data intensive (e.g., multiple years of data needed).

The EPA does develop implementation guidance and solicits public comment on the guidance. The implementation guidance for methylmercury (to generate a total mercury water column concentration for a water body) was issued 9 years after the final fish tissue methylmercury criterion was issued[8]. EPA has not yet finalized the implementation (and monitoring) guidance for selenium fish-tissue; work that began in 2004[9]. Point being: the amount of data and information needed takes time to generate, validate, and utilize to be able to develop the sound models and recommendations to translate the direct effect (methylmercury in fish tissue or selenium in fish tissue) to water column concentrations (mercury in water column or selenium in water column). Implementation of criteria has enforcement implications for both a state agency and a discharger. It is difficult to develop the confidence in a model as one would expect for incorporation into regulation, one size does not fit all for implementation or translation of fish-tissue criteria to water column concentrations. As presented by MPCA, the MBLR sulfate equation (which is a model) is not aligning with porewater sulfide or wild rice health (MPCA uses the term “misclassification”) for an alarming number of waterbodies (TSD, pg 48 to 62, 67 to 83; SONAR pg 77 to 79) as one considers the regulatory impact on agency decisions and actions.

MPCA should take a page from EPA and use guidance to implement the porewater sulfide threshold. Certainly MPCA would have far more flexibility to allow implementation of the porewater sulfide threshold concentration into water column sulfate concentrations to exist as guidance, and not regulation. This would also allow MPCA the nimbleness needed to respond to additional data, evolving understanding the geochemistry of wild rice waters, and improved statistical methods.

Others will be commenting on the errors underlying the development of the MBLR sulfate equation including the selection of field results, the differences in the MPCA databases used to develop and validate the equation including how MPCA elected to use various databases, the high misclassification rate for the results of the MBLR equation, and MPCA’s dismissal of alternative statistical methods to evaluate the relationships between sulfide, sulfate and wild rice.

MPCA added in from the draft TSD to the final TSD reference to the state of Vermont phosphorus standard rulemaking process (TSD pg 55, 62-63, SONAR, pg 77 -78) as a comparison of false negatives and false positives or misclassification rate as found for the MBLR sulfate equation to the Vermont process.

However, MPCA neglected to explain the Vermont process and highlight how the process was very different from the MPCA approach for the MBLR sulfate equation. In particular, specific to the implementation of the Vermont nutrient criteria, an integrated approach to implementation is

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[8] EPA. 2010. Guidance for Implementing the January 2001 Methylmercury Water Quality Criteria. Available on-line: https://nepis.epa.gov/Exe/ZyPDF.cgi/P1007BKQ.PDF?Dockey=P1007BKQ.PDF

[9] EPA. 2016. Technical Support for Adopting and Implementing EPA’s 2016 Selenium Criterion in Water Quality Standards, Draft. Available on-line: https://www.epa.gov/sites/production/files/2016-10/documents/technical-support-adoption-implementation-selenium.pdf

also presented by Vermont[10]. The integrated approach used by Vermont allows for compliance with nutrient criteria to be evaluated by either comparison to nutrient criteria or by comparison to nutrient response variables (e.g., macroinvertebrate community health). This integrated approach is used because of the misclassification rates of 20 to 40%.

MPCA is not proposing an integrated approach to assessing compliance with the MBLR sulfate equation. Compliance is only evaluated based on comparing the equation water column sulfate and the monitoring results for sulfate. An integrated approach that might be considered is the presence and health of the wild rice in the wild rice water body and if the wild rice were present and healthy, then compliance is demonstrated. Given the amount of MPCA MBLR sulfate misclassification rate, an integrated approach is warranted.

Summary

The evolution to developing water quality criteria continues to focus on the direct effect and the dose-response, whether the direct effect impacting designated use of water body is found in the water column or not. EPA has water quality criteria that are based on fish-tissue and continue to be based on dose-response. MPCA's identification of porewater sulfide as the direct cause of an adverse impact on wild rice is similar to EPA's fish-tissue based criteria. However, EPA has taken the time to generate robust and valid data and methods to translate the fish-tissue criteria to water column chemical concentrations and the translation is adopted as guidance; not a water quality criterion or rule. MPCA, by adopting into rule the translation of the porewater sulfide to water column sulfate with the development of the MBLR sulfate equation, needs a level of confidence (e.g., far lower level of misclassifications) that is not currently shown. In addition, Vermont, in recognition of their high misclassification rate (similar to MPCA's misclassification rate) for nutrients, is using an integrated approach for implementation while MPCA is not.

Porewater Sulfide Analytical Method (SONAR E.7)

MPCA correctly notes that to use the MBLR sulfate equation, sediment porewater must be sampled and analyzed for sulfide. MPCA states that for new or expanding dischargers, the discharger must collect and analyze the samples as per the MPCA's "Sampling and Analytical Methods for Wild Rice", July 2017. On page 12 of this document, MPCA states that the analytical method for porewater sulfide is Standard Methods 4500-$S2^-$ E. Gas Dialysis, Automated Methylene Blue Method. Standard Methods presents limited data on precision and bias of the method (one single laboratory, spiked laboratory water at 4 concentrations for precision and two samples for bias). Standard Methods does not identify the method detection limit (MDL) nor the reporting limit (RL) expected for method 4500-$S2^-$ E Sulfide.

MPCA does list acceptable analytical performance but neglects to identify the required MDL. My opinion is given MPCA's use of a porewater sulfide threshold of 120 ug/L, the MDL should be at least 3 to 5 ug/L and the RL 10 to 15 ug/L to have confidence in using the data to derive an enforceable sulfate standard. The accuracy (bias) statement presented by MPCA is different than that included in Standard Methods. Further, no documentation or data on the development of an acceptable recovery of 80 to 100% (versus 97.6% to 104.2%) is provided by MPCA. The typical commercial lab quality assurance and quality control packages were not presented to the

[10] Vermont DEC. 2014 rev 2016. Nutrient Criteria for Vermont's Inland Lakes and Wadeable Streams: Technical Support Document. Available on-line: http://dec.vermont.gov/sites/dec/files/wsm/Laws-Regulations-Rules/2016_12_22-Nutrient_criteria_technical_support_document.pdf

Wild Rice Advisory Work Group in support of the Minnesota State health laboratory (state laboratory) that generated the porewater sulfide analytical results. Therefore, it is not known how they developed their MDL or RL, how they developed their calibration curve, what their quality control charts looked like, nor their overall precision and bias. Given the volume of analyses conducted, the details and quality control data would be most informative in having confidence in the selected analytical method and in the quality of data generated from the method. Finally, as this is not a routine method for dischargers, it would have been beneficial for MPCA to have split samples to understand interlaboratory variability (as of now MPCA, if they have any laboratory control data, only have data on intralaboratoy variability).

Finally, Ramboll has reached out to over 10 reputable certified (e.g., NELAC) commercial water testing laboratories and none of them either are set-up to run this method or routinely run this method to be confident in the quality of their results at a RL of 10 to 15 ug/L sulfide[11]. One commercial lab who has been a leader in AVS and sulfide analytical method development, Alpha Analytical, noted that colorimetric methods have a high potential for false positives due to naturally colored water. It is concerning that dischargers have limited knowledge on the accuracy and precision of the state laboratory execution of Method 4500-S2$^-$ E Sulfide and has no information on what to expect for interlaboratory variability. As I quoted Dr. Robert Hare[12] as part of my public comments during the Peer Review process[13]: "The key is measurement. Science cannot progress without reliable and accurate measurement of what it is they're trying to study. Simple as that."

<u>Summary</u>

MPCA needs to fully share all the laboratory quality control data and MDL studies conducted by the state lab to assure that MPCA, existing, new or expanding dischargers, and stakeholders are informed on the reliability and accuracy of Method 4500-S2$^-$ E Sulfide. As of now, neither MPCA nor other parties, can document the reliability and accuracy of a porewater sulfide result that will be key to deriving the enforceable sulfate standard.

In addition, as of today, no certified commercial water testing labs are available to conduct this method to a RL of 10 to 15 ug/L sulfide. As MPCA seems to have the most experience with this analytical method, they should engage in public outreach to share their knowledge with commercial labs on reliably and accurately conducting Method 4500-S2$^-$ E Sulfide.

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[11] Ramboll personal phone and email communications in August 2017.

[12] Dr. Hare is a researcher in the field of criminal psychology.

[13] ERG. 2014. Summary report of the Meeting to Peer Review MPCA's Draft Analysis of the Wild Rice Sulfate Standard Study. Pg E-17

Document type
Report

Date
April 7, 2017

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ATTACHMENT 1 - EC10 SULFIDE WILD RICE (HYDROPONICS) BY MBLR

RAMBOLL ENVIRON

Calculation of EC10 using Pastor Hydroponics data, Growth

The purpose of this analysis is to confirm the source and calculation methods associated with the EC10 presented both in the Pastor published paper and by MPCA based on the Pastor data. As per MPCA, a binary logistic regression (BLR) was fitted to the Pastor hydroponics data (growth versus sulfide).

The binary logistic model is used to estimate the probability of a binary response, in this case the probability of emergence, based on one or more predictor variables, in this case sulfide. It allows one to say that the presence of a risk factor (elevated sulfide) decreases the probability of emergence. Binary logistic regression is one of the most commonly applied statistical models. However, other binary models exist that for some data sets can provide a better fit for dose response modeling (for example 5 parameter log logistic regression). For binary logistic regression, one must make sure that there is sufficient data to fit the curve and the statistician must also verify the strength of the fit.

Our analyses were conducted using R.

Summary of Pastor Data

test	uM	Reps	TWA_SO4	TWGM_SO4	Weight_Change	Growth
definitive1	12.5	3	159.21817	78.32118	2.7809524	2.7809524
definitive1	25	3	579.20467	568.29462	-0.8460317	0.0904762
definitive1	50	3	1277.28133	1255.79182	-1.8047619	0.0000000
definitive1	6.25	3	75.20567	39.17295	3.2333333	3.2333333
definitive1	Control	3	11.05000	11.05000	4.5142857	4.5142857
definitive2	10	3	159.21600	70.76421	2.3666667	2.3666667
definitive2	20	3	509.98967	496.88179	-0.4047619	0.0000000
definitive2	40	3	1009.86167	991.23102	-0.3476190	0.0047619
definitive2	5	3	82.40233	41.20921	3.1619048	3.1619048
definitive2	Control	3	11.05000	11.05000	2.2523810	2.2523810
rangefinder	10	3	181.82417	87.49147	3.3666667	3.3666667
rangefinder	3	3	59.92333	34.57361	4.2000000	4.2000000
rangefinder	30	3	825.33067	787.34099	0.3071429	0.4023810
rangefinder	90	3	2633.64133	2529.31107	-0.6293651	0.0000000
rangefinder	Control	3	11.05000	11.05000	5.1063492	5.1063492

We calculated the EC10 using the resultant equation. The EC0, the baseline response associated with 10.5 sulfide, was used to define the EC10 and EC50 as per MPCA approach.

The equation is given as:

$$c + \frac{d - c}{1 + exp(b(log(sulfide) - log(e)))}$$

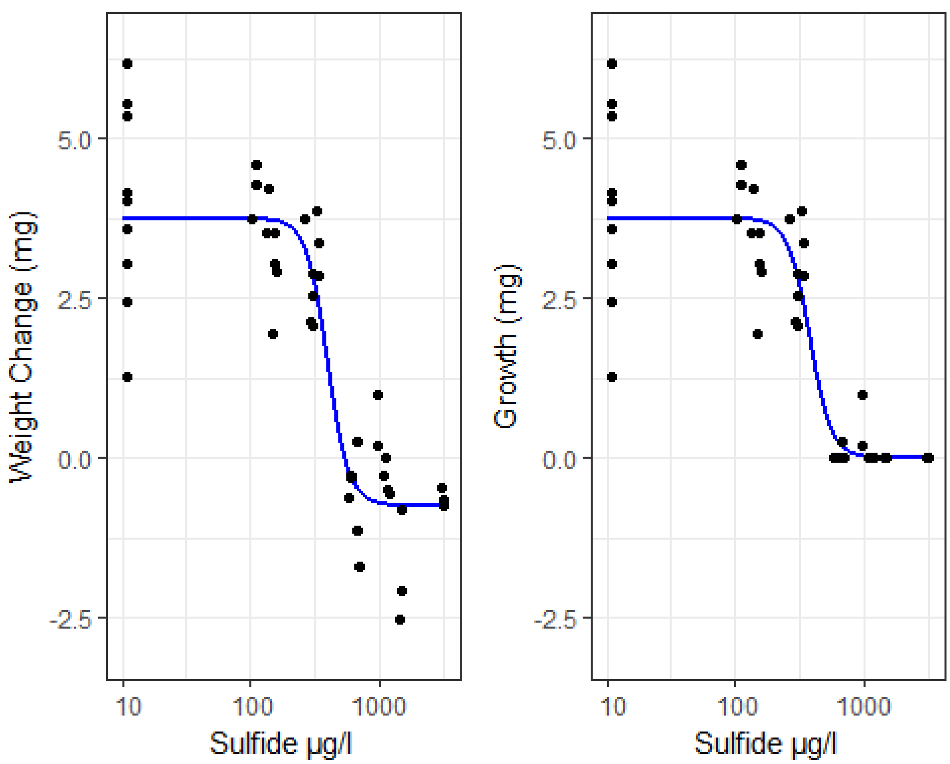
Initial Sulfide as Dependent variable

```
## [1] "Weight change based model"

##
## A 'drc' model.
##
## Call:
## drm(formula = weight_change_mg ~ meaninitialsulfide_ugL, data = FData,     fct = LL.4())
##
## Coefficients:
```

```
## b:(Intercept)  c:(Intercept)  d:(Intercept)  e:(Intercept)
##        5.2250        -0.7467         3.7427       403.0444

## [1] "Growth based model"

##
## A 'drc' model.
##
## Call:
## drm(formula = growth_mg ~ meaninitialsulfide_ugL, data = FData,     fct = LL.4())
##
## Coefficients:
## b:(Intercept)  c:(Intercept)  d:(Intercept)  e:(Intercept)
##      5.124624      -0.000693       3.748352     384.475375
```

Summary with Initial Average Sulfide				
EC	**Weight**	**Estimate**	**Growth**	**Estimate.1**
Control	3.742656	11.0500	3.748353	11.0500
EC10	3.368390	254.7301	3.373517	250.4055
EC20	2.994124	296.2232	2.998682	293.3359
EC50	1.871328	377.9573	1.874176	384.4476

Results



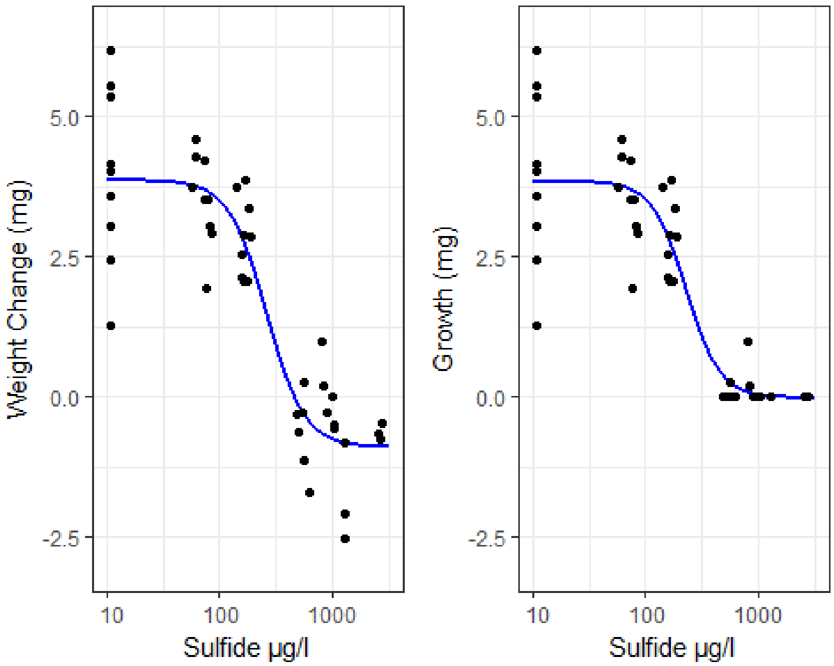
TWA Sulfide as Dependent variable

```
## [1] "Weight change based model"

##
## A 'drc' model.
##
## Call:
## drm(formula = weight_change_mg ~ arithmeticTWMsulfide_ugL, data = FData,     fct = LL.4())
##
## Coefficients:
## b:(Intercept)  c:(Intercept)  d:(Intercept)  e:(Intercept)
##        2.5874        -0.8957         3.8624       262.8643

## [1] "Growth based model"

##
## A 'drc' model.
##
## Call:
## drm(formula = growth_mg ~ arithmeticTWMsulfide_ugL, data = FData,     fct = LL.4())
##
## Coefficients:
## b:(Intercept)  c:(Intercept)  d:(Intercept)  e:(Intercept)
##       2.88824       -0.01358        3.84122      230.26192
```

Summary with TW Average Sulfide

EC	Weight	Estimate	Growth	Estimate.1
Control	3.861084	11.0500	3.840622	11.0500
EC10	3.474976	103.0407	3.456560	107.5173
EC20	3.088867	139.5059	3.072498	142.3063
EC50	1.930542	226.9197	1.920311	229.7257

Results



TW Geometric Mean Sulfide as Dependent variable

```
## [1] "Weight change based model"

##
## A 'drc' model.
##
## Call:
## drm(formula = weight_change_mg ~ geometricTWMsulfide_ugL, data = FData,     fct = LL.4())
##
## Coefficients:
## b:(Intercept)  c:(Intercept)  d:(Intercept)  e:(Intercept)
##         1.561         -1.001          4.028        162.497

## [1] "Growth based model"

##
## A 'drc' model.
##
## Call:
## drm(formula = growth_mg ~ geometricTWMsulfide_ugL, data = FData,     fct = LL.4())
##
## Coefficients:
## b:(Intercept)  c:(Intercept)  d:(Intercept)  e:(Intercept)
##       1.75964       -0.07114        3.99975      128.05931
```

Summary with TW Average Sulfide				
EC	Weight	Estimate	Growth	Estimate.1
Control	3.953667	11.05000	3.945855	11.05000
EC10	3.558301	37.89945	3.551269	39.06802
EC20	3.162934	59.39123	3.156684	59.71269
EC50	1.976834	127.98906	1.972927	127.44441

Results