Attachment 2

Water Quality Standards Stakeholders Meeting Agenda, dated October 19, 2009

Water Quality Standards Stakeholders Meeting Agenda
October 19, 2009
Illinois EPA HQ Training Room

10:00 Welcome and Introduction to the Rulemaking Proposal – Bob Mosher

10:15 Proposed Manganese Public Water Supply Intake Standard – Brian Koch

11:00 Derivation Process for Boron Aquatic Life Use Standards – Dave Soucek

11:45 Discussion

12:00 Lunch on your own

1:15 Derivation Process for Manganese and Fluoride Aquatic Life Use Standards – Brian Koch

2:00 Proposed Housekeeping Changes to the Water Quality Standards – Bob Mosher

2:30 Open Forum – Questions, Comments concerning the proposed standards

2:50 What's Next

3:00 Dismissal

The length of this meeting is somewhat dependent on the number of questions and comments the stakeholders have. We want to allow plenty of time for this interaction, which is the purpose of this meeting. If questions are few, we will probably get finished before 3:00.

Attachment 3

Water Quality Standards Stakeholders Meeting, Sign in Sheet, dated October 19, 2009

Illinois E P A

1021 North Grand Avenue, East
Springfield, IL

Meeting Sign-in

Meeting Date 10-19-09

Meeting Name Water Quality Standards Stakeholders

Participant Name	Representing	Telephone/FAX Number
Brian Koch	IEPA	785-4116
Mike Smallwood	Ameren	314-554-4521
Tom Davis	Dynegy	618-206-5931
Kay Anderson	ABTP, Sauget	618 337 1710
Amy Grady	IEPA	558-2012
Scott Twait	IEPA	558-2012
Mike Henebry	IEPA	785-3944
Sue Menon	WRP	757-8610 x1108
Cindy Skrukrud	Sierra Club	815-675-2544
Albert Ettinger	ELPA - Sierra	(312) 795-3707
Dave Soucek	INHS	217 265-5489
Carl Cannon	U.S. Steel	618-451-3013
Barbara Mazur	U.S. EPA	312-886-1491
Andrew Ditch	Spring. Coal	217-691-6696
Cory Schoonover	North Canton	309-824-1528
Brenda Carter	IERG	217 522 5512
Nancy Erickson	IFB	309-557-3153
Debbie Williams	IEPA	217 524-3332

Illinois E P A
1021 North Grand Avenue, East
Springfield, IL

Meeting Sign-in

Meeting Date 10-19-09 **Meeting Name** Water Quality Standards Stakeholders

Participant Name	Representing	Telephone/FAX Number
John Gefferth	Consol Energy	618-625-6850
Gregg Humphrey	SMSD	217-528-0491
Traci Barkley	Prairie Rivers Network	217/344-2371
Blake Anderson	SMSD	217-201-8857
Guy Hunt	MaRyan Mining	217/899-1926
Pat Giordano	DNR	
Zach Rasche	DNR	217/779-1984
JOHN DRAKE	CMT	787 8850
Toby Frevert	IERG	488-6499
BOB ELVERT	EXXONMOBIL	815.521.7580
CHRISTINE ZEMAN	CWLP	217/789-2116 ext 2628
Tim Kluge	San. Dist. Decatur	422-6931

32 2579
217 Mar-98

Attachment 4

Opinion and Order of the Illinois Pollution Control Board, dated March 6, 1975

ILLINOIS POLLUTION CONTROL BOARD
March 6, 1975

IN THE MATTER OF:)
)
PROPOSED AMENDMENTS TO RULES) R73-15
203 AND 408 OF THE ILLINOIS)
WATER POLLUTION CONTROL)
REGULATIONS

OPINION AND ORDER OF THE BOARD (by Mr. Henss):

Ozark-Mahoning Company and Minerva Oil Company filed a joint proposal seeking changes in Rules 203 and 408 of the Water Pollution Control Regulations as those Rules pertain to fluoride. The proposal was to relax the standard for mining companies by adding the sentence which has been underlined.

Rule 203(f) Water Quality Standards - General Standards

Constituent	Storet Number	Concentration (mg/l)
Fluoride	00950	1.4*

*<u>Except that fluoride derived from mining and concentrating the mineral fluorspar (CaF_2) shall not exceed 15 mg/l.</u>

Rule 408(a) Effluent Standards - Additional Contaminants

Constituent	Storet Number	Concentration (mg/l)
Fluoride (total)	00951	2.5*

*<u>Except that fluoride derived from mining and concentrating the mineral fluorspar (CaF_2) shall not exceed 15 mg/l.</u>

The proposed amendments and a statement of reasons supporting the proposal were published in Board Newsletter #78, dated December 29, 1973. Public hearings on the proposal were held in Elizabethtown on March 29, 1974 and in Chicago on April 19, 1974. Pursuant to its Petition to Intervene, Olin Corporation was designated a party in interest and granted leave to participate in the hearings. Other participants included the U. S. Environmental Protection Agency, the Illinois Environmental Protection Agency, Allied Chemical Company and private citizens.

The existing effluent limitation of 2.5 mg/l for fluoride was adopted by the Board on January 9, 1972 following extensive public hearings through the State. In setting this limitation the Board stated:

> "Fluoride. Our initial proposal for a fluoride effluent standard was 1.0 mg/l. This was somewhat tighter then the water quality standards we later proposed (1.4) for both aquatic life and public water supply, and it posed problems for municipal treatment plants whose influent has been deliberately dosed with as much as 1.0 mg/l of fluoride for dental purposes. Patterson reported that 1.0 mg/l was achievable only through relative exotic and costly methods, such as ion exchange, and that 10.0 mg/l was a more appropriate standard to achieve by ordinary precipitation. Weston and Dodge both said, however, that 1.0 was readily achievable, Weston specifying the use of alum at cost less than those for achieving most of the metals concentrations here proposed. The most specific information in the record came from Olin, which reports that its fertilizer works at Joliet consistently reduces fluoride concentrations by standard treatment from an influent of 15 mg/l to an effluent of 2.5, but that other ions present prevent reduction as low as 1.0.
>
> We have accepted Olin's figure of 2.5 mg/l, in recognition of the difficulties encountered in going lower and of the likelihood of dilution in many instances to achieve a relatively lenient stream quality standard."

A water quality standard of 1.4 mg/l fluoride was adopted on March 7, 1972, again following extensive public hearings throughout the State. On the fluoride water quality standard the Board stated:

> "Fluoride. Fluoride can delay the hatching of fish eggs and has been reported by McKee and Wolf to kill trout at concentrations ranging from 2.3 to 7.2 mg/l. They recommend a standard of 1.5 mg/l. The figure of 1.4, here repeated from the May 12 draft, is in line with that recommendation and also should assure a potable supply."

Both proponents in this matter are actively engaged in the mining and processing of fluorspar (also known as fluorite) for various industrial uses. Operating in Pope and Hardin Counties in Southern Illinois, Proponents extract the fluorspar from bedded and vertical vein deposits 350 to 850 feet below surface. They are the only fluorspar producers in Illinois and their combined production accounts for 80% of the entire amount produced in the United States. Ozark-Mahoning processes about 17,000 tons of crude ore per month at its Rosiclare mill. Minerva processes from 900 to 1300 tons of crude ore per month, from which about 157 tons of fluorspar concentrate, 20 tons of zinc concentrate and 30 tons of barite ($BaSO_4$) are extracted.

During the concentrating processes, part of the fluorspar in the crude ore is dissolved and discharged in the mill effluent. Some fluoride is also contained in the discharges from the fluorspar mines.

The two counties in which the fluorspar industries operate are described as two of the smallest and most sparsely populated counties in Illinois. The 1970 Census showed that Hardin County had 4914 people on 183 square miles while Pope County had 3,857 people on 381 square miles. Ozark-Mahoning employs 220 persons directly and another 55 to 60 on contract. Minerva employs 210 persons directly and 40 persons indirectly. The majority of the workers reside in either Hardin or Pope County. The only other industries in the two-county area are quarrying, farming and cattle raising. Proponents state that the economy of these two counties is largely dependent upon the fluorspar industry as are the users of the fluorspar product insofar as total domestic production is concerned.

Fluoride-bearing effluent from proponent's mines and mills is discharged to receiving streams which vary from intermittent drainage ditches or creeks to flowing rivers as follows:

<u>OZARK-MAHONING COMPANY</u>

Parkinson Mine - To Big Grand Pierre Creek to Ohio River.
Barnett Mine - To Big Grand Pierre Creek to Ohio River.
Barnett Air Shaft - To unnamed creek to Little Grand Pierre Creek to Big Grand Pierre Creek to Ohio River.
Oxford Mine #7 - To unnamed creek to Duck Creek to Rock Creek to Harris Creek to Saline River to Ohio River.
Knight Mine - To unnamed creek to Mud Creek to Three Mile Creek to Ohio River.
W. L. Davis Mine #1 - To Davis Branch to Big Sinks to Ohio River (possibly)*
Rosiclare Lead and Fluorspar Mine - To Willow Creek to Ohio River
Rosiclare Flotation Plant - To settling pond to Ohio River
North Green Mine** - To Sheridan Branch to Haney Creek to Ohio River
West Green Mine** - To Sheridan Branch to Haney Creek to Ohio River

MINERVA OIL COMPANY

Mine #1 - To Running Bear Creek to Rock Creek to Saline River.

MINERVA OIL COMPANY (continued)

Mill #1	- To Rock Creek to Saline River.
Crystal Mill	- To unnamed creek (sometimes called Davis Creek) to Big Sinks to Ohio River* (possibly)
Gaskins Mine	- To Big Grand Pierre Creek to Ohio River
Tucker Hill Area	- To unnamed creek to unnamed creek to Rock Creek to Harris Creek to Saline River to Ohio River.
Spivey Mine	- To Goose Creek to Harris Creek to Saline River to Ohio River.
Deardorff Mine	- No discharge***

*The Big Sinks, a natural sinkhole, drains periodically to an unknown receiving stream. It is believed that water drains from Big Sinks through an underground stream to the Ohio River, although dye tests have been unsuccessful in confirming the location of the ultimate receiving stream.

**Initial information showed that both the North and West Green Mines were not consistently discharging water. When operating conditions required the pumping of water from these mines, it was done on an intermittent basis only (1 to 4 hours per day) and the mine water was discharged to the streams shown. New information shows that these mines are now discharging water consistently at a combined rate of 100,800 gpd.

***Mine water from this mine flows underground through depleted excavations to Ozark-Mahoning's W. L. Davis Mine. Such flow is minimal.

The other industrial firms participating in this matter have fluoride problems significantly different from those of the mining companies and from each other. At its Blockson Works in Joliet, Olin imports calcium phosphate rock, soda ash and sulfuric acid which are used to manufacture sodium phosphate. Fluoride-based products are also produced at the Blockson Works through the reaction of sulfuric acid and fluorspar to form hydrofluoric acid. The hydrofluoric acid is then reacted with other materials to form the desired fluoride-based final product. Fluoride-bearing effluent from Olin's Blockson Works is discharged to the Des Plaines River.

Allied Chemical operates a facility for the production of uranium hexafluoride (UF_6), sulfur hexafluoride (SF_6), fluorene, antimony pentafluoride and iodine pentafluoride in Metropolis, Illinois. Allied's liquid discharge, which consists of spent ammonium sulfate solution, sulfide liquors, hydrofluoric acid solution, spent potassium hydroxide solution and uranium recovery leach liquors, flows to the Ohio River through two industrial ditches.

Corporate positions on these matters vary as widely as do the processes in which the fluoride bearing wastes are generated. As earlier noted, Ozark-Mahoning and Minerva propose to amend the standards only as those standards apply to the fluorspar industry. Olin's position was one of disagreement with Ozark-Mahoning and Minerva over the proposed changes in Rule 408. Olin proposes to change Rule 408 to allow a fluoride effluent concentration of 10 mg/l for all industries. Olin took no position on the proposed change in Rule 203.

Allied first contended that the effluent standard should be revised to allow 15 mg/l fluoride based on an average of 24 hour composite analysis for 30 consecutive days and 30 mg/l maximum for any one 24 hour composite. Allied took no position on the proposed revision of Rule 203. Neither the U. S. environmental Protection Agency nor the Illinois Environmental Protection Agency took a position on the proposed changes prior to the public hearings. Their post hearing comments will be discussed elsewhere in this Opinion. Of the two Agencies, only the U. S. EPA chose to present any testimony.

Fluorides are widely distributed in the earth's crust, occurring in both igneous and sedimentary rocks. Among the more common fluoride minerals are fluorspar (CaF_2), villiaumite (NaF), cryolite (Na_3AlF_6) and fluorapatite [$CaF_2.3Ca_3(PO_4)_2$]. Fluorides in high concentrations are not a common constituent of natural surface waters but they may be prevalent in detrimental concentrations in ground waters.

Small concentrations of fluoride (0.6 mg/l to 1.7 mg/l) in drinking water have been shown to effectively reduce the prevalence of dental carries while excessive amounts cause effects in humans varying from mottled teeth to death. When fluoride is 2.5 mg/l 75% to 80% of children have mottled teeth. In drinking water, fluoride of 180 mg/l is toxic and 2000 mg/l is lethal to man.

Solubility of a fluoride varies according to the nature, pH and temperature of the solvent, cationic partner and prevalence of other chemical constituents in the solvent. The two most discussed fluoride compounds during these proceedings, sodium fluoride and calcium fluoride, vary significantly in their solubility. The solubility of calcium fluoride at 18° C. (64.4° F) is 16 ppm (about 8 ppm fluoride ion) whereas the solubility of sodium fluoride is about 19,000 ppm. This means that sodium fluoride is inherently more soluble in water than is calcium fluoride.

Neither Ozark-Mahoning nor Minerva discharges any effluent that approaches the proposed effluent limit of 15 mg/l fluoride. Discharges from the mines and mills operated by these two companies are less than 5 mg/l, as shown below, with a single exception of the discharge from the Rosiclare flotation plant settling pond.

OZARK-MAHONING

	Fluoride, mg/l
Parkingson Mine - 40 gpm Barnett Mine - 50 gpm Barnett Air Shaft - 40 gpm	
Big Grand Pierre above discharge	0.28
Parkinson discharge to Big Grand Pierre	1.40
Barnett Mine discharge to Big Grand Pierre	2.40
Big Grand Pierre below Parkinson and Barnett	0.30
Barnett air shaft discharge to unnamed creek	3.10
Unnamed creek at confluence with Little Grand Pierre	0.50
Little Grand Pierre above confluence with unnamed creek	0.25
Little Grand Pierre below confluence with unnamed creek	0.40
Big Grand Pierre below all discharges	0.28
Oxford Mine #7 - 10 gpm	
Mine discharge to unnamed creek	2.20
Unnamed creek at confluence with Duck Creek	0.25
Duck Creek above confluence with unnamed creek	1.50
Duck Creek below confluence with unnamed creek	1.00
Duck Creek above confluence with Rock Creek	0.97
Rock Creek above confluence with Duck Creek	0.25
Rock Creek below confluence with Duck Creek	0.63
Knight Mine - 90 gpm	
Knight discharge to unnamed creek	1.40
Unnamed creek above confluence with Mud Creek	0.75
Mud Creek above confluence with unnamed creek	0.25
Mud Creek below confluence with unnamed creek	0.25
W. L. Davis Mine #1 - 1200 gpm	
Mine discharge to unnamed creek	1.4
Unnamed creek above entry to Big Sinks	1.2
Rosiclare Lead and Fluorspar Mine - 20 gpm	
Mine discharge to Willow Creek	1.3
Willow Creek above confluence with Ohio River	1.4
Rosiclare Flotation Plant - 650 gpm	
Plant discharge to settling pond	--
Settling pond discharge to Ohio River	10.0

MINERVA

	Fluoride, mg/l
Mine #1 and Mill - 368 gpm	
#3 pond discharge to Rock Creek	4.5 (avg.)
Rock Creek above #3 pond discharge	0.6 (avg.)
Rock Creek below #3 pond discharge	2.5 (avg.)
Harris Creek below confluence with Rock Creek	0.45
Saline River above confluence with Harris Creek	0.40
Saline River at confluence with Harris Creek	0.47
Crystal Mill - 52 gpm	
Heavy-media-separation tails	3.62 (avg.)
Unnamed creek above HMS tails	1.34 (avg.)
Big Sinks	1.51 (avg.)
Tucker Hill Area - 150 gpm	
Churn Drill Hole, underground water	3.02 (avg.)
Unnamed creek upstream	No flow
Unnamed creek downstream	1.14
Gaskins Mine - 875 gpm	
Gaskins Shaft	1.58 (avg.)
Big Grand Pierre Creek above discharge	0.39 (avg.)
Big Grand Pierre Creek below discharge	0.50 (avg.)
Spivey Mine - 80 gpm	
Spivey Shaft	2.75 (avg.)
Goose Creek above discharge	0.51 (avg.)
Goose Creek below discharge	0.66 (avg.)

C. B. Rash, Ozark-Mahoning's Superintendent of Milling, explained that the proposed 15 mg/l effluent limitation was necessary as a "safeguard" in the event recycling of effluent was imposed upon the industry (R. 41). Ozark-Mahoning's plant in Colorado attempted a waste water recycling effort when the Colorado Department of Public Health requested an effort to achieve "zero flow". Although "zero flow" was not achieved, the effort resulted in the recycling of 80% of the waste water--but at a price. This price was an increase in fluoride concentration to 32 ppm.

When the Board set the effluent standard at 2.5 mg/l it relied heavily upon the testimony of an Olin employee, Emil Stoltz, regarding the technology available to reduce fluoride in waste water. Stoltz had testified that while Olin had not been able to "obtain it in our specific effluent" they did have the technology to "get down to 2 to 2 1/2 mg/l."

Stoltz testified in the current proceedings that he had meant to inform the Board that this level of fluoride reduction was only a technical feasibility based on laboratory studies made at the corporation's research headquarters in New Haven, Connecticut. This research was primarily based on a lime treatment process which Olin has not used at its Blockson Works. Stoltz testified that, based on the research program, he now believes that Olin could reduce the fluoride in waste water from 15 mg/l to 2.5 mg/l. Blockson Works waste water currently contains about 20 mg/l fluoride before treatment (R. 224).

At this point, it is necessary to review the health related information about fluorides in order to provide a balance to the later discussion on feasibility and economic reasonableness of fluoride treatment.

In setting a 1.4 mg/l fluoride water quality standard, the Board cited a report by McKee and Wolf (McKee, J. E., and Wolf, H. W., Water Quality Criteria, California State Water Resources Control Board, Second Edition, 1963) showing that fluoride can delay the hatching of fish eggs and that concentrations ranging from 2.3 to 7.2 mg/l can kill trout. These references, p. 191 of the McKee-Wolf report, also show that in 15 studies the majority involved the use of sodium fluoride and none of the studies is shown to have involved calcium fluoride.

Under Sodium Fluoride, McKee-Wolf cite research showing the following effects of sodium fluoride on certain aquatic bacteria, algae and small crustaceans:

Species	Results
Daphnia (an order of crustaceans which includes water fleas, found everywhere in fresh waters)	Threshold of NaF at 23° C. was found to be 270 mg/l for a 2 day exposure.
Scenedesmus (a fresh water algae, most common and best known of all algaes, found almost anywhere algae grows)	Threshold of toxic effect was 95 mg/l during 4 days at 24° C.
Microregina (A single cell protozoan often found in fresh water)	Threshold of toxic effect was 226 mg/l during 4 days at 24° C.

Species	Results
Escherichia coli (a bacteria found abundantly in vertebrate intestine)	Threshold of toxic effect was 180 mg/l during 4 days at 27° C.
Free-living protozoa and fresh water rotifers	Survived and reproduced in water containing 1000 mg/l but were killed at 1700 mg/l.

This information tends to show that low concentrations of sodium fluoride probably would not present any significant toxicological difficulties for at least some of the more common lower aquatic organisms expected to inhabit Illinois streams. Based on research reported in McKee-Wolf, the same is not true for higher aquatic organisms. This research reported the following effects of sodium fluoride on fish:

Concentration, mg/l	Type Fish	Effect
2.3 to 7.3	Trout	TL_m at 18° C. in soft water
2.6 to 6.0	Trout	TL_m at 13° C. in soft water
2.7 to 4.7	Trout	TL_m
5.9 to 7.5	Trout	TL_m at 7.5° C. in soft water

Thus, it would appear that some lower aquatic organisms are able to tolerate sodium fluoride concentrations on the order of 100 times that tolerated by trout. Although time of exposure for determining TL_m is usually specified, this parameter was not provided for the data above, making comparison of results impracticable. Reasons for these phenomenal differences in survivability (for example, osmotic capabilities of membranes of lower aquatic forms vs. higher aquatic forms, significant physiological differences, etc.) were not stated.

In this proceeding, expert testimony indicates that sodium fluoride concentrations in natural waters should be minimal in comparison to concentrations of calcium fluoride. Dr. W. F. Sigler, head of the Wildlife Science Department at Utah State University, testified that all research conducted in the U. S. on fish fluorosis "was done by me and under my direction". Dr. Sigler noted that while small amounts of sodium fluoride might exist, larger amounts do not exist naturally because it dissociates to form calcium fluoride.

A number of opinions on the relative toxicities of sodium and calcium fluoride were aired during the hearings. C. B. Rash testified that his opinion of available research was that sodium fluoride "would be more toxic than calcium fluoride even at the same concentration, because there is indication that the calcium present with the fluoride ion reduces the toxicity" (R. 45).

Dr. Sigler first testified that sodium fluoride and calcium fluoride have equal toxicities at equal concentrations. (R. 120) Admittedly not a chemist, Dr. Sigler later qualified this statement by testifying that the toxicities would be equal except when other positive ions were present (R. 155). Then later, Dr. Sigler testified that calcium fluoride would be the less toxic of the two fluorides because "calcium and the fluoride have an affinity for each other and reduces the toxicity" (R. 206). Dr. Sigler indicated his preference to let Franklin Davis of the Colorado School of Mines Research Institute answer the questions relating to the chemistry of fluorides. When called upon, Davis testified that he could not "answer that with the proper credentials" because he was not a toxicologist (R. 164).

Significant testimony on fluoride toxicity was produced by Dr. Leonard Krause of Olin Chemical Company. Dr. Krause testified that fluoride entering the system of any living organism will combine with the most prevalent tissue around it, usually tissue containing calcium such as cartilage or bony tissue. Such a combination is known as fluorosis. Fluoride interferes with enzyme systems at the cellular level and interferes with the oxygen uptake in organisms by some mechanism that toxicologists don't yet understand (R. 322).

Fluoride taken into a body in the form of calcium fluoride tends to be excreted almost exclusively as calcium fluoride. This occurs, according to Dr. Krause, because very little, if any, of the fluoride will combine with the body calcium since sufficient calcium is already available for combining with the fluoride.

Dr. Krause testified that his research work involving humans showed that 14 mg/l of calcium fluoride was not toxic to humans. He did not think a toxic level of calcium fluoride in solution could be reached because it would be precipitating out. Dr. Krause stated that he would not hesitate to drink water containing 14 mg/l of calcium fluoride but would never put the same amount of sodium fluoride into his body (R. 332). Fluoride in water containing sodium fluoride would not be excreted as would the calcium fluoride. It would be available to bony tissues and kidneys.

Another of the body elements that could be affected by the ingestion of calcium fluoride is potassium, an essential element in nerve tissues. At first Dr. Krause stated unequivocally that potassium in the body would not be replaced by the calcium in calcium fluoride because of the tight chemical bond found in calcium fluoride (R. 335). He later acknowledged that such a replacement possibility did exist (R. 342) although the fluoride itself is more available to cartilaginous and bony tissue than for nerve tissue (R. 351).

Table 6-5 of the McKee-Wolf report shows various levels of fluoride concentrations that caused mottled teeth. In the range from 0.2 to 1.0 mg/l fluoride the mottling is mild with a concentration of 1.0 mg/l listed as the "threshold for mottling". One study reveals a mild to moderate degree of mottling from 1.0 to 2.0 mg/l fluoride. At 6.0 mg/l the references reported pitting and chipping of teeth and that 100% of children had mottled teeth.

E. F. Carter, Jr., Rosiclare postmaster, testified that he knew of no mottling of teeth in the Pope-Hardin County area caused by the discharges of Ozark-Mahoning or Minerva. W. W. Fowler, Ozark-Mahoning Vice President and General Manager, testified that he knew of no adverse effects, including mottling of teeth, that had been suffered by any of his employees. He added that miners have drunk water from the mine seams and walls for a number of years. The highest fluoride concentration in such water was found to be 2.5 mg/l. C. B. Rash also testified that he had observed no ill effects or mottling of teeth in the area.

Rash testified that several farmers in the area depend on the mine discharge water as a source of water for their livestock. The farmers had informed Rash that they had never observed any ill effects in their cattle as a result of drinking the mine discharge water.

Proponents submitted a letter from Truman Louderbach, a Research Biologist at the Colorado School of Mines Research Institute (CSMRI), reporting on results of bioassay testing conducted at CSMRI at the request of Ozark-Mahoning Company (Petitioner's Exhibit 4). For the test, samples were drawn from the tailings dam effluent of Ozark-Mahoning's Cowdrey, Colorado operation and from Pinkham Creek above the confluence with the tailings dam effluent. These samples had the following properties:

	Tailings Dam Effluent	Pinkham Creek
Temperature	7° C. (44.6° F.)	6.5° C. (43.7° F.)
pH	7.6	7.5
D.O., ppm	8.3	7.0
F^-, ppm	32	2.6

Six-month-old fingerling rainbow trout were acclimatized for 10 days in Pinkham Creek water at 15° C. ± 2° (59° F.) with a dissolved oxygen concentration above 7 ppm. Following the acclimatization the trout were subjected to testing using various mixtures of Pinkham Creek water and tailings dam effluent up to 100% tailings dam effluent. The tests showed a 100% survival of trout for 96 hours in all mixtures including the undiluted tailings dam effluent. No evidence of distress in the behavior of fish specifimens was observed.

Also submitted by proponents was a report by CSMRI's Senior Research Biologist, Dr. Gary D. Boss, in which Boss summarized his findings on fluoride toxicity based on published reports. According to the Boss report, assignment of specific toxic levels is difficult because of the following major factors:

1. Fish species, race, or strain
2. Fish size and stage of development
3. Physiological state, including age of fish
4. Level, type and solubility of fluoride and fluoride containing compounds
5. Water temperature
6. Individual biological response
7. Composition of the water, in particular the content of calcium, magnesium and chloride

Boss cites a Utah State University study (Neuhold and Sigler, 1960) conducted on carp and rainbow trout, using fluoride containing water with a calcium and magnesium content of less than 3 ppm. Results were reported as follows:

Species	Temperature, °F.	TL_{50}* at F^- ion conc. (ppm)
Trout	55	2.7 to 4.7
Carp	65-75	75.0 to 91.0

*TL_{50} - Tolerance limit at which 50% of the fish survived and is nearly equal to LD_{50} (lethal dose) and LC_{50} (lethal concentration)

Boss qualifies the above results by stating "Fish populations including rainbow trout flourish in Wyoming and Nevada where fluoride concentrations are 13.0 - 14.0 ppm. Yet reared trout have displayed TL_{50}'s of about 3.0 ppm of fluoride (Sigler and Neuhold, 1972)".

The Boss report cites another study of response of rainbow trout eggs in water containing less than 3.0 ppm of calcium and magnesium under varying temperatures (Neuhold and Sigler, 1960). Reported results were as follows:

Temperature, °F	TL_{50}, ppm F^-	Hours
46	222-273	424
55	242-261	214
60	237-281	167

These data show that fluoride toxicity increases for trout eggs with increasing temperature.

Information was also reported on efforts to determine the effect of chloride concentration on rainbow trout (Neuhold and Sigler, 1962). In water containing measured amounts of fluoride and chloride ions, the following results were obtained:

F^- ion, ppm	Cl^- ion, ppm	
	0	9
	(Deaths)	
0	0	0
4	0	0
7	1	0
13	6	1
25	10	1

Boss states that such evidence indicates that the presence of either calcium, magnesium or chloride ion decreases the toxic level of fish to the fluoride ion. While admitting that the effect of the chloride ion is conditional, Boss asserts that "the weight of the experimental evidence supports the contention that fish acclimated to moderate concentrations of chloride ion have increased resistance to fluoride toxicity."

Summarizing, Boss states: "Fluoride ion has a high affinity for calcium and its presence in the water in significant amounts seems to reduce the effective concentration of calcium in the body of the fish. CaF_2, however, dissociates to form so few fluoride ions that evidently only light symptoms of fluorosis are produced. Moreover, the calcium ion made available by the dissociation of CaF_2 would seem to provide a replacement for any calcium extracted from the body of the fish."

Boss's overall conclusion based on available information was that "in our opinion, data on fluoride toxicity are too general and vague to establish a valid toxicity level for aquatic life at this time".

As will be noted in the following table, waters used in the tests just described bear little, if any, resemblance to stream conditions applicable to the parties in this proceeding. This table provides definitive stream values in relation to various streams receiving proponents effluent, the Des Plaines River near Olin's Blockson Works and the Ohio River near Allied's Metropolis plant:

Average Stream value (1)	Big Grand (2) Pierre Creek, AL01	Saline River South Fork (3)	Saline (4) River, AT04
ph	7.5	---	6.5
D.O.	7.9	---	8.0
Fluoride	0.6	0.3	0.4
Chloride	13	---	49
Hardness	---	160	---

	Saline (5) River, AT02	Ohio (6) River, A08	Ohio (7) River, A07
ph	7.6	7.9	7.8
D.O.	8.2	8.4	8.6
Fluoride	0.2	0.2	0.1
Chloride	23	100	22
Hardness	---	---	---

	Ohio (8) River	Ohio (9) River A01	Ohio (10) River, A02
ph	---	7.7	7.6
D.O.	---	8.6	8.7
Fluoride	0.6	0.2	0.1
Chloride	---	24	19
Hardness	160	---	---

	Ohio (11) River, A06	Ohio (12) River, A04	Ohio (13) River
ph	7.5	7.6	---
D.O.	7.6	7.8	---
Fluoride	0.2	0.2	0.3
Chloride	22	20	---
Hardness	---	---	178

	Des Plaines (14) River, G12	Des Plaines (15) River, G01
ph	7.3	7.4
D.O.	7.0	7.3
Fluoride	0.8	0.8
Chloride	120	165
Hardness	320	290

(1) Stream identification followed by an "A" or "G" identification number (i.e., AL01, G12) represents data taken from Illinois EPA Water Quality Network, Summary of Data, 1972. Stream identification without an "A" or "G" identification number represents data taken from Illinois EPA Public Water Supplies Data Book, 1973 (Allied Exhibit #2). Values reported in mg/l.
(2) Below discharge from Minerva's Gaskins Mine. At or near discharges from Barnett Air Shaft, Barnett Mine and Ozark-Mahoning's Parkinson Mine.
(3) Above fluorspar mine discharges.
(4) Far above fluorspar mine discharges.
(5) Mouth of River below fluorspar mine and mill discharges.
(6) Near Shawneetown, above fluorspar mine and mill discharges.
(7) Near Cave-In-Rock, below confluence of Saline and Ohio Rivers.
(8) Rosiclare water intake below discharge from Ozark-Mahoning's Rosiclare Mill.
(9) Golconda water intake below fluorspar mine and mill discharges.
(10) Brookport below all fluorspar mine and mill discharges but above discharge from Allied plant.
(11) Olmsted below Allied plant discharge.
(12) Cairo water intake.
(13) Cairo water intake.
(14) Above discharge from Olin's Blockson Works.
(15) Below discharge from Olin's Blockson Works.

From the record it is apparent that the determination of toxicity in this matter depends largely upon the concentration of ions in the receiving waters, particularly calcium and magneisum ions. The reports refer to the concentration of these ions as hardness. (Water hardness in the Des Plaines River near Olin's Blockson Works is about 90% calcium and 10% magnesium [R. 222]). As the above Table shows,Illinois streams are not deficient in calcium and magnesium ion concentrations.

On this basis, toxicity data submitted by Allied Chemical appear to be more pertinent to this proceeding than any other data submitted. Allied contracted Industrial Bio-Test Laboratories Inc. to conduct a 4-day static fish toxicity study using bluegill sunfish (Lepomis macrochirus) and channel catfish (Ictalorus punctatus). A test solution was prepared by using de-ionized water and measured amounts of calcium and magnesium sulfate, sodium bicarbonate and potassium chloride. Water taken from the Ohio River near Metropolis was used as a dilutant.

Sodium fluoride, calcium fluoride and hydrofluosilicic acid were added at test concentrations of 2.5, 10.0 and 20.0 ppm fluorine to separate vessels, each containing 10 specimens of each species of fish. An untreated sample containing only river water was used as a control. Water temperature was maintained at about 18° C. (64.4° F.).

In the test using sodium and calcium fluoride no fish fatalities had occurred after 96 hours exposure to the calcium fluoride test solution. One bluegill died after 24 hours exposure to the 10.0 ppm sodium fluoride solution and another died after 72 hours exposure to the 20.0 ppm sodium fluoride solution. No catfish fatalities occurred in the sodium or calcium fluoride solutions. Investigators concluded that the 96-hour TL_{50} of both sodium and calcium fluoride for unacclimated native fish is in excess of 20 mg/l

These results are particularly important and directly relatable to Illinois streams. They again point to the importance of associating fluoride toxicity levels with calcium and magnesium concentrations in surface streams.

Another document which provides additional insight into the effect of fluoride on stream quality was submitted as Proponent's Exhibit #14. This document reports the results of a biological survey conducted by the Illinois EPA on February 6-7, 1974 to determine the condition of stream environments relative to discharges from Minerva's Gaskins Mine. The survey reveals well balanced benthic invertebrate populations both upstream and downstream from the mine discharge. (An unnamed tributary receiving effluent from the mine was reported to be "semi-polluted" with the cause appearing to be of an "organic origin"). Although fluoride concentrations are not reported in the biological survey, data reported earlier in this Opinion indicate that the fluoride water quality is being met and this receiving stream is adequately protected.

Turning now to the question of economic reasonableness and technical feasibility, we shall first review Proponents' Exhibit #8. Under the direction of Franklin T. Davis, CSMRI, a report titled "Capital and Operating Cost of a Suggested Process for the Removal of Fluoride Ion from Tailings Water" was prepared. The report shows applicability of currently available methods of fluoride removal and also details an as yet unproven method which has a potential of reducing fluoride content from 10 ppm to about 1 ppm at a rate of one million gallons per day.

The Davis report disposes of "state-of-the-art" systems as follows:

A. CaF_2 precipitation - economically unreasonable because of excessive calcium requirements.
B. Contacting beds of activated alumina, calcium phosphate, calcium super phosphate or bauxite - prohibitively large bed volume required to treat large amounts of 10 ppm fluoride water, loss of bed material in regeneration and probable addition of phosphate ion to water.
C. Combined magnesia-lime system - restricted to water containing less than 3 ppm fluoride, large amounts of magnesium co-precipitated.
D. Carbon, zeolites and activated bone - best suited for low volume of water with a fluoride concentration of less than 5 ppm and a pH of 7 or less, regeneration losses.
E. Ion exchange - low capacity, slow exchange, low fluoride selectivity and economics.
F. Reverse osmosis and ion selective membrane - economically unattractive and not proven technology.

An alternate method proposed by Davis, but not yet tested, could be labeled as the "Hydroxyapatite Method". In that method water and lime are mixed to produce a 10% slurry which is reacted with 85% phosphoric acid to produce hydroxyapatite by the following reaction:

$$5Ca(OH)_2 + 3H_3PO_4 \rightarrow Ca_5(OH)(PO_4)_3 + 9H_2O$$

Twice the stoichiometric amount of hydrated lime is added to favor complete reaction of the phosphoric acid in the 1 hour reaction time.

Hydroxyapatite slurry is then pumped to an agitated reaction vessel where it contacts the incoming fluoride-bearing waste water. Reaction tank volume allows 1 hour for reaction of the fluoride to fluorapatite. From the reaction vessel the slurry flows to a floculator tank where a flocculating polymer is added. After 15 minutes the treated slurry flows to a clarifier where suspended solids are settled. Overflow from the clarifier is discharged from the plant at a rate of 693 gpm. Sludge from the clarifier is pumped to the tailings dam but can be recirculated in varying amounts to the reaction tank in order to react any remaining unreacted hydroxyapatite. Sludge generation is small for this process and should not present any major disposal problem.

While Davis thinks the method looks good on paper, he quickly adds that additional laboratory studies are required to finalize a numer of parameters before final evaluation is possible. Among the parameters to be determined are:

1. Ratio of lime to phosphoric acid and required reaction time,
2. Rate and absorbtion capacity of the hydroxyapatite, and
3. Optimum quantity of flocculant, flocculating time and settling time in the clarifier.

Capital investment for use of the hydroxyapatite method to treat one million gallons per day would be $287,300, exclusive of roads, power lines and pipe lines. Operating costs for the plant were listed as $11,278 per month or $0.376 per 1,000 gallons.

Davis testified that Ozark-Mahoning would require three such plants since 3 million gallons of waste water must be treated (R. 172). Therefore, capital cost for Ozark-Mahoning would be in excess of $1 million and operating costs would be $45,000 per month (R. 171). Similar costs on a percentage basis would apply to Minerva's operations (R. 172).

Full-scale laboratory testing remains to be done for the hydroxyapatite method. Davis has performed some laboratory experiments using "artificial hydroxyapatite" with the result being a reduction to less than 1 ppm fluoride (R. 172).

As to other processes for removal of fluorides as described in Waste Water Treatment Technology, Second Edition, IIEQ Document 73-1 (Petitioner's Exhibit #9) Davis testified that none of the processes would be effective on mill tailings water. Davis stated that the processes would not be effective because most of the processes treat water that is relatively free of turbidity. Mill tailings water would have to be clarified or filtered in order to use the process and this "is expensive" (R. 168). Another reason for nonacceptance, according to Davis, was "although they don't say this, ...it is pretty obvious that after they removed it [fluoride] they dumped it back into the river downstream" (R. 167). This option is not open to Proponents.

After reviewing the various methods in the IIEQ document, the Board agrees that they do not directly relate to the fluorspar industry. However, a possible exception might be the use of contact beds of activated alumina. Without committing to the applicability of this process, the Board notes that one such unit in Bartlett, Texas has operated since 1952 on a municipal water plant to reduce fluoride from 8 mg/l to 1 mg/l. Noticeably absent from discussion on the Bartlett plant are flow rates and cost data. According to the report, two investigators experimented with an alumina bed as a polishing unit following lime precipitation. They found that a 30 mg/l residual fluoride concentration could be reduced to 2 mg/l. At a pH of 11.0 to 11.5 they were able to reduce fluoride from 9 mg/l to 1.3 mg/l. Regenerative losses were cited as 4% alumina lost per 100 regenerative cycles.

While such information is far too skimpy, it certainly raises the possibility of use on Proponent's mine waters, which are "reasonably clear" (R. 200), or on mill tailings water after clarification. Further, the Board finds nothing in the IIEQ document to indicate that any of the methods discussed involves subsequent dumping of removed contaminants "back into the river downstream".

In his letter dated April 26, 1974 (Petitioner's Exhibit #11), Davis said that new information supplied to Davis showed the mine waters to be free of turbidity. On this basis Davis states that the best process would be the one reported in "Defluoridation of Municipal Water Supplies", by F. J. Maier in the Journal of the American Water Works, August 1953. This is the same alumina contact bed process used in Bartlett, Texas and discussed just above. Davis states the process has a potential for lower capital cost than the hydroxyapatite method but laboratory verification would be required.

A set of figures based on the alumina bed process for mine waters and the hydroxyapatite method for tailings water, adjusted to 1974 prices, was supplied by Davis. These figures show a one million gpd tailings treatment plant with a fixed capital investment of $298,000 and operating costs of $12,800 per month. A 650,000 gpd mine water treatment plant to treat water from #7 Oxford Shaft, North Green Mine and West Green Mine and a 650,000 gpd mine water treatment plant to treat water from the Parkinson Mine and Barnett Air Shaft would require a fixed capital investment of $568,800. Adjusted operating costs are shown as $0.251 per 1000 gallons for the two mine water treatment plants and $0.427 per 1000 gallons for the tailings plant for a total of $0.328 per 1000 gallons. These costs exclude about 10,100 feet of right-of-way for pipeline which Davis warns may be "very substantial".

James N. Pappas, a Sanitarian with the U. S. EPA, attacked Davis' estimates of capital operating cost for the hydroxyapatite method. Pappas testified that these costs most likely would be considerably different if Proponents only treated the blow-down from a recycling process and where fluoride concentration was to be reduced to 2.5 mg/l rather than 1 mg/l. He stated that Proponents had not proved that recycling would be required and had failed to provide data relative to marketing of recovered fluorides as a possible cost reduction.

Davis responded (Petitioner's Exhibit #11) by stating that prior testimony had established "that recycling of tailings water in this type of flotation system is not compatible with the flotation system". He admitted that the water could be purified for recycling purposes but added that such a process would probably be more expensive than the hydroxyapatite method because sodium ions and organics would have to be removed. C. B. Rash had testified that recycling adversely affected the efficiency of the flotation process (R. 41). Davis added that recycling efforts at the Colorado plant were not very successful. Solar evaporation ponds were required, which Davis adds, would not be practical in Illinois.

As to the possible sale of recovered fluoride, Davis responded that recovery of acid grade CaF_2 from two million gallons of water would amount to about 240 pounds per day with a market value of about $10.00. He added that he knows of no process from which CaF_2 is recovered in a marketable form and that the whole idea is "a most impractical consideration".

In a letter dated May 16, 1974 Chris Potos, Chief of Water Quality Standards, U. S. EPA, suggested several possible methods of treatment which, in his opinion, raised doubts regarding the claim of economic hardship. Responding to the concern that during periods of low flow the water quality standard of 1.4 mg/l could be violated by an effluent which would be acceptable during periods of normal flow, Potos suggests that retention basins or lagoons could be utilized to store mine waters until sufficient flows upstream are available to allow release of mine waters without contravention of water quality standards. Potos hastens to add that the U. S. EPA does not necessarily recommend such a solution but merely raises the question "as to consideration of alternatives".

Other alternatives suggested by Potos included relocation of mills to sites near the Saline or Ohio Rivers and transmission of mill waste water from existing sites to the larger receiving streams.

Petitioner's Exhibit #11 was of particular concern to Potos. He questions whether generalized cost figures are applicable for specific projects. He states that treatment costs for reducing fluoride in mill tailings from 5 mg/l at the Minerva Mine #1 Mill to 2.5 mg/l at 580,000 gpd would probably be different than the cost of reducing fluoride in mill tailings from 10 mg/l to 2.5 mg/l at Ozark-Mahoning's Rosiclare Mill at 980,000 gpd. Further reduction of fluoride to 1 mg/l could amount to 90% of the total treatment cost according to Potos.

In his statement of treatment cost, Davis assumed that mine water flows from the Oxford, North Green and West Green Mines were 650,000 gpd. Potos states that Federal NPDES files show the flows to be only about 116,000 gpd. U. S. EPA files containing this information were not made a part of the record.

The Davis estimate also cited a 650,000 gpd flow to the "Barnett area waste treatment plant" from the Parkinson Mine and the Barnett air shaft. As Potos points out, Petitioner's Exhibit #13 shows flows from the Parkinson Mine, Barnett Mine and Barnett air shaft as 187,200 gpd.

If we were dealing with another type of industry it would be a simple matter at this point to combine the flows each proposed plant was to receive. These figures would show that the two proposed 650,000 gpd plants are substantially larger than required thus showing that the estimates of cost are overstated.

However, this industry must contend with substantial changes in mine discharges. In their Supplemental Submission Petitioners insist that a plant capacity of 650,000 gpd is necessary. Assuming for purposes of argument that it were both possible and practical to combine mine discharges from several mines at one (or more) location, Petitioner states that history would show the inability of the fluorspar industry, or anyone else for that matter, to anticipate increases in mine water as new veins are mined and new faces opened. For examples of the above, Petitioner cites the current discharge from Ozark-Mahoning W. L. Davis Mine which is now three times larger than the original discharge level. Minerva's older Gaskins Mine has a 1,260,000 gpd discharge as opposed to the 115,000 gpd discharge from the new Spivey Mine. Another example is the Crystal Mill facility which has a current discharge of 75,000 gpd during intermittent operations. If both the heavy media separation and flotation mill were placed into operation, this discharge would increase to as much as 480,000 gpd. Thus, Petitioners argue, it would be sheer folly to construct a treatment plant based on current operating requirements when these requirements might increase two, three, or more times in the months and years to come.

The basic premise necessary for such regional treatment plants is that the discharge flows from several points must be combined. Petitioner's concede that a project of this type might be accomplished if reasonableness and ability to finance the project were not to be considered.

Hurdles to be overcome by Petitioners in such a project are numerous and varied. Petitioners would have to commit finances covering the cost of land, easements, pipelines, electrical distribution lines, storage facilities, buildings, labor and maintenance for a theoretical process without any reasonable assurance that compliance would be achieved.

Pipelines and electrical distribution lines would have to cross land in the Shawnee National Forest. Petitioners state that past experiences considered, the U. S. Forest Service would be reluctant and probably unwilling to issue the permits necessary for such a project.

Petitioners also believe the concept of ponding or lagooning mine discharge is not a feasible alternative. Of the 15 discharge points from Petitioners mines and mills, one flows to the Ohio River and the remaining 14 flow to streams classified as intermittent streams. These discharge points are widely separated in the rock and hill terrain of Hardin and Pope Counties making centralization or combining of discharges impracticable. Numerous small treatment plants would have to be built. Petitioners state that 10 of the 15 discharges are currently in violation of the effluent or water quality standards.

As an example of the problems to be encountered if the ponding concept were implemented, Petitioners cite the following estimated cost for impoundment of discharge water from the Gaskins Mine for a 90 day period:

Total discharge for period = 113,400,000 gallons
Estimated evaporation = 22,000,000 gallons
Volume to be retained = 91,400,000 gallons
Requires a 60 acre pond with average depth of 4.67 feet.
Estimate need to purchase or lease 180 acres for pond site.
Levee requires two feet of freeboard - 6.67 feet levee height.
Requires moving approximately 31,000 cubic yards of dirt.

Cost:

Building levee at 60¢ per yard = $ 18,400
180 acres of land at $300/acre = 54,000
Cost of pipeline and pumps = 55,000
Major expense total = $127,000

In addition to the above estimated cost Petitioners would incur fees of $200 per acre for land leased from the U. S. Forest Service (assuming such leases could be arranged) as well as cost for seed and fertilizer, pipeline right-of-way and maintenance.

However, Petitioner states that the major problem in ponding is that they are simply unaware of any land in the area suitable for ponds or lagoons.

One alternative available to Petitioner is to pump the discharge waters from Gaskin's Mine to the Ohio River, a distance of 7 miles. This project would require a 10" pipe, 40,000 feet long, costing $320,000 according to Petitioner's estimates. Estimated total cost of this alternative including right-of-way, survey costs, legal fees, leases, piping, pumps and installation is in excess of $420,000.

A second alternative would be to pump the Gaskin's Mine discharge to a central treatment plant serving all Minerva discharges. If this central plant were located at the Minerva Mill, the cost of pipe alone for the 15 mile project would be in excess of $600,000 at $8 per foot. Petitioners believe that a project of this magnitude would take longer than the reamining productive life of the Gaskin's Mine.

Responding to the suggestion that Petitioners consider relocation of mills near the Saline or Ohio Rivers, Petitioners state that they have no way of estimating the cost of such a project and that the project would be comparable in difficulty to relocating the Sears Tower.

The Board feels that Petitioners have shown that the many alternatives suggested are not practicable or economically feasible solutions to this complex problem. Hillsides blighted with pipelines and electrical power lines, especially in a national forest, makes these alternatives particularly displeasing from an aesthetic viewpoint in addition to the other drawbacks.

Olin's fluoride problem, as earlier noted, is substantially different from that of Ozark-Mahoning, Minerva or Allied Chemical. Nicholas J. Barone testified that Olin had investigated numerous fluoride removal techniques which were found to be unacceptable from an economic consideration. Olin's corporate engineering department devoted the efforts of some 50 people over a period of years on scaling up laboratory data to a full-scale operation intended for purchase and installation if the effluent standard was not changed.

Waste water from Olin's plant contains phosphate in proportions which enhance utilization of the lime process. Barone testified that the Olin fluoride removal process requires a ratio of phosphate to fluoride of 20 to 1 or greater or the process will fail to achieve the desired reduction (R. 232). An excess of lime of about 200% over stoichiometric is required to reach 2.5 mg/l fluoride.

The Olin process will require a capital investment of $1.4 million and annual operating costs are estimated to be $450,000 (R. 238). When operating, the Olin process will require 7 tons of lime and 28 tons of phosphate per day to treat the 1200 gpm waste water flow. About 70,000 lbs. of 35% solid sludge per day will be generated which will either be impounded or hauled to a landfill. Sludge disposal will cost an estimated $80,000 to $90,000 a year exclusive of land requirement cost (R. 240). Weighed against these factors will be the removal of an estimated 100 to 200 lbs. per day of fluoride (R. 288). Even with these process disadvantages, Olin believes it has a significant economic advantage over the other parties in this matter because of the phosphate content of its waste water. The other parties would have to add phosphate to their waste water to make them treatable. Barone estimated that phosphate addition would increase operating cost by an additional 10 to 20% (R. 257).

The U. S. EPA's criteria for best practicable treatment of fertilizer industry effluent calls for achieving 15 ppm fluoride or a maximum of 30 mg/l fluoride for any 24 hour period (R. 243). The U. S. EPA's best available technology for the steel industry calls for reduction to levels of 4.2 to 8.3 mg/l fluoride on a 30-day average and 10 to 20 mg/l as maximum allowable for a 24-hour period (R. 246).

However, if the effluent standard were changed to Olin's proposed level of 10 mg/l, Olin could reach this level through "in-process controls" (i.e. pump leakage control, recycling, etc.). Fluoride in Olin's waste water comes in large part from leakage from over 800 pumps at the Blockson Works (R. 241). Barone testified that the reduction to 10 mg/l is a "very reliable number" (R. 269) based on actual experience at the plant (R. 254). Obviously the cost for in-process control would be far cheaper than installation and operation of a lime treatment process.

Allied Chemical's Metropolis plant effluent currently contains about 410 mg/l fluoride which is equivalent to a discharge of 7,000 lbs. per day fluoride (R. 375). Richard J. Sobel, Director of Environmental and Process Technology for Allied's Special Chemicals Division, testified that it is Allied's belief that technology is available to achieve 15 mg/l fluoride levels in the presence of calcium (R. 370). Allied is committed to a program aimed at an over-all level of 7 mg/l fluoride in the Metropolis plant effluent (R. 370).

Allied presented testimony in 1971 when the Board was considering the fluoride effluent standard. A. J. von Frank, Allied's Director of Air and Water Pollution Control, testified that it was a practical impossibility to achieve a fluoride level of less than 8.3 mg/l. This level represents the theoretical minimum that can be achieved in a water solution of calcium fluoride from the conventional lime method of fluoride removal (R. 371).

Sobel testified that Allied began a search of technical literature and an intensive in-house development program immediately after the Board adopted the 2.5 mg/l standard. This effort was directed toward discovery of a technically feasible and economically reasonable method of achieving the 2.5 mg/l standard. After two years of research and thousands of manhours, Allied concluded that there was no such method available.

Allied sought and was granted a variance from the fluoride effluent standard (and others) on February 28, 1974 upon satisfying the Board that it was diligently working on fluoride abatement technology. Sobel testified that the abatement program approved in that variance will require about two years for completion at a cost in excess of $4 million (R. 375). Research on fluoride removal technology will continue during the two year period.

Allied Chemical estimates that it would remove 6,880 lbs. per day of fluoride to achieve 7 mg/l. The capital investment for doing this would be $2,683,200 and the operating costs would be $660,000 per year. If the control equipment had a life expectancy of 10 years then capital costs would be approximately $.107 per lb. of fluoride removed. Operating costs would be approximately $0.26 per lb. of fluoride removed.

If Allied Chemical then used the most promising and technically feasible method to achieve 4.1 mg/l fluoride (filtration) an additional 33 lbs. of fluoride per day would be removed at a capital cost of $220,110 ($1.83 per lb. over a 10 year period) and an operating cost of $73,000 per year (R. 377). If Allied then used a fixed alumina bed process to reach 2.5 mg/l, an additional 25 lbs. of fluoride per day would be removed at a capital cost of $330,000 ($3.62 per lb. over a 10 year period) and operating cost of $99,000 per year (R. 378).

If the life expectancy of the abatement equipment is 10 years Allied Chemical would have capital costs of $0.127 per lb. of fluoride removed. If the life expectancy of the equipment is 20 years then the capital costs for fluoride removal would be just $0.064 per lb. The claim of Allied Chemical that capital costs would amount to $9,480 per lb. per day is absurd. Allied's mistake was in failing to allocate the cost of the plant over the entire life expectancy. It seems obvious that the entire cost of the capital outlays should not be assigned to the first day of operation. The other companies which were participating in the hearings did not make this same mistake, but Allied Chemical made the mistake for them. (See Appendix A attached to Allied's final position paper). For instance, Allied claimed that capital costs for Ozark-Mahoning would amount to $11,110 per pound of fluoride removed, apparently assigning a life expectancy of only one day for that proposed facility. Franklin Davis, the designer of the proposed Ozark-Mahoning system indicated that it would have a life expectancy of 20 years. Over a 20-year period the Ozark-Mahoning capital costs per pound of fluoride removed would be around $1.50.

Allied Chemical did not tell us what the useful life of its control equipment will actually be. We doubt that the equipment installed at the Allied plant would have a life expectancy of 20 years. The U. S. EPA allows a depreciation factor of 10 years, and we have already noted that capital costs over a 10-year period would be less than $.13 per pound of fluoride removed.

The Internal Revenue Code allows companies to take depreciation deductions for pollution control facilities over a five year period instead of the "estimated useful life" of the equipment. This practice inflates the cost figures attributable to the equipment during the period of depreciation, a fact Allied Chemical readily concedes. However, such costs could not under any acceptable accounting practice reach $9,480 per pound.

Proponents mine water discharges do not appear in danger of violating the Mine Related effluent criteria of 8 mg/l. No testimony relating to the Mine Related Pollution Control Regulation was presented by proponents.

A remaining problem unique to Ozark-Mahoning and Minerva comes about as a result of mine discharges. Proponents contend that Rule 302(k) of the Water Pollution Control Regulations "proceeds to designate" as Secondary Contact and Indigenous Aquatic Life Waters "all waters in which, by reason of low flow or other conditions, a diversified aquatic biota cannot be satisfactorily maintained even in the absence of contaminants".

Rule 302(k) (As amended February 14, 1974) states:

> "Secondary Contact and Indigenous Aquatic Life Waters"
>
> Secondary contact and indigenous aquatic life waters are those waters which will be appropriate for all secondary contact uses and which will be capable of supporting an indigenous aquatic life limited only by the physical configuration of the body of water, characteristics and origin of the water, and the presence of contaminants in amounts that do not exceed the applicable standards.
>
> The following are designated as secondary contact and indigenous aquatic life waters;
>
> (k) All waters in which by reason of low flow or other conditions, a diversified aquatic biota cannot be satisfactorily maintained even in the absence of contaminants."

In its Opinion on this matter the Board stated:

> "Part III contains water use designations. All waters are designated for general use except those in the restricted category, which has here been broadened in response to testimony to include waters whose flow is too low to support aquatic life. This should relieve the burden of treatment beyond the effluent standards

> for discharges to intermittent streams. Such extra effort is difficult to justify when it will not result in a satisfactory aquatic life because of insufficient flow." (Vol. 3, p. 765).

The request of the mining companies that certain waters be designated "Secondary Contact and Indigenous Aquatic Life Waters" is important, because such designation would substantially increase the allowable fluoride levels in the stream.

Rule 402 of the Water Pollution Regulations provides:

> "In addition to the other requirements of this Part, no effluent shall, alone or in combination with other sources, cause a violation of any applicable water quality standard. When the Agency finds that a discharge that would comply with effluent standards contained in this Chapter would cause or is causing a violation of water quality standards, the Agency shall take appropriate action under Section 31 or Section 39 of the Act to require the discharge to meet whatever effluent limits are necessary to ensure compliance with the water quality standards. When such a violation is caused by the cumulative effect of more than one source, several sources may be joined in an enforcement or variance proceeding, and measures for necessary effluent reductions will be determined on the basis of technical feasibility, economic reasonableness, and fairness to all discharges."

Therefore, if we adopt an effluent standard of 15 mg/l, the discharges must meet that effluent standard and also must not cause a violation of the Water Quality Standard beyond the mixing zone. The mining companies could meet a Water Quality Standard of 5 mg/l fluoride.

If on the other hand, the water quality standards were held at the present 1.4 mg/l criteria while the effluent standard is changed to 15 mg/l, the mining companies would still have a problem during periods of low flow when effluent from the mines is proportionately a larger part of the stream. Several alternatives would have to be considered by the mining companies:

1. The mining companies could petition to have the stream declared a "Secondary Contact and Indigenous Aquatic Life Water" under Rule 302(k). Water so designated would have a water quality standard identical to the new 15 mg/l effluent standard (See Rule 205).

2. Ponding--This concept has already been discussed and found to be impracticable for the mining companies.

3. Treat the effluent down to the water quality standard of 1.4 mg/l. This alternative would cause undue hardship on the mining companies.

4. Variance--This is available only on a temporary basis while permanent solutions to the problem are brought into play.

The record for reclassification of the streams is woefully inadequate. While numerous streams are known to be receivers of the mine water discharges, proponents sole presentation on the issue is a copy of an Agency report on biological samples taken on Big Grand Pierre Creek. As earlier noted, results of this survey indicate well-balanced benthic invertebrate populations both upstream and downstream from the mine discharge. One stream was found to be "semi-polluted".

If the Board were to act at this time on the information presented, the obvious decision would be to deny the "secondary contact" classification. However, the Board feels that no decision is required at this time on the Rule 302(k) matter simply because Rule 302(k) was not adequately addressed as an issue during these proceedings. Our ruling does not preclude Proponents from raising the Rule 302(k) issue at some later date. Our decision only relates to the inadequacy of the record now before the Board on that matter.

It is the Board's finding that Proponents, with the aid of Olin and Allied, have presented proof sufficient to warrant changing the fluoride effluent limit from 2.5 mg/l to 15 mg/l. Effluent of that quality should be acceptable in Illinois waters. The Water Quality Standard for fluoride remains unchanged at 1.4 mg/l for all dischargers other than the fluorspar mining and concentrating industry. The Water Quality Standard becomes 5 mg/l fluoride in waters which receive effluent from the mines and mills of the fluorspar mining and concentrating industry, and have been designated by the Illinois State Water Survey as streams which once in 10 years have an average minimum seven day low flow of zero.

Throughout these proceedings some degree of importance was attached to information in the Illinois EPA's Public Water Supplies Data Book, July 1973. In that document, fluoride levels in drinking water as high as 7.7 mg/l fluoride for Bureau Junction and 5.8 mg/l for Parkersberg are shown. Proponents state that they are not aware of any Agency initiated proceedings, enforcement or otherwise, because of the fluoride level in these public water supplies. However no evidence was introduced regarding the impact of these fluoride levels in these communities, and we certainly do not infer from the lack of legal action that 5.8 mg/l - 6.6 mg/l is an appropriate standard for the entire state.

It is the responsibility of this Board, as charged by the Environmental Protection Act, to protect the quality of the environment. Having reviewed all aspects of these proceedings, the Board feels that an increase in the general water quality standard for streams receiving fluoride containing discharges from the fluorspar mining and concentrating industry, without change for other streams in the State, would not create significant and unwarranted effects on the environment. Unrefuted testimony and evidence in the record shows that no apparent environmental damage has occurred in these streams because of continuous mine discharges over a number of years.

In raising the water quality standard and the effluent limitation for fluoride, the Board has carefully taken into consideration the expected impact upon the receiving streams and the economic impact of the Regulation. Ozark-Mahoning and Minerva will receive relief for operation of their mines and concentrating mills. Ozark-Mahoning's current discharge level of 10 mg/l is below the new effluent limit and should not require any additional treatment barring a major process upset. Minerva, on the other hand, discharges water from its Mill #1 and Crystal Mill that are well within the 15 mg/l limit. Thus, Minerva will not be required to provide any additional fluoride control treatment unless process changes cause the fluoride concentration to increase significantly above the current concentrations.

In those instances where Proponent's mines discharge to flowing streams, current effluent levels appear to be low enough to preclude violation of the 15 mg/l effluent criteria. A different situation confronts Proponents when and if their mine discharges go to dry or intermittent streams. For the most part, mine discharges are well below the new 5 mg/l water quality standard for such streams. Pond #3 of Minerva's Mine #1 and Mill now average 4.5 mg/l and Minerva will have to monitor this discharge closely to insure that this discharge does not violate the new standard. With proper chemical treatment Minerva should be able to maintain this discharge concentration within the new limits.

Increasing the effluent limit to 15 mg/l will provide significant relief for Olin since that level can be reached by implementing "in process controls". In process controls, according to Barone's testimony, will involve some repiping, recycling of certain waste streams, elimination of chronic leaks and possibly some equipment modifications or replacement. Barone testified that Olin considered in process controls to be "a very attractive thing" since the operating costs would be so low as to not even show up as a separate cost (R. 242).

Through the recycling effort Olin would actually receive some benefit since phosphate materials now being discharged would be recovered and end up as product instead of waste. Olin did not have any cost figures relating to in process control but Barone testified that the capital investment would be "much lower" than installing a lime treatment plant (R. 242).

This change in effluent criteria for fluoride affects Allied differently since the current fluoride concentration in Allied's effluent is significantly higher than that of any other party in this matter.

At one time in these proceedings Allied sought to change the effluent standard to allow 15 mg/l fluoride based on the average of 24 hour composite analysis for thirty consecutive days and 30 mg/l maximum for any one 24 hour composite. In its last submission Allied states that its recommended standard of 30 mg/l for any one 24 hour composite may prove to be too restrictive for some industries such as hydrofluoric acid manufacturers. Allied now seeks to change the effluent limit to 30 mg/l as the average of 24 hour composites for 30 consecutive days and 60 mg/l for any one 24 hour period.

Allied's original recommendation was based upon criteria published in Volume 39, No. 49 of the Federal Register on March 12, 1974 by the U. S. Environmental Protection Agency. The reasonableness of this U. S. EPA criteria was challenged by the hydrofluoric acid manufacturers in the Fourth Circuit Court of Appeal. One result of this action, according to Allied, is that the U. S. EPA now plans to revise the fluoride effluent limitations to the same limits Allied now seeks in this matter. Although the U. S. EPA has not yet proposed any new limits, Allied states that Region VI of the U. S. EPA granted Allied a permit for its Baton Rouge Works on December 9, 1974 using the new limit.

Allied is now committed to a fluoride reduction program designed to achieve a fluoride concentration in its effluent of 7 mg/l. Undoubtedly, Allied will modify this program to meet the fluoride level now permitted and we would expect this modification to reduce cost.

Having considered all information in this record concerning the technical feasibility and economic reasonableness of alternative methods of fluoride abatement in conjunction with the data from a commercial lime treatment facility now in operation at another Allied facility it is our finding that the 15 mg/l fluoride is both economically reasonable and technically feasible when applied to Allied Chemical.

ORDER

It is the Order of the Pollution Control Board that the Water Quality Standards and the Effluent Standards of the Illinois Water Pollution Control Regulations be amended to specify the following limitations for fluoride:

PART II WATER QUALITY STANDARDS

203.1 Exceptions to Rule 203

(a) The fluoride standard of Rule 203(f) shall not apply to waters of the State which:

(1) receive effluent from the mines and mills of the fluorspar mining and concentrating industry, and

(2) have been designated by the Illinois State Water Survey as streams which once in ten years have an average minimum seven day low flow of zero.

Such waters shall meet the following standard with regard to fluoride:

Constituent	Storet Number	Concentration (mg/l)
Fluoride	00950	5

PART IV EFFLUENT STANDARDS

408 - Additional Contaminants

(a) The following levels of contaminants shall not be exceeded by any effluent:

Constituent	Storet Number	Concentration (mg/l)
Fluoride (total)	00951	15

I, Christan L. Moffett, Clerk of the Illinois Pollution Control Board, hereby certify the above Opinion and Order was adopted this 6th day of March, 1975 by a vote of 4 to 0.

Christan L Moffett

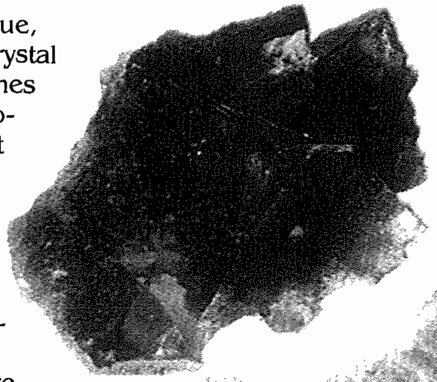
Attachment 5

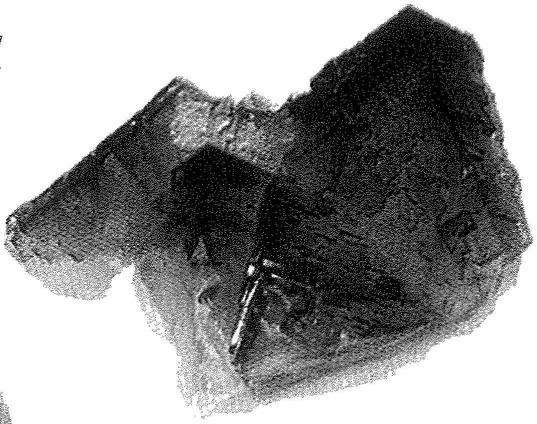
Information from the Illinois State Geological Survey

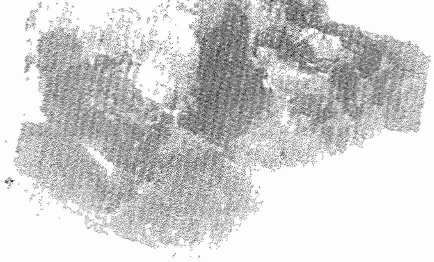
Illinois State Geological Survey

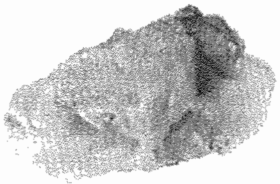
Geobit 4

Fluorite—Illinois' State Mineral









Deep purple, amethyst, sky blue, sea green, sunny yellow, and crystal clear—the mineral fluorite comes in all colors. Many types of fluorite even glow under ultraviolet light. They're "fluorescent."

Pure fluorite (CaF_2), made of the elements calcium (Ca) and fluorine (F), is colorless. The various colors result from tiny amounts of other elements substituting for the calcium in the crystalline structure.

Transparent to translucent, this glass-like mineral may be found as irregular masses filling veins that cut through rocks, or in flat-lying bands or layers parallel with the bedding planes of sedimentary rocks. As the photos show, fluorite also forms as clusters of beautiful cubic crystals.

Light reflects strongly from fluorite's crystal faces and cleavage surfaces, which can be polished to a high luster. As lovely as a gemstone, fluorite is brittle and relatively soft (4 on Moh's hardness scale), so it's unsuitable for ring settings. Brooches and pendants must be handled carefully to avoid scratching or fracturing the mineral specimens in these settings.

Just for display, miners chipped octahedrons out of coarse crystals of the mineral known to the mining industry as *fluorspar*. They called the eight-sided crystals "diamonds."

How did Illinois' fluorite deposits form?

Hot water containing fluorine and other dissolved chemicals rose from deep in the earth during the Jurassic Period, about 150 to 200 million years ago. The water flowed through northeast-trending faults and fractures in limestones laid down earlier in the Mississippian Period, about 330 million years ago.

When the hot brines reached the calcium-rich Mississippian rocks, the temperature and other conditions were just right for crystallizing fluorite along the walls of the faults and in flat-lying layers parallel to the beds of limestone. These host rocks dissolved and were replaced with the fluorite.

Country's leading producer of fluorspar

Since the early 1800s, fluorite has been mined in southeastern Illinois. The fluorspar-rich region, which reaches from southeastern Illinois into parts of Kentucky, was called the Illinois–Kentucky Fluorspar Mining District.

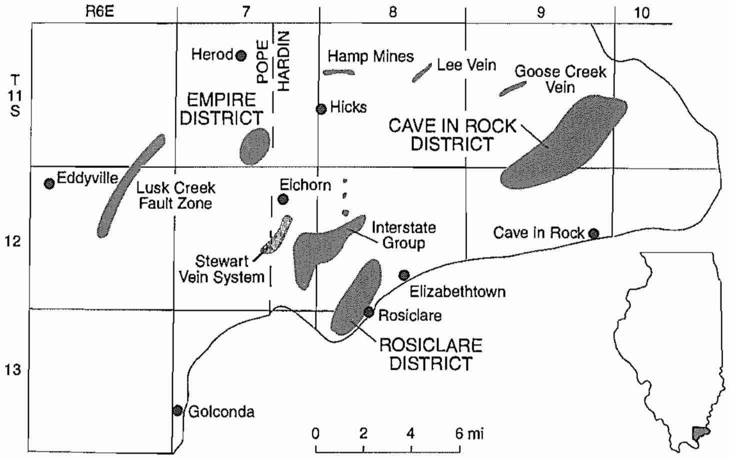
In Illinois, fluorite was mined almost exclusively in Hardin and Pope Counties. The main production came from fissure-vein deposits in the Rosiclare district, and stratiform (bedding plane) deposits in the Cave in Rock district (*map, p. 2*). Other areas in the two counties yielded smaller amounts of the mineral.

Most mining was underground, as much as 1,300 feet deep. But open-pit mines operated where fluorite deposits intersected land surface.

Illinois displaced Kentucky as the country's leading producer of fluorite in 1942. For many years, Illinois accounted for more than 50% of total U.S. fluorspar production. But by 1990, more than 90% of the fluorite used in the U.S. was imported. Illinois was the only remaining domestic producer.

Competition from foreign producers coupled with high costs of underground operations made Illinois' fluorspar mining unprofitable. The last fluorspar mine in Illinois closed in December 1995. Fluorspar is no longer mined anywhere in the United States.

Illinois' State Mineral The General Assembly made fluorite the State Mineral in 1965, when fluorspar mining was a multimillion-dollar-per-year industry in Illinois. Over the years, much more fluorite has been mined in Illinois than in any other state.



Principal mining areas in the southeastern Illinois part of the Illinois–Kentucky Fluorspar Mining District.

The many uses for fluorite

Native Americans carved fluorspar to make artifacts, but the first recorded use of Illinois' fluorite was in 1823, when fluorspar mined near Shawneetown in Gallatin County was used to manufacture hydrofluoric acid.

The mineral, fluorite, is vital to the nation's economy. Its uses:

Mineral

- smelting iron, aluminum, and other metal alloys,
- manufacturing glass, enamel glazes, ceramics, portland cement, and many chemical compounds.

Hydrofluoric acid

- refining aluminum,
- refining uranium fuel for nuclear reactors,
- making rocket fuel and metal plating.

Inorganic fluoride chemicals

- toothpastes, special fluxes for welding rods, optical lenses, and concrete hardeners.

Organic fluoride chemicals

- Plastics, refrigerants, nonstick coatings, lubricants, stain repellents, dyes, herbicides, medicines and anesthetics, cleaning solvents, degreasing agents and foaming agents.

One of the most widely used organic fluoride compounds, the refrigerant Freon 12®, is no longer produced in the United States. The chlorine in the compound is thought to damage the protective ozone layer that shields the earth from ultraviolet radiation.

Contributed by D.L. Reinertsen and J.M. Masters

ILLINOIS STATE GEOLOGICAL SURVEY
615 East Peabody Drive
Champaign, IL 61820-6964
217/333-4747 FAX 217/244-7004

ILLINOIS
DEPARTMENT OF NATURAL RESOURCES

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Attachment 6

Great Lakes Environmental Commission Final Report
(October 22, 2010)
(Excerpts pertaining to boron, manganese
and fluoride tests only)

Final Report on Acute and Chronic Toxicity of Nitrate, Nitrite, Boron, Manganese, Fluoride, Chloride and Sulfate to Several Aquatic Animal Species

Prepared for:

United States Environmental Protection Agency
Office of Science and Technology
Health and Ecological Criteria Division

EPA Contract: EP-C-09-001
Work Assignments: B-12 and 1-12
Contacts: Tom Poleck and Brian Thompson

Prepared by:

GLEC

Great Lakes Environmental Center
739 Hastings Street
Traverse City, Michigan 49686
Phone: (231) 941-2230
Facsimile: (231) 941-2240
Contacts: Jamie Saxton and Mailee Garton

SUBMISSION DATE: October 22, 2010

Boron

Table 60 provides a summary of estimated LC_{50} values for the nine toxicity tests performed using boron. LC_{50} values ranged between 28.4 and >544 mg B/L.

Table 60. LC_{50} estimates for toxicity tests performed using boron.

Test Species and Duration	LC_{50} (mg B/L)
Lampsilis siliquoidea 96 hr	137
Pimephales promelas - 96 hr	101
Pimephales promelas - 32 day	28.4
Ceriodaphnia dubia - 48 hr (pH 7.75)	76.9
Pimephales promelas - 96 hr (pH 6.75)	70.6
Pimephales promelas - 96 hr (pH 7.75)	137
Pimephales promelas - 96 hr (pH 8.75)	133
Ligumia recta - 96 hr	147
Megalonaias nervosa - 96 hr	> 544

For each of the acute toxicity tests completed using boron, two tables were generated: the first summarizes the test results for each toxicity test, including nominal and analytical test concentration and LC_{50} estimates with confidence intervals; the second table summarizes analytical chemistry data collected throughout the toxicity tests. The results of chronic tests performed with boron were summarized in three tables: the first summarizes nominal and analytical test concentrations, LC_{50} estimates with confidence intervals, NOEC and LOEC estimates, mean survival and mean biomass; the second table summarizes replicate-specific survival and growth data and the third table summarizes analytical chemistry data collected throughout the toxicity tests. Also discussed, if applicable, are deviations from the guidance provided in the ASTM method used to complete the toxicity testing.

96-hr Toxicity of Boron on Lampsilis siliquoidea

The 96-hr test to determine the toxicity of boron on *L. siliquoidea* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Missouri State University laboratory culture, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 500, 250, 125, 62.5, and 31.3 mg B/L.

Testing was conducted at 20 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 61; test results are provided in Table 62. Analytical chemistry data are provided in Table 63. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 18.

Table 61. Test conditions for 96-hour toxicity test on *Lampsilis siliquoidea* with boron.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Lampsilis siliquoidea,* juveniles <5 days old (Missouri State University)
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	June 03 - 07, 2009
4. Test Temperature (°C):	20 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	50 mL beaker
9. Volume of Test Solutions:	40 mL
10. No. of Test Organisms per Test Vessel:	5
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	20
13. Test Concentrations (mg B/L):	500, 250, 125, 62.5, and 31.3
14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg B/L):	524, 260, 140, 72, and 34
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	USEPA MHRW
17. Test Material:	Boric acid: Acros Organics, 99.6%, ACS Reagent (crystals) Cas. No. 10043-35-3, Lot # B0124654 and Borax (sodium tetraborate decahydrate), 99.5 +% (for analysis ACS), Cas. No. 1303-96-5, Lot # A0256722
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 62. Test results for 96-hour toxicity test on *Lampsilis siliquoidea* with boron.

Results of a *Lampsilis siliquoidea* 96-Hour Static Acute Toxicity Test

Conducted 06/03/09 - 06/07/09 Using: Boric acid Cas. No. 10043-35-3 and Borax Cas. No. 1303-96-5

Nominal (Measured) Concentrations	**Cumulative Percent Affected [a]**				**LC$_{50}$ Values* (mg/L)**			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	5	5	5	5	>524	>524	181	137
31.3 (34) mg/L	0	10	25	35	**96-Hour LC$_{50}$* = 137 mg/L**			
62.5 (72) mg/L	0	25	30	35	LC$_{50}$ 95% Confidence Limits			
125 (140) mg/L	10	15	35	45	24-Hr	48-Hr	72-Hr	96-Hr
250 (260) mg/L	15	25	60	90	LL NR UL NR	NR NR	110 296	86 220
500 (524) mg/L	15	20	90	95				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC$_{50}$ and EC$_{50}$ Confidence Limit Values: Spearman-Karber			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC$_{50}$ and EC$_{50}$ values are determined based on measured concentrations.

Table 63. Analytical chemistry data for 96-hour toxicity test on *Lampsilis siliquoidea* with boron.

Nominal (Measured) Test Concentration		Boron[a] (mg/L)	Temperature (°C)	pH (s.u.)	D.O. (mg/L)	Conductivity (µmhos)	Alkalinity (mg/L)	Hardness (mg/L)
Dilution water/Control	Day 0	0.1	20.0	8.0	7.81	305	60	90
	Day 1		19.9					
	Day 2		20.0					
	Day 3		20.1					
	Day 4	1.1	19.9	8.0	7.10	305	62	90
		0.3						
31.3 (34) mg/L	Day 0	35	20.1	8.0	7.80	322	82	90
	Day 1		19.8					
	Day 2		20.0					
	Day 3		20.1					
	Day 4	33	19.9	8.0	7.05	320	82	92
		34						
62.5 (72) mg/L	Day 0	69	20.0	8.0	7.80	344	90	90
	Day 1		19.8					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	76	20.0	8.0	6.99	350	92	92
		72						
125 (140) mg/L	Day 0	130	20.2	8.0	7.92	385	116	90
	Day 1		19.7					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	150	19.9	8.0	6.97	390	120	90
		140						
250 (260) mg/L	Day 0	250	20.0	8.0	7.96	464	164	88
	Day 1		19.9					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	270	19.9	8.0	6.92	465	164	90
		260						
500 (524) mg/L	Day 0	500	20.1	8.1	7.99	619	272	86
	Day 1		20.0					
	Day 2		20.1					
	Day 3		20.1					
	Day 4	550	20.0	8.0	6.89	625	270	90
		524						

[a] Boron Analysis Method 200.7

96-hr Toxicity of Boron on Megalonaias nervosa

The 96-hr test to determine the toxicity of boron on *M. nervosa* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Genoa National Fish Hatchery, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. In one replicate of the 250 mg/L treatment, a test organism was inadvertently crushed, but this was accounted for in the LC_{50} calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 500, 250, 125, 62.5, and 31.3 mg B/L.

Testing was conducted at 20 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 64; test results are provided in Table 65. Analytical chemistry data are provided in Table 66. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 19.

Table 64. Test conditions for 96-hour toxicity test on *Megalonaias nervosa* with boron.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Megalonaias nervosa,* juveniles <5 days old, Genoa National Fish Hatchery
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	October 16 - 20, 2009
4. Test Temperature (°C):	20 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	50 mL beaker
9. Volume of Test Solutions:	40 mL
10. No. of Test Organisms per Test Vessel:	5
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	20
13. Test Concentrations (mg B/L):	500, 250, 125, 62.5, and 31.3
14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg B/L):	544, 275, 140, 74, and 37
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	USEPA MHRW
17. Test Material:	Boric acid: Acros Organics, 99.6%, ACS Reagent (crystals) Cas. No. 10043-35-3, Lot # B0124654 and Borax (sodium tetraborate decahydrate), 99.5 +% (for analysis ACS), Cas. No. 1303-96-5, Lot # A0256722
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 65. Test results for 96-hour toxicity test on *Megalonaias nervosa* with boron.

Results of a *Megalonaias nervosa* 96-Hour Static Acute Toxicity Test

Conducted 10/16/09 - 10/20/09 Using: Boric acid Cas. No. 10043-35-3 and Borax Cas. No. 1303-96-5

Nominal (Measured) Concentrations	**Cumulative Percent Affected [a]**				**LC$_{50}$ Values* (mg/L)**			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0	0	0	0	>544	>544	>544	>544
31.3 (37) mg/L	0	0	0	0	**96-Hour LC$_{50}$* = >544mg/L**			
62.5 (74) mg/L	0	0	5	10	LC$_{50}$ 95% Confidence Limits			
125 (140) mg/L	0	0	5	15	24-Hr	48-Hr	72-Hr	96-Hr
250 (275) mg/L	0	5	10	20	LL NR UL NR	NR NR	NR NR	NR NR
500 (544) mg/L	0	0	0	5				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC$_{50}$ and EC$_{50}$ Confidence Limit Values: Spearman-Karber			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC$_{50}$ and EC$_{50}$ values are determined based on measured concentrations.

Table 66. Analytical chemistry data for 96-hour toxicity test on *Megalonaias nervosa* with boron.

Nominal (Measured) Test Concentration		Boron[a] (mg/L)	Temperature (°C)	pH (s.u.)	D.O. (mg/L)	Conductivity (mmos)	Alkalinity (mg/L)	Hardness (mg/L)
Dilution water/Control	Day 0	<0.02	21.0	7.8	7.95	300	60	88
	Day 1		20.8	7.7	8.27	303		
	Day 2		20.5	7.8	8.21	305		
	Day 3		20.3	8.0	8.21	290		
	Day 4	<0.02	20.6	8.0	7.62	337	68	90
		na						
31.3 (37) mg/L	Day 0	36	21.0	8.0	8.21	320	70	88
	Day 1		20.5	7.9	8.32	320		
	Day 2		20.5	7.9	8.22	320		
	Day 3		20.4	8.0	8.28	330		
	Day 4	38	20.6	8.0	8.15	351	72	88
		37						
62.5 (74) mg/L	Day 0	72	21.0	8.0	8.20	340	86	88
	Day 1		20.8	7.9	8.34	343		
	Day 2		20.5	7.9	8.22	345		
	Day 3		20.3	8.0	8.27	347		
	Day 4	76	20.5	8.0	8.25	364	90	88
		74						
125 (140) mg/L	Day 0	140	21.0	7.9	8.25	381	110	88
	Day 1		20.9	8.0	8.38	389		
	Day 2		20.6	8.0	8.23	390		
	Day 3		20.5	8.1	8.29	401		
	Day 4	140	20.7	8.1	8.27	417	115	88
		140						
250 (275) mg/L	Day 0	270	21.0	7.9	8.23	460	160	88
	Day 1		20.8	8.0	8.40	461		
	Day 2		20.6	8.0	8.21	461		
	Day 3		20.5	8.1	8.25	488		
	Day 4	280	20.8	8.1	8.28	504	178	88
		275						
500 (544) mg/L	Day 0	520	21.0	7.9	8.24	613	266	86
	Day 1		20.9	8.0	8.32	616		
	Day 2		20.6	8.0	8.20	618		
	Day 3		20.4	8.1	8.25	638		
	Day 4	570	20.8	8.2	8.23	654	276	88
		544						

[a] Boron Analysis Method 200.7

na=not applicable

96-hr Toxicity of Boron on Ligumia recta

The 96-hr test to determine the toxicity of boron on *L. recta* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Missouri State University laboratory culture, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. One replicate was mistakenly loaded with only four individuals, but this was accounted for in the LC_{50} calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 500, 250, 125, 62.5, and 31.3 mg B/L.

Testing was conducted at 20 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 67; test results are provided in Table 68. Analytical chemistry data are provided in Table 69. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 20.

Table 67. Test conditions for 96-hour toxicity test on *Ligumia recta* with boron.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Ligumia recta,* juveniles <5 days old, Missouri State University
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	September 10 - 14, 2009
4. Test Temperature (°C):	20 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	50 mL beaker
9. Volume of Test Solutions:	40 mL
10. No. of Test Organisms per Test Vessel:	5
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	20
13. Test Concentrations (mg B/L):	500, 250, 125, 62.5, and 31.3
14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg B/L):	510, 255, 130, 64, and 33
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	USEPA MHRW
17. Test Material:	Boric acid: Acros Organics, 99.6%, ACS Reagent (crystals) Cas. No. 10043-35-3, Lot # B0124654 and Borax (sodium tetraborate decahydrate), 99.5 +% (for analysis ACS), Cas. No. 1303-96-5, Lot # A0256722
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 68. Test results for 96-hour toxicity test on *Ligumia recta* with boron.

Results of a *Ligumia recta* **96-Hour Static Acute Toxicity Test**

Conducted 09/10/09 - 09/14/09 Using: Boric acid Cas. No. 10043-35-3 and Borax Cas. No. 1303-96-5

Nominal (Measured) Concentrations	**Cumulative Percent Affected [a]**				**LC_{50} Values* (mg/L)**			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0	0	0	0	>510	>510	>510	147
31.3 (33) mg/L	0	0	0	30	**96-Hour LC_{50}* = 147 mg/L**			
62.5 (64) mg/L	0	0	0	30	LC_{50} 95% Confidence Limits			
125 (130) mg/L	0	0	0	50	24-Hr	48-Hr	72-Hr	96-Hr
250 (255) mg/L	0	0	0	58	LL NR UL NR	NR NR	NR NR	88 246
500 (510) mg/L	0	0	0	100				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC_{50} and EC_{50} Confidence Limit Values: Spearman-Karber			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC_{50} and EC_{50} values are determined based on measured concentrations.

Table 69. Analytical chemistry data for 96-hour toxicity test on *Ligumia recta* with boron.

Nominal (Measured) Test Concentration		Boron[a] (mg/L)	Temperature (°C)	pH (s.u.)	D.O. (mg/L)	Conductivity (µmhos)	Alkalinity (mg/L)	Hardness (mg/L)
Dilution water/Control	Day 0	<0.02	20.4	7.9	8.18	301	60	92
	Day 1		19.5					
	Day 2		19.1					
	Day 3		19.2					
	Day 4	<0.02	19.2	8.1	8.14	312	60	92
		na						
31.3 (33) mg/L	Day 0	33	20.5	8.0	8.14	320	68	92
	Day 1		19.5					
	Day 2		19.0					
	Day 3		19.3					
	Day 4	34	19.3	8.1	8.10	334	68	92
		33						
62.5 (64) mg/L	Day 0	62	20.5	8.0	8.13	341	90	90
	Day 1		19.5					
	Day 2		19.0					
	Day 3		19.3					
	Day 4	66	19.3	8.1	8.11	353	90	90
		64						
125 (130) mg/L	Day 0	130	20.4	8.0	8.12	382	112	90
	Day 1		19.5					
	Day 2		19.0					
	Day 3		19.3					
	Day 4	130	19.3	8.1	8.05	394	112	90
		130						
250 (255) mg/L	Day 0	250	20.5	8.1	8.12	460	170	90
	Day 1		19.5					
	Day 2		19.2					
	Day 3		19.3					
	Day 4	260	19.3	8.1	8.03	472	170	90
		255						
500 (510) mg/L	Day 0	500	20.5	8.1	8.11	616	270	90
	Day 1		19.5					
	Day 2		19.4					
	Day 3		19.1					
	Day 4	520	19.1	8.1	8.12	634	270	90
		510						

[a] Boron Analysis Method 200.7

na = not applicable

96-hr Toxicity of Boron on Pimephales promelas

The 96-hr test to determine the toxicity of boron on *P. promelas* was completed by GLEC. Test organisms, collected from the GLEC laboratory culture, were acclimated to the dilution water (de-chlorinated Lake Michigan water), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); two replicates were used per treatment with ten organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 38.9, 64.8, 108, 180, 300 and 500 mg B/L.

Testing was conducted at 25 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Probit method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 70; test results are provided in Table 71. Analytical chemistry data are provided in Table 72. Accompanying information, including raw laboratory data, analytical chemistry data, reference toxicant data and statistical analyses, is provided in Appendix 21.

Table 70. Test conditions for 96-hour toxicity test on *Pimephales promelas* with boron.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Pimephales promelas*, (weight 0.12g and 19.8 mm length), GLEC Culture
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	September 2-September 6, 2009
4. Test Temperature (°C):	25 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	4000 mL beaker
9. Volume of Test Solutions:	3500 mL
10. No. of Test Organisms per Test Vessel:	10
11. No. of Test Vessels per Treatment:	2
12. Total No. of Test Organisms per Treatment:	20
13. Target or Nominal Test Concentrations (mg B/L):	500, 300, 180, 108, 64.8, and 38.9
14. Analytical Test Concentrations (average of samples collected at test initiation and termination-mg B/L):	546, 352, 200, 123, 71.5, and 46.1
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	De-chlorinated Lake Michigan Water
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 71. Test results for 96-hour toxicity test on *Pimepehales promelas* with boron.

Results of a *Pimephales promelas* 96-Hour Static Acute Toxicity Test

Conducted 09/02/09 - 09/06/09 Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043 35-3)
(Borax: Sigma Aldrich Cas No. 1303-96-4)

Nominal (Measured) Concentrations	Cumulative Percent Affected [a]			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)
38.9 (46.1) mg/L	0 (0)	0 (0)	0 (0)	0 (0)
64.8 (71.5) mg/L	0 (0)	0 (0)	0 (5)	15 (15)
108 (123) mg/L	0 (0)	0 (0)	20 (20)	70 (70)
180 (200) mg/L	0 (0)	5 (5)	60 (60)	100 (100)
300 (352) mg/L	0 (0)	65 (65)	100 (100)	100 (100)
500 (546) mg/L	45 (45)	100 (100)	100 (100)	100 (100)

LC_{50}* Values (mg/L)			
24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
>546	312	173	101

96-Hour LC_{50} *= 101 mg/L

LC_{50} *95% Confidence Limits			
24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
LL NA	271	150	88.3
UL NA	353	200	116

LL = Lower Limit
UL = Upper Limit
NR = Confidence Intervals are not reliable

Method(s) Used to Determine LC_{50} Confidence Limit Values: Probit

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[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC_{50} values are determined based on measured concentrations.

Table 72. Analytical chemistry data for 96-hour toxicity test on *Pimephales promelas* with boron.

Nominal (and Measured) Test Concentrations		Boron (mg/L)	Temperature (°C)	pH (s.u.)	D.O. (mg/L)		Conductivity (mmos)	Alkalinity (mg/L)	Hardness (mg/L)	Ammonia (mg/L)
Dilution water/Control	Day 0	0.03	25.0	8.01	8.0		290	104	144	ND
	Day 1		24.7	7.73	6.3	6.0				
	Day 2		24.8	7.54	5.9	6.1				
	Day 3		24.8	7.65	5.7	5.5				
	Day 4	0.04	24.7	7.95	6.0	6.1	278	100	132	
		0.035								
38.9 (46.1) mg/L	Day 0	54.7	25.0	8.16	8.0		318			
	Day 1		24.6	8.12	6.7	6.8				
	Day 2		24.8	8.02	6.4	6.5				
	Day 3		24.9	8.05	6.0	5.9				
	Day 4	37.4	24.7	8.07	6.4	6.5	323			
		46.1								
64.8 (71.5) mg/L	Day 0	82	25.0	8.14	7.9		335			
	Day 1		24.7	8.17	7.7	6.7				
	Day 2		24.8	8.05	4.6	6.4				
	Day 3		24.8	8.11	6.3	4.5				
	Day 4	61	24.7	8.02	5.4	6.4	346			
		71.5								
108 (123) mg/L	Day 0	146	25.0	8.18	8.0		362			
	Day 1		24.8	8.18	7.0	6.4				
	Day 2		25.0	8.15	6.5	6.2				
	Day 3		24.9	8.13	5.9	6.4				
	Day 4	99.1	24.8	8.15	6.7	6.0	376			
		123								
180 (200) mg/L	Day 0	223	25.0	8.18	8.0		406			
	Day 1		25.1	8.17	6.2	6.3				
	Day 2		24.9	8.14	6.1	5.8				
	Day 3		24.9	8.11	5.9	4.7				
	Day 4	176	24.8	8.13	5.3	5.3	422	146	136	
		200								
300 (352) mg/L	Day 0	388	25.0	8.17	8.0		476			
	Day 1		24.7	8.17	7.0	6.3				
	Day 2		24.8	8.17	6.8	5.4				
	Day 3		24.8	8.15	5.8	5.0				
	Day 4	316								
		352								
500 (546) mg/L	Day 0	585	25.0	8.14	8.0		597	316	168	ND
	Day 1		24.7	8.15	7.0	7.1				
	Day 2		24.8	8.13	7.4	7.5				
	Day 3									
	Day 4	507								
		546								

[a] Boron Analysis EPA 200.8
ND Not Detect; below detection limit

32-day Toxicity of Boron on Pimephales promelas

The 32-day test to determine the toxicity of boron on *P. promelas* was completed by GLEC. The fish were continuously exposed for 32 days to five concentrations of boron (nominal concentrations of 6.3, 12.5, 25, 50 and 100 mg B/L) and to a dilution water control using a continuous flow-through system (Benoit et al. 1982). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were four replicate test chambers for each treatment. The flow through test was conducted at 25 ± 1 °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with < 24 hour old fertilized embryos. The embryos were randomly assigned to incubation cups until each incubation cup contained 30 embryos. The incubation cups were randomly assigned to the 2.5 L glass test chambers (1 cup per chamber) and suspended in the test solutions from a rocker arm assembly. The rocker arm assembly moves the incubation cups in a reciprocal motion within each test chamber. Embryos were inspected on a daily basis and the number of live, hatched and dead embryos was recorded. On Day 8 of the test (four days after first hatch), the surviving fish were randomly thinned to achieve 20 fish in each test chamber. The remainder of the surviving fish was discarded. The number of surviving fish was recorded at test termination (32 days). In addition, the wet weights were recorded for each fish at test termination. Because of the size range of fish in each test chamber, all of the fish from each test chamber were weighed together to determine average dry weight.

Instantaneous water temperature measurements made on September 12 (Day 2: 23.5°C-24.5°C), October 1 (Day 21: 23.7°C-24.3°C), October 9 (Day 29: 25.9°C-26.5°C), October 11 (Day 31: 23.7°C-24.1°C), and October 12 (Day 32: 23.8°C-24.5°C) exceeded the allowable range of 25 ± 1 °C in the toxicity testing method. However, the overall average water temperatures (across the duration of the test) in each replicate were within ± 0.5 °C of the target test temperature (25 °C) in all treatments. Therefore, the water temperature exceedances noted above likely had no effect on the results of this study.

On September 19 and 20, 2009 (test days 9 and 10) 60-90 percent mortality occurred in replicates one and two of the laboratory control. It is of GLEC's opinion that the equipment used during the thinning procedure on test day 8 contributed toxicity to these two control replicates, resulting in the high fish mortality. This high mortality was communicated to the EPA Work Assignment Manager and GLEC was advised to continue the test with the assumption that further control mortality would result in test failure. No further toxicity was observed in the remaining control fish throughout the test. However, because of the mortality observed in the control treatments, only replicates three and four of the laboratory control were used in the survival and growth statistical comparisons.

Once the test was complete, the LC_{50}, NOEC and LOEC values were determined using the average measured concentrations with the Spearman Karber and ANOVA methods. LC_{25}, LC_{20} and LC_{10} values were determined using the Probit method and EC_{50}, EC_{25}, EC_{20} and EC_{10} values were estimated using EPA's TRAP.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 73; test results are provided in Table 74. Survival and growth data are provided in Table 75 and analytical chemistry data are provided in Table 76. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 22.

Table 73. Test conditions for 32-day toxicity test on *Pimephales promelas* with boron.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Pimephales promelas*, (<24 hour fertilized embryos), GLEC Culture
2. Test Type and Duration:	Continuous flow-through; 32 days
3. Test Dates:	September 10-October 12, 2009
4. Test Temperature (°C):	25 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	Live Brine Shrimp (*Artemia nauplii*) Twice daily
8. Size of Test Vessel:	2.5 Liter glass Tank
9. Volume of Test Solutions:	2000 mL
10. No. of Test Organisms per Test Vessel:	30 eggs, thinned to 20 larvae after hatch
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	120 eggs, thinned to 80 larvae after hatch
13. Target or Nominal Test Concentrations (mg/L):	100, 50.0, 25.0, 12.5 and 6.25 mg/L-boron
14. Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):	112, 56.5, 27.4, 12.9, and 5.90 mg/L-nitrate
15. Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day
16. Dilution and Primary Control Water:	De-Chlorinated Lake Michigan Water
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Survival (LC_{50}, LC_{25}, LC_{20} and LC_{10}, NOEC and LOEC) and Growth (EC_{50}, EC_{25}, EC_{20} and EC_{10}, NOEC and LOEC)

Table 74. Test results for 32-day toxicity test on *Pimepehales promelas* with boron.

Results of a *Pimephales promelas* **32-Day Continuous Flow Chronic Toxicity Test**

Conducted 09/10/09 - 10/12/09 **Using: Boron (Boric Acid: Sigma Aldrich Cas. No. 10043-35-3**
Borax: Sigma Aldrich Cas. No. 1303-96-4)

Test Solution Concentrations Measured	**Primary Control/ Dilution Water**	**5.90 mg/L**	**12.9 mg/L**	**27.4 mg/L**	**56.5 mg/L**	**112 mg/L**
Embryo Percent Hatch (%)	100	100	100	100	100	100
32-Day Mean Survival (%)	97.5	93.8	92.5	58.8[a]	1.3[a]	0[a]
32-Day Average Biomass[1] (mg)	10.1	7.27 [a]	7.37 [a]	4.29[a]	0.06[a]	0[a]

Embryo Percent Hatch NOEC:	100 mg/L	32-Day LC_{20}:	18.6 mg/L (15.9 mg/L – 21.3 mg/L)
32-Day LC_{50}*:	28.4 mg/L (25.5 mg/L-31.7 mg/L)	32-Day LC_{10}:	12.8 mg/L (9.6 mg/L – 16.0 mg/L)
32-Day Survival NOEC:	12.9 mg/L	32-Day EC_{50}:	28.7 mg/L (23.7 mg/L – 34.6 mg/L)
32-Day Survival LOEC:	27.4 mg/L	32-Day EC_{25}:	25.1 mg/L (17.1 mg/L – 36.7 mg/L)
32-Day Growth NOEC:	<5.9 mg/L	32-Day EC_{20}:	24.2 mg/L (14.3 mg/L – 41.0 mg/L)
32-Day Growth LOEC:	5.9 mg/L	32-Day EC_{10}:	21.9 mg/L (8.6 mg/L – 56.0 mg/L)
32-Day LC_{25}:	20.8 mg/L (18.2 mg/L – 23.4 mg/L)		

*: All LC, EC, NOEC and LOEC values are determined based on the average measured boron concentration.

NOEC: No-Observed-Effect-Concentration
LOEC: Lowest-Observed-Effect-Concentration

[1] Biomass: Biomass is the average dry weight of the four replicates calculated by the total dry weight of surviving organisms divided by the initial number of organisms (20).

Table 75. Survival and growth data for 32-day toxicity test on *Pimephales promelas* with boron.

Nominal (and Measured) Test Concentration		Number of Eggs at Test Initiation	Number of Hatched Larvae	Number of Dead Eggs	Percent Hatched Larvae	Number of Larvae at Test Termination	Percent Survival at Test Termination*	Biomass[1] (mg)
Dilution water/Control	Replicate # 1	30	30	0	100.0	1	5.0	1.27
	Replicate # 2	30	30	0	100.0	4	20.0	4.83
	Replicate # 3	30	30	0	100.0	19	95.0	9.62
	Replicate # 4	30	30	0	100.0	20	100.0	10.52
Average					**100.0**		**97.5**[2]	**10.07**[2]
6.25 (5.9) mg/L	Replicate # 1	30	30	0	100.0	17	85.0	6.69
	Replicate # 2	30	30	0	100.0	18	90.0	6.84
	Replicate # 3	30	30	0	100.0	20	100.0	7.34
	Replicate # 4	30	30	0	100.0	20	100.0	8.21
Average					**100.0**		**93.8**	**7.27**
12.5 (12.9) mg/L	Replicate # 1	30	30	0	100.0	17	85.0	6.99
	Replicate # 2	30	30	0	100.0	19	95.0	8.53
	Replicate # 3	30	30	0	100.0	19	95.0	6.77
	Replicate # 4	30	30	0	100.0	19	95.0	7.17
Average					**100.0**		**92.5**	**7.37**
25.0 (27.4) mg/L	Replicate # 1	30	30	0	100.0	10	50.0	3.76
	Replicate # 2	30	30	0	100.0	10	50.0	3.62
	Replicate # 3	30	30	0	100.0	13	65.0	5.05
	Replicate # 4	30	30	0	100.0	14	70.0	4.73
Average					**100.0**		**58.8**	**4.29**
50.0 (56.5) mg/L	Replicate # 1	30	30	0	100.0	0	0.0	0.00
	Replicate # 2	30	30	0	100.0	0	0.0	0.00
	Replicate # 3	30	30	0	100.0	1	5.0	0.25
	Replicate # 4	30	30	0	100.0	0	0.0	0.00
Average					**100.0**		**1.3**	**0.06**
100 (112) mg/L	Replicate # 1	30	30	0	100.0	0	0.0	0.00
	Replicate # 2	30	30	0	100.0	0	0.0	0.00
	Replicate # 3	30	30	0	100.0	0	0.0	0.00
	Replicate # 4	30	30	0	100.0	0	0.0	0.00
Average					**100.0**		**0.0**	**0.00**

* On Day 8 of the test, (four days after first hatch) the surviving fish were randomly thinned to 20 fish in each test chamber. Percent survial at test termination is the number of surviving at test termination divided by 20.

[1] Biomass: Biomass is the total dry weight of surviving organisms divided by the initial number of organisms (20)

[2] Due to a technician error on day 8, only replicates 3 and 4 were used in the growth and survival analysis for the laboratory control

Table 76. Analytical chemistry data for 32-day toxicity test on *Pimephales promelas* with boron.

Nominal (and Measured) Test Conentrations		Boron (mg/L)	Temperature (°C)	pH (s.u.)	DO (mg/L)	SC (mmos)	Hardness (mg/L)	Alkalinity (mg/L)	Ammonia (mg/L)
Dilution Water/Control	Replicate # 1	0.0 (0.0-0.1)	25.1 (23.7-26.0)	8.08	7.5 (7.0-8.1)	290	112	100	ND
	Replicate # 2	0.0 (0.0-0.1)	24.9 (23.8-26.3)	8.11	7.3 (6.8-7.8)	296	132	99	ND
	Replicate # 3	0.1 (0.0-0.4)	24.9 (24.2-26.0)	8.16	7.2 (6.9-7.4)	300	NM	NM	ND
	Replicate # 4	0.0 (0.0-0.1)	24.9 (24.1-25.5)	8.17	7.3 (7.1-7.5)	304	NM	NM	ND
Average Boron mg/L		**0**							
6.25 mg/L (5.9 mg/L)	Replicate # 1	5.8 (4.9-6.6)	24.6 (23.9-25.6)	8.16	7.5 (7.0-7.9)	301	128	106	ND
	Replicate # 2	6.0 (4.9-7.0)	24.9 (24.0-26.1)	8.17	7.3 (6.7 8.0)	305	132	104	ND
	Replicate # 3	6.0 (5.0-7.1)	25.0 (24.1-26.2)	8.21	7.1 (6.6-7.5)	305	NM	NM	ND
	Replicate # 4	5.9 (5.1-6.6)	24.8 (23.9-25.4)	8.18	7.1 (6.7-7.6)	309	NM	NM	ND
Average Boron mg/L		**5.9**							
12.5 mg/L (12.9 mg/L)	Replicate # 1	13.0 (11.6-13.9)	24.8 (23.7-25.9)	8.17	7.4 (6.8-8.1)	307	132	110	ND
	Replicate # 2	13.1 (11.5-14.2)	24.9 (24.1-26.1)	8.20	7.5 (6.9-8.0)	308	130	107	ND
	Replicate # 3	13.1 (11.6-14.7)	25.1 (24.2-26.5)	8.25	7.3 (7.0-7.6)	310	NM	NM	ND
	Replicate # 4	12.3 (11.0-13.8)	25.0 (24.2-25.6)	8.21	7.4 (7.1-7.8)	313	NM	NM	ND
Average Boron mg/L		**12.9**							
25.0 mg/L (27.4 mg/L)	Replicate # 1	28.6 (23.8-34.3)	24.6 (23.7-25.7)	8.18	7.5 (7.1-8.0)	314	128	116	ND
	Replicate # 2	28.0 (25.1-32.5)	24.7 (23.8-26.2)	8.21	7.5 (7.2-8.0)	316	128	113	ND
	Replicate # 3	26.2 (22.7-31.1)	24.8 (23.8-26.1)	8.12	7.4 (7.1-7.8)	317	NM	NM	ND
	Replicate # 4	26.3 (22.9-31.2)	24.5 (23.5-25.2)	8.22	7.4 (7.1-7.7)	320	NM	NM	ND
Average Boron mg/L		**27.4**							
50.0 mg/L (56.5 mg/L)	Replicate # 1	56.1 (48.2-65.4)	24.8 (23.9-25.7)	8.21	7.6 (7.1-8.5)	331	132	128	ND
	Replicate # 2	56.4 (46.9-63.3)	24.7 (24.1-26.0)	8.23	7.5 (7.2-8.1)	333	130	127	ND
	Replicate # 3	57.4 (45.8-67.9)	24.8 (23.9-26.0)	8.27	7.5 (7.1-8.3)	351	NM	NM	ND
	Replicate # 4	56.2 (45.3-63.4)	24.8 (23.9-25.4)	8.24	7.5 (7.3-8.0)	337	NM	NM	ND
Average Boron mg/L		**56.5**							
100 mg/L (112 mg/L)	Replicate # 1	111 (98.9-117)	24.8 (23.6-26.0)	8.22	7.6 (7.0-8.4)	363	128	152	ND
	Replicate # 2	111 (93.7-117)	25.0 (23.9-26.2)	8.23	7.6 (7.2-8.4)	367	130	150	ND
	Replicate # 3	111 (97.2-122)	25.0 (24.4-26.2)	8.27	7.4 (7.0-8.0)	367	NM	NM	ND
	Replicate # 4	113 (103-123)	24.9 (24.0-25.6)	8.24	7.6 (7.3-8.0)	370	NM	NM	ND
Average Boron mg/L		**112**							

DO: Dissolved Oxygen
SC: Specific Conductance

ND: Non Detect; below detection limit.
NM: Not Measured

48-hr Toxicity of Boron (pH 7.75) on Ceriodaphnia dubia

The 48-hr test to determine the toxicity of boron (pH 7.75) on *C. dubia* was completed by GLEC. The *C. dubia* were continuously exposed for 48-hours to five concentrations of boron and to a dilution water control with a target test pH of 7.75 (range of 7.65 to 7.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/metering unit. The pH of both the dilution water and stock solution were adjusted by using a 1:1 ratio of sulfuric acid and hydrochloric acid (acid solution). The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted, de-chlorinated Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 7.75.

Due to the buffering capacity of the borax and boric acid solution used to prepare the boron concentrations, EPA agreed that GLEC should target the dilution water control pH at 7.75 (range of 7.65 to 7.85). The five test concentrations were targeted to a pH of ± 0.1 pH unit from the pH value defined at test initiation in each test concentration (i.e. regardless of whether or not the pH in the test concentrations were ± 0.1 pH units from that observed in the control water). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were four replicate test chambers for each treatment. The flow through test was conducted at 25 ± 1 °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with < 24 hour old *C. dubia* collected from the GLEC laboratory culture. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *C. dubia* in de-chlorinated Lake Michigan water maintained at a pH of 7.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water would have jeopardized the age requirement (< 24 hour old at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *C. dubia* were randomly assigned to test cups until each test cup contained five *C. dubia.* The test cups were randomly assigned to the 2.5 L glass test chambers (1 cup per chamber) and suspended in the test solutions from a rod. *C. dubia* were counted on a daily basis and the number of live *C. dubia* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e. morning and evening). The number of surviving *C. dubia* was recorded at test termination (48-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L. The average pH for the dilution water control measured in the *C. dubia* toxicity test for the 48-hour test period was 7.77. The average pH over the 48-hour test duration for the five test concentrations of 27.6, 49.8, 118, 223, and 391 mg/L was 7.92, 8.03, 8.03, 8.07, and 8.06, respectively.

Once the test was complete, the LC_{50} was determined using the average measured test concentrations with the Probit method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 77; test results are provided in Table 78. Analytical chemistry data are provided in Table 79. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 23.

Table 77. Test conditions for 48-hour toxicity test on *Ceriodaphnia dubia* with boron (pH 7.75).

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Ceriodaphnia dubia,* < 24 hours old, GLEC Culture
2. Test Type and Duration:	Continuous flow-through, 48 hours
3. Test Dates:	October 23-October 25, 2009
4. Test Temperature (°C):	25 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	2.5 Liter glass Tank
9. Volume Test Solutions:	2000 mL
10. No. of Test Organisms per Test Vessel:	10
11. No. of Test Vessels per Treatment:	2
12. Total No. of Test Organisms per Treatment:	20
13. Target or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0
14. Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):	391, 223, 118, 49.8, and 27.6
15. Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day
16. Dilution and Primary Control Water:	De-Chlorinated Lake Michigan Water
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 78. Test results for 48-hour toxicity test on *Ceriodaphnia dubia* with boron at pH 7.75.

Results of a ***Ceriodaphnia dubia*** **48-Hour Static Acute Toxicity Test**

Conducted 10/23/09 - 10/25/09 **Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043-35-3)**
(Borax: Sigma Aldrich Cas No. 1303-96-4)

Nominal (Measured) Concentrations	**Cumulative Percent Affected** [a]			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0 (0)	0 (0)		
25.0 (27.6) mg/L	0 (0)	0 (0)		
50.0 (49.8) mg/L	0 (0)	25 (25)		
100 (118) mg/L	15 (15)	75 (75)		
200 (223) mg/L	75 (75)	100 (100)		
400 (391) mg/L	100 (100)	100 (100)		

LC$_{50}$ Values* (mg/L)			
24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
172	76.9	NA	NA

48-Hour LC$_{50}$* = 76.9 mg/L

LC$_{50}$* 95% Confidence Limits			
24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
LL 144	62.3	NA	NA
UL 202	94.9	NA	NA

LL = Lower Limit
UL = Upper Limit
NR = Confidence Intervals are not reliable

Method(s) Used to Determine LC$_{50}$ Confidence Limit Values: Probit

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC$_{50}$ values are determined based on measured concentrations.

Table 79. Analytical chemistry data for 48-hour toxicity test on *Ceriodaphnia dubia* with boron at pH 7.75.

Nominal (and Measured) Test Conentrations		Boron (mg/L)	Temperature (°C)	pH (s.u.)	DO (mg/L)	SC (mmos)	Hardness (mg/L)	Alkalinity (mg/L)	Ammonia (mg/L)
Dilution Water/Control	Replicate # 1	0.05 (0.04-0.05)	24.6 (24.5-24.6)	7.65 (7.53-7.75)	7.7 (6.5-8.7)	320	146	47	ND
	Replicate # 2	0.06 (0.04-0.07)	25.0 (24.9-25.1)	7.75 (7.70-7.82)	7.9 (6.9-8.6)	328			
	Replicate # 3	0.05 (0.03-0.07)	25.2 (25.1-25.2)	7.84 (7.77-7.94)	7.7 (7.1-8.1)	322			
	Replicate # 4	0.06 (0.05-0.07)	24.9 (24.9-24.9)	7.71 (7.56-7.79)	7.5 (6.4-8.1)				
Average		**0.05**	**24.8**	**7.77**	**7.7**	**323**	**146**	**47**	**ND**
25 mg/L (27.6 mg/L)	Replicate # 1	27.9 (27.2-28.5)	24.7 (24.5-24.8)	7.89 (7.80-7.95)	7.3 (5.9-8.1)	346	138	50	ND
	Replicate # 2	27.3 (25.9-28.0)	25.1 (25.0-25.2)	7.96 (7.89-8.06)	7.7 (7.2-8.0)	350			
	Replicate # 3	27.4 (27.1-27.8)	24.8 (24.7-24.9)	7.92 (7.87-8.00)	7.6 (6.7-8.1)				
	Replicate # 4	28.0 (27.2-28.9)	24.8 (24.8-24.9)	7.97 (7.89-8.06)	7.7 (7.1-8.1)				
Average		**27.6**	**24.7**	**7.92**	**7.6**	**348**	**138**	**50**	**ND**
50 mg/L (49.8 mg/L)	Replicate # 1	49.8 (47.4-52.9)	24.6 (24.5-24.7)	8.00 (7.96-8.07)	7.4 (6.4-7.9)	364	144	57	ND
	Replicate # 2	50.1 (47.8-53.7)	24.8 (24.7-24.8)	8.04 (7.97-8.11)	7.7 (7.1-8.0)	365			
	Replicate # 3	49.3 (47.5-52.7)	24.8 (24.7-24.9)	8.00 (7.96-8.07)	7.8 (7.3-8.1)				
	Replicate # 4	49.8 (47.8-53.5)	24.8 (24.7-24.9)	8.04 (7.97-8.11)	7.5 (6.5-8.1)				
Average		**49.8**	**24.6**	**8.03**	**7.6**	**364**	**144**	**57**	**ND**
100 mg/L (118 mg/L)	Replicate # 1	120 (111-127)	24.8 (24.6-24.9)	8.07 (8.03-8.13)	7.5 (6.7-8.0)	411	128	77	ND
	Replicate # 2	120 (112-124)	24.9 (24.8-25.0)	8.08 (8.02-8.14)	7.6 (6.8-8.0)	412			
	Replicate # 3	118 (110-130)	24.8 (24.7-24.9)	8.07 (8.02-8.13)	7.5 (6.4-8.1)				
	Replicate # 4	115 (105-125)	24.9 (24.6-25.1)	8.07 (8.00-8.14)	7.6 (6.9-8.0)				
Average		**118**	**24.8**	**8.03**	**7.5**	**411**	**128**	**77**	**ND**
200 mg/L (223 mg/L)	Replicate # 1	227 (221-232)	24.7 (24.6-24.8)	8.08 (8.02-8.15)	7.7 (7.2-8.0)	475	132	105	ND
	Replicate # 2	222 (216-228)	24.5 (24.4-24.7)	8.08 (8.03-8.15)	7.5 (6.6-8.0)	477			
	Replicate # 3	218 (211-224)	24.7 (24.6-24.8)	8.09 (8.02-8.15)	7.7 (7.1-8.0)				
	Replicate # 4	225 (222-228)	25.1 (24.8-25.3)	8.07 (8.01-8.14)	7.6 (6.7-8.1)				
Average		**223**	**24.8**	**8.07**	**7.6**	**476**	**132**	**105**	**ND**
400 mg/L (391 mg/L)	Replicate # 1	392 (381-399)	24.5 (24.4-24.6)	8.06 (8.00-8.13)	7.6 (6.7-8.0)	587	132	167	ND
	Replicate # 2	391 (386-396)	24.6 (24.4-24.8)	8.07 (8.01-8.14)	7.7 (7.1-8.0)	589			
	Replicate # 3	391 (388-394)	24.5 (24.3-24.8)	8.07 (8.01-8.14)	7.7 (7.2-8.0)				
	Replicate # 4	391 (384-401)	24.6 (24.4-24.7)	8.06 (8.01-8.13)	7.5 (6.4-8.0)				
Average		**391**	**24.6**	**8.06**	**7.6**	**588**	**132**	**167**	**ND**

DO: Dissolved Oxygen
SC: Specific Conductance

ND: Non Detect; below detection limit.
NM: Not Measured

96-hour Toxicity of Boron (pH 6.75) on Pimephales promelas

The 96-hr test to determine the toxicity of boron (pH 6.75) on *P. promelas* was completed by GLEC. The *P. promelas* (collected from the GLEC laboratory culture) were continuously exposed for 96-hours to five concentrations of boron and to a dilution water control with a target test pH of 6.75 (range of 6.65 to 6.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/ metering unit. The pH of both the dilution water and stock solution were adjusted by using a 1:1 ratio sulfuric acid and hydrochloric acid (acid solution). The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 6.75. Due to the buffering capacity of the borax and boric acid solution used to prepare the boron concentrations, EPA agreed that GLEC should target to a pH of ± 0.1 pH unit from the pH value defined at test initiation in each test concentration (i.e. regardless of whether or not the pH in the test concentrations were ± 0.1 pH unit from that observed in the control water). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were two replicate test chambers for each treatment. The flow through test was conducted at 25 ± 1 °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with the *P. promelas*. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *P. promelas* in de-chlorinated Lake Michigan water maintained at a pH of 6.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water may have jeopardized the weight requirement (0.1–5 g at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *P. promelas* were randomly assigned to the 2.5 L glass test chambers until each test chamber contained ten *P. promelas*. *P. promelas* were counted on a daily basis and the number of live *P. promelas* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e., morning and evening). The number of surviving *P. promelas* was recorded at test termination (96-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L (dilution factor of 0.5). The average pH for the dilution water control measured in the *P. promelas* toxicity test for the 96-hour test period was 6.67. The average pH over the 96-hour test duration for the five test concentrations of 32.9, 55.2, 122, 224, and 394 mg/L was 6.85, 6.93, 7.18, 7.28, and 7.33, respectively.

The hourly water temperatures as recorded by the continuous temperature logger did show that test temperatures fell outside the temperature allowance in the early morning on October 29, 2009 (readings of 23.8 and 23.9 °C). However, instantaneous water temperatures measured by GLEC technicians on October 28 and later in the morning on October 29 never fell outside the acceptable range as outlined in the method. Therefore, these water temperature exceedances were very brief and likely had no effect on the results of this study.

Once the test was complete, the LC_{50} was determined using the average measured test concentrations with the Probit and Spearman method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 80; test results are provided in Table 81. Analytical chemistry data are provided in Table 82. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 24.

Table 80. Test conditions for 96-hour toxicity test on *Pimephales promelas* with boron (pH 6.75).

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Pimephales promelas*, (weight 0.11g and18.4 mm length), GLEC Culture
2. Test Type and Duration:	Continuous flow-through, 96 hours
3. Test Dates:	October 29-November 02, 2009
4. Test Temperature (°C):	25 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	2.5 Liter glass Tank
9. Volume of Test Solutions:	2000 mL
10. No. of Test Organisms per Test Vessel:	10
11. No. of Test Vessels per Treatment:	2
12. Total No. of Test Organisms per Treatment:	20
13. Target or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0
14. Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):	394, 224, 122, 55.2, and 32.9
15. Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day
16. Dilution and Primary Control Water:	De-chlorinated Lake Michigan Water
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 81. Test results for 96-hour toxicity test on *Pimephales promelas* with boron at pH 6.75.

Results of a *Pimephales promelas* **96-Hour Static Acute Toxicity Test**

Conducted 10/29/09 - 11/02/09 **Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043-35-3)**
(Borax: Sigma Aldrich Cas No. 1303-96-4)

Nominal (Measured) Concentrations	Cumulative Percent Affected [a]			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)
25.0 (32.9) mg/L	5 (5)	5 (5)	10 (10)	10 (10)
50.0 (55.2) mg/L	5 (5)	5 (5)	5 (5)	20 (20)
100 (122) mg/L	0 (0)	0 (0)	30 (30)	90 (90)
200 (224) mg/L	5 (5)	5 (5)	65 (65)	100 (100)
400 (394) mg/L	10 (10)	95 (95)	100 (100)	100 (100)

LC$_{50}$* Values (mg/L)			
24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
>394	297	163	70.6

96-Hour LC$_{50}$ *= 70.6 mg/L

LC$_{50}$ *95% Confidence Limits				
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**
LL	NA	285	130	58.3
UL	NA	310	205	86.3

LL = Lower Limit
UL = Upper Limit
NR = Confidence Intervals are not reliable

Method(s) Used to Determine LC$_{50}$ Confidence Limit Values: Probit and Spearman

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC$_{50}$ values are determined based on measured concentrations.

Table 82. Analytical chemistry data for 96-hour toxicity test on *Pimephales promelas* with boron at pH 6.75.

Nominal (and Measured) Test Conentrations		Boron (mg/L)	Temperature (°C)	pH (s.u.)	DO (mg/L)	SC (mmos)	Hardness (mg/L)	Alkalinity (mg/L)	Ammonia (mg/L)
Dilution Water/Control	Replicate # 1	0.04 (0.04-0.04)	24.4 (24.0-24.8)	6.62 (6.48-6.69)	7.3 (6.7-8.0)	327	130	70	ND
	Replicate # 4	0.06 (0.05-0.06)	24.5 (24.0-24.8)	6.73 (6.63-6.88)	7.2 (6.7-8.3)	343			
Average		**0.05**	**24.5**	**6.67**	**7.3**	**332**	**130**	**7**	**ND**
25 mg/L (32.9 mg/L)	Replicate # 1	32.8 (30.3-38.6)	24.5 (24.1-24.9)	6.82 (6.73-6.93)	7.2 (6.6-8.0)	362	130	10	ND
	Replicate # 3	33.1 (30.4-39.0)	24.6 (24.1-25.0)	6.88 (6.80-6.98)	7.6 (7.2 8.2)	365			
Average		**32.9**	**24.5**	**6.85**	**7.4**	**363**	**130**	**10**	**ND**
50 mg/L (55.2 mg/L)	Replicate # 1	55.2 (51.8-62.5)	24.4 (24.0-24.6)	6.94 (6.86-7.04)	7.4 (6.8-8.0)	382	126	11	ND
	Replicate # 3	55.2 (51.1-61.1)	24.6 (24.3-24.8)	6.93 (6.83-7.04)	7.3 (6.5-8.1)	351			
Average		**55.2**	**24.5**	**6.93**	**7.4**	**371**	**126**	**11**	**ND**
100 mg/L (122 mg/L)	Replicate # 1	120 (112-126)	24.5 (24.0-25.1)	7.17 (7.11-7.25)	7.4 (6.8-8.1)	440	130	18	ND
	Replicate # 3	123 (119-129)	24.5 (24.1-24.9)	7.19 (7.13-7.26)	7.6 (7.1-8.3)	447			
Average		**122**	**24.5**	**7.18**	**7.5**	**442**	**130**	**18**	**ND**
200 mg/L (224 mg/L)	Replicate # 2	223 (219-234)	24.3 (24.0-24.9)	7.28 (7.22-7.35)	7.2 (6.5-8.2)	518	128	25	ND
	Replicate # 4	224 (217-232)	25.3 (25.1-25.5)	7.28 (7.20-7.35)	7.3 (6.8-8.2)	527			
Average		**224**	**24.8**	**7.28**	**7.2**	**521**	**128**	**25**	**ND**
400 mg/L (394 mg/L)	Replicate # 1	391 (372-407)	24.3 (24.0-24.6)	7.33 (7.26-7.38)	7.4 (6.9-8.1)	670	126	41	ND
	Replicate # 4	397 (368-426)	24.6 (24.3-25.1)	7.32 (7.24-7.37)	7.5 (7.1-8.2)	696			
Average		**394**	**24.4**	**7.33**	**7.4**	**678**	**126**	**41**	**ND**

DO: Dissolved Oxygen
SC: Specific Conductance

ND: Non Detect; below detection limit.
NM: Not Measured

96-hr Toxicity of Boron (pH 7.75) on Pimephales promelas

The 96-hr test to determine the toxicity of boron (pH 7.75) on *P. promelas* was completed by GLEC. The *P. promelas* (collected from the GLEC laboratory culture) were continuously exposed for 96-hours to five concentrations of boron and to a dilution water control with a target test pH of 7.75 (range of 7.65 to 7.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/metering unit. The pH of both the dilution water and stock solution were adjusted by using a 1:1 ratio of sulfuric acid and hydrochloric acid (acid solution). The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 7.75. Due to the buffering capacity of the borax and boric acid solution used to prepare the boron concentrations, EPA agreed that GLEC should target the dilution water control pH at 7.75 (range of 7.65 to 7.85). The five test concentrations were targeted to a pH of ± 0.1 pH unit from the pH value defined at test initiation in each test concentration (i.e. regardless of whether or not the pH in the test concentrations were ± 0.1 pH unit from that observed in the control water). The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were two replicate test chambers for each treatment. The flow through test was conducted at 25 ± 1 °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with the *P. promelas*. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *P. promelas* in de-chlorinated Lake Michigan water maintained at a pH of 7.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water may have jeopardized the weight requirement (0.1–5 g at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *P. promelas* were randomly assigned to the 2.5 L glass test chambers until each test chamber contained ten *P. promelas*. *P. promelas* were counted on a daily basis and the number of live *P. promelas* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e. morning and evening). The number of surviving *P. promelas* was recorded at test termination (96-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L (dilution factor of 0.5). The average pH for the dilution water control measured in the *P. promelas* toxicity test for the 96-hour test period was 7.68. The average pH over the 96-hour test duration for the five test concentrations of 28.6, 50.9, 121, 223, and 392 mg/L was 7.88, 7.98, 8.05, 8.07, and 8.06, respectively.

Once the test was complete, the LC_{50} was determined using the average measured test concentrations with the Spearman method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 83; test results are provided in Table 84. Analytical chemistry data are provided in Table 85. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 25.

Table 83. Test conditions for 96-hour toxicity test on *Pimephales promelas* with boron (pH 7.75).

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Pimephales promelas*, (weight 0.12g and 22.0 mm length), GLEC Culture
2. Test Type and Duration:	Continuous flow-through, 96 hours
3. Test Dates:	October 23-October 27, 2009
4. Test Temperature (°C):	25 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	2.5 Liter glass Tank
9. Volume of Test Solutions:	2000 mL
10. No. of Test Organisms per Test Vessel:	10
11. No. of Test Vessels per Treatment:	2
12. Total No. of Test Organisms per Treatment:	20
13. Target or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0
14. Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):	392, 223, 121, 50.9, and 28.6
15. Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day
16. Dilution and Primary Control Water:	De-chlorinated Lake Michigan Water
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 84. Test results for 96-hour toxicity test on *Pimephales promelas* with boron at pH 7.75.

Results of a _*Pimephales promelas*_ 96-Hour Static Acute Toxicity Test

Conducted 10/23/09 - 10/27/09 Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043-35-3) (Borax: Sigma Aldrich Cas No. 1303-96-4)

Nominal (Measured) Concentrations	**Cumulative Percent Affected [a]**				**LC_{50}* Values (mg/L)**			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)	>392	289	202	137
25.0 (28.6) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	**96-Hour LC_{50} *= 137 mg/L**			
50.0 (50.9) mg/L	0 (0)	0 (0)	0 (0)	0 (5)	**LC_{50} *95% Confidence Limits**			
100 (121) mg/L	0 (0)	0 (0)	0 (0)	21 (21)	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
200 (223) mg/L	5 (5)	10 (10)	65 (65)	100 (100)	LL NA UL NA	260 320	178 229	118 158
400 (392) mg/L	10 (10)	95 (95)	100 (100)	100 (100)				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC_{50} Confidence Limit Values: Spearman			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC_{50} values are determined based on measured concentrations.

Table 85. Analytical chemistry data for 96-hour toxicity test on *Pimephales promelas* with boron at pH 7.75.

Nominal (and Measured) Test Conentrations		Boron (mg/L)	Temperature (°C)	pH (s.u.)	DO (mg/L)	SC (mmos)	Hardness (mg/L)	Alkalinity (mg/L)	Ammonia (mg/L)
Dilution Water/Control	Replicate # 1	0.05 (0.04-0.05)	24.8 (24.5-25.3)	7.63 (7.49-7.75)	7.3 (6.5-8.0)	351	146	41	ND
	Replicate # 4	0.06 (0.05-0.07)	25.1 (24.9-25.4)	7.72 (7.56-7.85)	7.3 (6.4-8.1)	321			
Average		**0.05**	**24.9**	**7.68**	7.3	**336**	**146**	**41**	**ND**
25 mg/L (28.4 mg/L)	Replicate # 1	28.7 (27.2-31.2)	24.9 (24.5-25.4)	7.86 (7.70-7.95)	7.2 (5.9-8.1)	375	138	49	ND
	Replicate # 3	28.6 (27.1-32.1)	25.0 (24.7-25.4)	7.91 (7.80-8.00)	7.4 (6.7-8.1)	345			
Average		**28.6**	**24.9**	**7.88**	**7.3**	**360**	**138**	**49**	**ND**
50 mg/L (50.9 mg/L)	Replicate # 1	51.0 (47.4-54.7)	24.7 (24.5-25.1)	7.97 (7.80-8.07)	7.2 (6.4-8.0)	393	146	57	ND
	Replicate # 3	50.9 (47.5-55.5)	24.9 (24.7-25.4)	7.98 (7.90-8.07)	7.4 (6.7-8.1)	361			
Average		**50.9**	**24.8**	**7.98**	7.3	377	**146**	**57**	**ND**
100 mg/L (121 mg/L)	Replicate # 1	121 (111-127)	24.9 (24.6-25.3)	8.06 (7.97-8.13)	7.4 (6.7-8.1)	436	128	77	ND
	Replicate # 3	121 (110-130)	25.0 (24.7-25.4)	8.05 (7.96-8.13)	7.1 (6.2-8.1)	410			
Average		**121**	**24.9**	**8.05**	7.3	**423**	**128**	77	**ND**
200 mg/L (223 mg/L)	Replicate # 2	222 (216-228)	24.6 (24.4-25.1)	8.07 (7.99-8.15)	7.3 (6.6-8.0)	500	132	107	ND
	Replicate # 4	225 (222-228)	25.2 (24.8-25.6)	8.07 (8.00-8.14)	7.3 (6.7-8.1)	476			
Average		**223**	**24.9**	**8.07**	**7.3**	**488**	**132**	**107**	**ND**
400 mg/L (392 mg/L)	Replicate # 1	392 (381-399)	24.7 (24.4-25.1)	8.07 (8.00-8.13)	7.3 (6.7-8.0)	616	128	168	ND
	Replicate # 4	392 (384-401)	24.7 (24.4-25.2)	8.06 (8.01-8.13)	7.3 (6.4-8.0)	590			
Average		**392**	24.7	**8.06**	7.3	**603**	**128**	**168**	**ND**

DO: Dissolved Oxygen
SC: Specific Conductance

ND: Non Detect; below detection limit.
NM: Not Measured

96-hr Toxicity of Boron (pH 8.75) on Pimephales promelas

The 96-hr test to determine the toxicity of boron (pH 8.75) on *P. promelas* was completed by GLEC. The *P. promelas* (collected from the GLEC laboratory culture) were continuously exposed for 96-hours to five concentrations of boron and to a dilution water control with a target test pH of 8.75 (range of 8.65 to 8.85) using a continuous flow-through system (modified Benoit mini-dilutor) and an in-line pH adjustment/metering unit. The stock solutions used for the test were prepared by diluting a known weight (grams) of borax and boric acid to a known volume of dilution water (de-chlorinated Lake Michigan water). The pH of both the dilution water and stock solution were adjusted by using sodium hydroxide. The pH adjusted stock solution was delivered to mixing cells and diluted with pH adjusted Lake Michigan water to achieve target nominal concentrations of boron and a target test pH of 8.75. The temperature-controlled test concentration solutions were supplied to each test chamber via the continuous flow-through system at a rate of approximately four turnovers a day. There were two replicate test chambers for each treatment. The flow through test was conducted at 25 ± 1 °C with a photoperiod of 16 hr light and 8 hr darkness (ambient laboratory light).

After test concentrations had achieved steady state in the flow through system, the test was initiated with the *P. promelas*. Although these organisms were cultured in, and acclimated to, the dilution water's "natural" pH (typically between a pH of 7.9 and 8.2), they were not acclimated to the pH-adjusted dilution water prior to test initiation for two reasons: 1) GLEC does not maintain a laboratory culture of *P. promelas* in de-chlorinated Lake Michigan water maintained at a pH of 8.75 (and this was outside the scope of work for this Work Assignment) and 2) adequate acclimation of the organisms to the pH-adjusted dilution water may have jeopardized the weight requirement (0.1–5 g at test initiation) for test organisms required under the toxicity testing method (ASTM 2007).

The *P. promelas* were randomly assigned to the 2.5 L glass test chambers until each test chamber contained ten *P. promelas*. *P. promelas* were counted on a daily basis and the number of live *P. promelas* was recorded. pH was recorded twice a day, at a minimum of eight hours apart (i.e., morning and evening). The number of surviving *P. promelas* was recorded at test termination (96-hours).

The test was completed at the following nominal boron concentrations: 25.0, 50.0, 100, 200, and 400 mg/L (dilution factor of 0.5). The average pH for the dilution water control measured in the *P. promelas* toxicity test for the 96-hour test period was 8.75. The average pH over the 96-hour test duration for the five test concentrations of 21.1, 42.4, 112, 219, and 376 mg/L was 8.72, 8.70, 8.70, 8.70, and 8.67, respectively.

Instantaneous water temperature measurements made on February 13, 14, 15 and 16 (Days 1-4: 23.6°C-24.7°C) exceeded the allowable range of 25 ± 1 °C outlined in the toxicity testing method. However, the average water temperatures across the duration of the test in each replicate were always ± 0.9 °C of the target test temperature (25 °C) in all treatments. In addition, the water temperature as recorded by the continuous temperature logger did show that the test temperature fell outside the temperature allowance during the times of 0430 to 0830 on those days. However, due to the relatively small number of temperature readings measured outside the range, these water temperature exceedances likely had little effect on the results of this study.

Once the test was complete, the LC_{50} was determined using the average measured test concentrations with the Probit method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 86; test results are provided in Table 87. Analytical chemistry data are provided in Table 88. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 26.

Table 86. Test conditions for 96-hour toxicity test on *Pimephales promelas* with boron (pH 8.75).

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Pimephales promelas*, (weight 0.13g and 22.8 mm length), GLEC Culture
2. Test Type and Duration:	Continuous flow-through, 96 hours
3. Test Dates:	February 12-February 16, 2010
4. Test Temperature (°C):	25 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	2.5 Liter glass Tank
9. Volume of Test Solutions:	2000 mL
10. No. of Test Organisms per Test Vessel:	10
11. No. of Test Vessels per Treatment:	2
12. Total No. of Test Organisms per Treatment:	20
13. Target or Nominal Test Concentrations (mg/L):	400, 200, 100, 50.0, and 25.0
14. Analytical Test Concentrations (average of samples collected at test initiation and termination-mg/L):	376, 219, 112, 42.4, and 21.1
15. Renewal of Test Solutions:	Continuous flow through, 4 turnovers per day
16. Dilution and Primary Control Water:	De-chlorinated Lake Michigan Water
17. Test Material:	Boric Acid: Sigma Aldrich, ACS Reagent >= 99.5% Cas. No. 10043-35-3, Batch 118K0007 and Borax (sodium tetraborate decahydrate) Sigma Aldrich, ≥ 99.5%, ACS reagent, Cas. No. 1303-96-4, Lot # 118K0172
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 87. Test results for 96-hour toxicity test on *Pimephales promelas* with boron at pH 8.75.

Results of a _*Pimephales promelas*_ 96-Hour Static Acute Toxicity Test

Conducted 02/12/10 - 02/16/10 Using: Boron (Boric Acid: Sigma Aldrich Cas No. 10043-35-3) (Borax: Sigma Aldrich Cas No. 1303-96-4)

Nominal (Measured) Concentrations	**Cumulative Percent Affected [a]**				**LC_{50}* Values (mg/L)**			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	5 (5)	>373	325	195	133
25.0 (21.1) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	**96-Hour LC_{50} *= 133 mg/L**			
50.0 (42.4) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	**LC_{50} *95% Confidence Limits**			
100 (112) mg/L	0 (0)	0 (0)	5 (5)	30 (30)	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
200 (219) mg/L	5 (5)	15 (15)	60 (60)	95 (95)	LL NA UL NA	276 412	165 227	110 157
400 (376) mg/L	10 (10)	65 (65)	100 (100)	100 (100)				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC_{50} Confidence Limit Values: Probit			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC_{50} values are determined based on measured concentrations.

Table 88. Analytical chemistry data for 96-hour toxicity test on *Pimephales promelas* with boron at pH 8.75.

Nominal (and Measured) Test Conentrations		Boron (mg/L)	Temperature (°C)	pH (s.u.)	DO (mg/L)	SC (mmos)	Hardness (mg/L)	Alkalinity (mg/L)	Ammonia (mg/L)
Dilution Water/Control	Replicate # 1	0.04 (0.03-0.04)	24.2 (23.8-24.6)	8.74 (8.68-8.83)	7.6 (7.4-7.7)	290	126	111	ND
	Replicate # 2	0.03 (0.03-0.03)	24.4 (23.8-25.2)	8.75 (8.71-8.81)	7.4 (7.1-7.6)	299			
Average		**0.03**	**24.3**	**8.75**	**7.5**	**295**	**126**	**111**	**ND**
25 mg/L (21.1 mg/L)	Replicate # 1	21.9 (21.7-22.2)	24.3 (23.8-24.8)	8.72 (8.67-8.77)	7.5 (7.4-7.7)	331	110	133	ND
	Replicate # 3	20.2 (18.0-21.7)	24.2 (23.8-25.2)	8.71 (8.63-8.77)	7.3 (7.1-7.4)	336			
Average		**21.1**	**24.2**	**8.72**	7.4	**333**	**110**	**133**	**ND**
50 mg/L (42.4 mg/L)	Replicate # 2	42.6 (37.0-46.0)	24.3 (23.8-25.2)	8.69 (8.62-8.72)	7.1 (6.8-7.6)	368	116	156	ND
	Replicate # 4	42.7 (36.3-45.4)	24.3 (23.8-24.8)	8.72 (8.67-8.77)	7.2 (7.1-7.4)	370			
Average		**42.4**	**24.3**	**8.70**	**7.2**	**369**	**116**	**156**	**ND**
100 mg/L (112 mg/L)	Replicate # 1	111 (93.5-125)	24.3 (23.9-25.2)	8.71 (8.65-8.76)	7.3 (7.1-7.5)	472	116	235	ND
	Replicate # 4	113 (96.3-128)	24.3 (23.6-25.0)	8.70 (8.63-8.76)	7.4 (7.2-7.6)	464			
Average		**112**	**24.3**	**8.70**	**7.3**	**471**	**116**	**235**	**ND**
200 mg/L (219 mg/L)	Replicate # 1	207 (185-254)	24.3 (23.6-24.8)	8.70 (8.64-8.73)	7.3 (7.0-7.6)	628	120	340	ND
	Replicate # 4	231 (202-264)	24.5 (23.9-25.2)	8.70 (8.64-8.74)	7.2 (7.1-7.4)	638			
Average		**219**	**24.4**	**8.70**	**7.3**	**633**	**120**	**340**	**ND**
400 mg/L (376 mg/L)	Replicate # 1	382 (359-412)	24.1 (24.0-24.5)	8.67 (8.62-8.73)	7.2 (6.9-7.3)	844	108	510	ND
	Replicate # 4	370 (353-399)	24.1 (23.8-24.8)	8.67 (8.62-8.71)	7.2 (7.1-7.3)	845			
Average		**376**	**24.1**	**8.67**	**7.2**	**845**	**108**	**510**	**ND**

DO: Dissolved Oxygen
SC: Specific Conductance

ND: Non Detect; below detection limit.
NM: Not Measured

Manganese

Table 89 provides a summary of estimated LC_{50} values for the two toxicity tests performed using manganese. LC_{50} values ranged between 31.5 and 43.3 mg Mn/L.

Table 89. LC_{50} estimates for toxicity tests performed using manganese.

Test Species and Duration	**LC_{50} (mg Mn/L)**
Lampsilis siliquoidea 96 hr	43.3
Megalonaias nervosa - 96 hr	31.5

For each of the acute toxicity tests completed using manganese, two tables were generated: the first summarizes the test results for each toxicity test, including nominal and analytical test concentration and LC_{50} estimates with confidence intervals; the second table summarizes analytical chemistry data collected throughout the toxicity tests. Also discussed, if applicable, are deviations from the guidance provided in the ASTM method used to complete the toxicity testing.

96-hr Toxicity of Manganese on Lampsilis siliquoidea

The 96-hr test to determine the toxicity of manganese on *L. siliquoidea* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Missouri State University laboratory culture, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. One replicate was mistakenly loaded with only 4 individuals, but this was accounted for in the LC_{50} calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 150, 75, 37, 18.8, 9.4, and 4.7 mg Mn/L.

Testing was conducted at 20 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 90; test results are provided in Table 91. Analytical chemistry data are provided in Table 92. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 27.

Table 90. Test conditions for 96-hour toxicity test on *Lampsilis siliquoidea* with manganese.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Lampsilis siliquoidea,* juveniles <5 days old, Missouri State Univbersity
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	September 8 - 12, 2009
4. Test Temperature (°C):	20 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	50 mL beaker
9. Volume of Test Solutions:	40 mL
10. No. of Test Organisms per Test Vessel:	5
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	20
13. Test Concentrations (mg Mn/L):	150, 75, 37, 18.8, 9.4, and 4.7
14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination- Mn mg/L):	154.9, 72.5, 34.5, 18.5, 10.1 and 4.5
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	USEPA MHRW
17. Test Material:	Manganese sulfate monohydrate: Fisher Scientific, ACS grade assay, 98.7%, Cas. No. 7785-87-7, Lot # 086316 and manganese chloride tetrahydrate, Fisher Scientific, certified ACS Assay 99.8% Cas. No. 7773-01-5, Lot # 081484
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 91. Test results for 96-hour toxicity test on *Lampsilis siliquoidea* with manganese.

Results of a *Lampsilis siliquoidea)* **96-Hour Static Acute Toxicity Test**

Conducted 09/08/09 - 09/12/09 **Using: Manganese sulfate Cas. No. 7785-87-7 &manganese chloride Cas. No. 7773-01-5**

Nominal (Measured) Concentrations	**Cumulative Percent Affected [a]**				**LC_{50} Values* (mg/L)**			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0	0	0	0	62.8	50.0	48.2	43.3
4.7 (4.5) mg/L	0	0	0	0	**96-Hour LC_{50}* = 43.3 mg/L**			
9.4 (10.1) mg/L	0	0	0	0	LC_{50} 95% Confidence Limits			
18.8 (18.5) mg/L	0	0	0	0	24-Hr	48-Hr	72-Hr	96-Hr
37 (34.5) mg/L	0	0	5.2	21.0	LL 52.7 UL 74.9	NR NR	45.0 51.7	38.1 49.2
75 (72.5) mg/L	70	100	100	100				
150 (154.9) mg/L	95	100	100	100	LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC_{50} and EC_{50} Confidence Limit Values: Spearman-Karber			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC_{50} and EC_{50} values are determined based on measured concentrations.

Table 92. Analytical chemistry data for 96-hour toxicity test on *Lampsilis siliquoidea* with manganese.

Nominal (Measured) Test Concentration		Manganese[a] (mg/L)	Temperature (°C)	pH (s.u.)	D.O. (mg/L)	Conductivity (mmos)	Alkalinity (mg/L)	Hardness (mg/L)
Dilution water/Control	Day 0	<0.01	20.4	7.9	7.08	316	62	92
	Day 1		20.3					
	Day 2		20.4					
	Day 3		19.6					
	Day 4	<0.01	19.1	7.9	7.50	322	62	90
		na						
4.7 (4.5) mg/L	Day 0	4.5	20.5	7.9	7.83	324	62	98
	Day 1		20.2					
	Day 2		20.3					
	Day 3		19.5					
	Day 4	4.6	19.1	7.9	7.69	328	62	100
		4.5						
9.4 (10.1) mg/L	Day 0	9.2	20.4	7.9	7.87	341	62	110
	Day 1		20.3					
	Day 2		20.4					
	Day 3		19.6					
	Day 4	11.0	19.0	7.9	7.65	345	62	110
		10.1						
18.8 (18.5) mg/L	Day 0	19.0	20.4	7.7	7.83	376	62	120
	Day 1		20.2					
	Day 2		20.3					
	Day 3		19.6					
	Day 4	18.0	19.0	7.9	7.72	380	62	124
		18.5						
37 (34.5) mg/L	Day 0	34.0	20.5	7.7	7.94	447	62	152
	Day 1		20.2					
	Day 2		20.3					
	Day 3		19.6					
	Day 4	35.0	19.0	7.9	7.76	450	62	152
		34.5						
75 (72.5) mg/L	Day 0	73.0	20.5	7.7	7.88	582	62	220
	Day 1		20.2					
	Day 2		20.3					
	Day 3		19.7					
	Day 4	72.0	19.1	7.9	7.70	590	62	224
		72.5						
150 (154.9) mg/L	Day 0	150.0	20.5	7.7	7.88	840	62	*
	Day 1		20.3					
	Day 2		20.4					
	Day 3		19.7					
	Day 4	160.0	19.2	7.9	7.80	850	62	*
		154.9						

[a] Manganese Analysis Method 200.7

* interference in hardness measurement

na = not applicable

96-hr Toxicity of Manganese on Megalonaias nervosa

The 96-hr test to determine the toxicity of manganese on *M. nervosa* was completed by INHS. Test organisms, < 5-day old juveniles collected from the Genoa National Fish Hatchery, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate. In one replicate of the control a test organism was inadvertently crushed, but this was accounted for in the LC_{50} calculation.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 300, 150, 75, 37.5, and 18.8 mg Mn/L.

Testing was conducted at 20 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 93; test results are provided in Table 94. Analytical chemistry data are provided in Table 95. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 28.

Table 93. Test conditions for 96-hour toxicity test on *Megalonaias nervosa* with manganese.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Megalonaias nervosa,* juveniles <5 days old (Genoa National Fish Hatchery)
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	October 23 - 27, 2009
4. Test Temperature (°C):	20 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 $\mu E/m^2/s$
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	50 mL beaker
9. Volume of Test Solutions:	40 mL
10. No. of Test Organisms per Test Vessel:	5
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	20
13. Test Concentrations (mg Mn/L):	300, 150, 75, 37.5, and 18.8
14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg Mn/L):	290, 140, 72, 34, and 18
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	USEPA MHRW
17. Test Material:	Manganese sulfate monohydrate: Fisher Scientific, ACS grade assay, 98.7%, Cas. No. 7785-87-7, Lot # 086316 and manganese chloride tetrahydrate, Fisher Scientific, certified ACS Assay 99.8% Cas. No. 7773-01-5, Lot # 081484
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 94. Test results for 96-hour toxicity test on *Megalonaias nervosa* with manganese.

Results of a *Megalonaias nervosa* **96-Hour Static Acute Toxicity Test**

Conducted 10/23/09 - 10/27/09 **Using: Manganese sulfate Cas. No. 7785-87-7 & manganese chloride Cas. No. 7773-01-5**

Nominal (Measured) Concentrations	**Cumulative Percent Affected [a]**				**LC_{50} Values* (mg/L)**			
	24-Hr	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0	0	0	0	41.6	37.5	31.5	31.5
18.8 (18) mg/L	0	0	0	0	**96-Hour LC_{50}* = 31.5 mg/L**			
37.5 (34) mg/L	30	40	65	65	LC_{50} 95% Confidence Limits			
75 (72) mg/L	95	100	100	100	24-Hr	48-Hr	72-Hr	96-Hr
150 (140) mg/L	100	100	100	100	LL 35.6 UL 48.8	32.2 43.6	27.2 36.6	27.2 36.6
300 (290) mg/L	100	100	100	100				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC_{50} and EC_{50} Confidence Limit Values: Spearman-Karber			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC_{50} and EC_{50} values are determined based on measured concentrations.

Table 95. Analytical chemistry data for 96-hour toxicity test on *Megalonaias nervosa* with manganese.

Nominal (Measured) Test Concentration		Manganese[a] (mg/L)	Temp. (°C)	pH (s.u.)	D.O. (mg/L)	Cond. (mmos)	Alkalinity (mg/L)	Hardness (mg/L)	Ammonia (mg/L)
Dilution water/Control	Day 0	<0.01	20.8	8.0	8.34	303	60	90	<0.05
	Day 1		20.9	8.1	8.04	297			
	Day 2		20.9	8.1	8.13	303			
	Day 3		20.9	8.0	8.31	305			
	Day 4 total	0.1	20.8	8.0	8.00	315	60	92	<0.05
	Day 4 dissolved	0.7							
		0.1							
18.8 (18.0) mg/L	Day 0	18.0	20.8	7.9	8.33	375	60	112	<0.05
	Day 1		20.9	7.9	8.10	362			
	Day 2		20.9	7.7	8.19	371			
	Day 3		20.9	7.6	8.31	379			
	Day 4 total	18.0	20.8	7.8	7.92	383	60	112	<0.05
	Day 4 dissolved	16.0							
		18.0							
37.5 (34.0) mg/L	Day 0	33.0	20.8	7.9	8.26	445	60	*	<0.05
	Day 1		20.9	7.7	8.11	442			
	Day 2		20.9	7.6	8.25	458			
	Day 3		20.9	7.6	8.42	478			
	Day 4 total	35.0	20.8	7.7	7.94	495	60	*	<0.05
	Day 4 dissolved	34.0							
		34.0							
75 (72.0) mg/L	Day 0	70.0	20.8	7.9	8.29	582	60	*	<0.05
	Day 1		20.9	7.7	8.07	569			
	Day 2		20.8	7.6	8.26	567			
	Day 3		20.9	7.5	8.32	581			
	Day 4 total	74.0	20.9	7.7	7.91	589	62	*	<0.05
	Day 4 dissolved	68.0							
		72.0							
150 (140.0) mg/L	Day 0	140.0	20.8	7.8	8.33	841	60	*	<0.05
	Day 1		20.9	7.7	8.03	826			
	Day 2		20.8	7.6	8.22	817			
	Day 3		20.9	7.5	8.40	851			
	Day 4 total	140.0	20.9	7.6	7.88	848	62	*	<0.05
	Day 4 dissolved	150.0							
		140.0							
300 (290) mg/L	Day 0	290.0	20.8	7.7	8.27	1333	60	*	<0.05
	Day 1		20.9	7.7	7.98	1301			
	Day 2		20.8	7.6	8.20	1300			
	Day 3		20.9	7.5	8.22	1325			
	Day 4 total	290.0	20.9	7.6	7.94	1340	62	*	<0.05
	Day 4 dissolved	290.0							
		290.0							

[a] Manganese Analysis Method 200.7

*interference in hardness measurement

Temp. = temperature; Cond. = conductivity

Fluoride

Table 96 provides a summary of estimated LC_{50} values for the two toxicity tests performed using fluoride. LC_{50} values ranged between 13.4 and 62.0 mg F/L.

Table 96. LC_{50} estimates for toxicity tests performed using fluoride.

Test Species and Duration	**LC_{50} (mg F/L)**
Sphaerium simile - 96 hr	62.0
Hyalella azteca - 96 hr	13.4

For each of the acute toxicity tests completed using fluoride, two tables were generated: the first summarizes the test results for each toxicity test, including nominal and analytical test concentration and LC_{50} estimates with confidence intervals; the second table summarizes analytical chemistry data collected throughout the toxicity tests.

96-hr Toxicity of Fluoride on Sphaerium simile

The 96-hr test to determine the toxicity of fluoride on *S. simile* was completed by INHS. Test organisms, juveniles released from field collected adults, were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 800, 400, 200, 100, and 50 mg F/L.

Testing was conducted at 22 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Spearman-Karber method.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 97; test results are provided in Table 98. Analytical chemistry data are provided in Table 99. Accompanying information, including raw laboratory data, analytical chemistry data and statistical analyses, is provided in Appendix 29.

Table 97. Test conditions for 96-hour toxicity test on *Sphaerium simile* with fluoride.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Sphaerium simile*, juveniles (released from field-collected adults)
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	July 13 - 17, 2009
4. Test Temperature (°C):	22 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	150 mL beaker
9. Volume of Test Solutions:	120 mL
10. No. of Test Organisms per Test Vessel:	5
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	20
13. Test Concentrations (mg F/L):	800, 400, 200, 100, and 50
14. Analytical Test Concentrations (geometric mean of samples collected at test initiation and termination-mg F/L):	800, 390, 185, 88 and 44
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	USEPA MHRW
17. Test Material:	Sodium fluoride: Acros Organics, 99+% for analysis ACS, Cas. No. 7681-49-5, Lot # A0243428
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 98. Test results for 96-hour toxicity test on *Sphaerium simile* with fluoride.

Results of a *Sphaerium simile* **96-Hour Static Acute Toxicity Test**

Conducted 07/13/09 - 07/17/09 **Using: Sodium fluoride: Sigma Aldrich Cas. No. 7681-49-5**

Nominal (Measured) Concentrations	Cumulative Percent Affected [a]				LC$_{50}$ Values* (mg/L)			
	24-Hr	48-Hr	72-Hr	96-Hr	24-Hr	48-Hr	72-Hr	96-Hr
Primary Control/ Dilution Water	0	0	0	5	>800	>800	>800	62
50 (44) mg/L	0	0	0	25	96-Hour LC$_{50}$* = 62 mg/L			
100 (88) mg/L	0	0	0	75	LC$_{50}$ 95% Confidence Limits			
200 (185) mg/L	0	0	35	100	24-Hr	48-Hr	72-Hr	96-Hr
400 (390) mg/L	0	5	50	100	LL NR UL NR	NR NR	NR NR	51 75
800 (800) mg/L	0	5	25	100				
					LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC$_{50}$ and EC$_{50}$ Confidence Limit Values: Spearman-Karber			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

- All LC$_{50}$ and EC$_{50}$ values are determined based on measured concentrations.

Table 99. Analytical chemistry data for 96-hour toxicity test *Sphaerium simile* with fluoride.

Nominal (Measured) Test Concentration		Fluoride[a] (mg/L)	Temperature (°C)	pH (s.u.)	D.O. (mg/L)	Conductivity (µmhos)	Alkalinity (mg/L)	Hardness (mg/L)
Dilution water/Control	Day 0	<0.5	22.9	8.0	7.76	307	62	96
	Day 1		22.9		7.97			
	Day 2		22.8		8.00			
	Day 3		22.7		7.63			
	Day 4	<0.5	22.9	8.0	8.05	310	62	96
		na						
50 (44) mg/L	Day 0	45	22.8	8.1	7.82	555	64	94
	Day 1		22.8		7.92			
	Day 2		22.8		7.83			
	Day 3		22.7		7.70			
	Day 4	44	22.9	8.0	8.04	558	68	80
		44						
100 (88) mg/L	Day 0	86	22.8	8.1	7.80	785	66	80
	Day 1		22.9		8.11			
	Day 2		22.8		7.95			
	Day 3		22.7		7.67			
	Day 4	91	22.9	8.1	7.53	784	68	62
		88						
200 (185) mg/L	Day 0	190	22.9	8.0	7.82	1264	78	76
	Day 1		22.8		8.05			
	Day 2		22.9		7.82			
	Day 3		22.8		6.63			
	Day 4	180	22.9	8.1	6.03	1268	82	50
		185						
400 (390) mg/L	Day 0	400	22.9	8.2	7.78	2210	120	64
	Day 1		22.9		7.99			
	Day 2		22.8		7.79			
	Day 3		22.8		4.88			
	Day 4	380	23.0	7.9	5.49	2210	120	30
		390						
800 (800) mg/L	Day 0	800	23.0	8.2	7.82	4050	160	14
	Day 1		22.9		8.05			
	Day 2		22.9		8.02			
	Day 3		22.8		6.51			
	Day 4	800	22.9	8.0	6.89	4090	160	2
		800						

[a] Fluoride Analysis Method 300.0

na = not applicable

96-hr Toxicity of Fluoride on Hyalella azteca

The 96-hr test to determine the toxicity of fluoride on *H. azteca* was completed by GLEC. *H. azteca* were collected from GLEC's laboratory culture. These organisms are maintained in 10 gallon glass aquaria; plastic artificial turf and screen mesh serve as a substrate for the culture. The tanks are filled with de-chlorinated Lake Michigan water (City of Traverse City, Michigan water passed through an activated carbon filter). Cultures are fed 50 mL of 4 g/L Tetrafin slurry daily. When visible algae are not observed within the glass aquaria, algae (*Selenastrum* sp.) are used as a supplement to the Tetrafin slurry. Additionally, on occasion, dried Aspen (*Populus* sp.) leaves are prepared as a food supplement. The culture is maintained in a 16-hour light: 8-hour dark photoperiod at a temperature between 23 and 26 °C.

Test organisms were acclimated to the dilution water (MHRW), test temperature and other test conditions prior to test initiation. Once acclimated, test organisms were examined for any disease, stress, parasites, etc. If free from ailments, test organisms were randomly assigned to the test chambers (which were randomly assigned to testing locations); four replicates were used per treatment with five organisms per replicate.

Organisms were exposed to a dilution water control and the test chemical at varying concentrations under static conditions. Serial dilutions of the highest test concentration (known weight of test chemical dissolved in a known volume of dilution water) were made to prepare the following nominal test concentrations: 7.8, 12.9, 21.6, 36.0, 60.0, and 100 mg F/L.

Testing was conducted at 22 ± 1 °C with a photoperiod of 16 hr light and 8 hr dark (ambient laboratory light). Organisms were not fed for the duration of the test and were examined daily for mortality. Once the test was complete, the LC_{50} value was determined using the Probit and Spearman Karber methods.

A summary of the toxicity test conditions present throughout the assessment are provided in Table 100; test results are provided in Table 101. Analytical chemistry data are provided in Table 102. Accompanying information, including raw laboratory data, analytical chemistry data, reference toxicant data and statistical analyses, is provided in Appendix 30.

Table 100. Test conditions for 96-hour toxicity test on *Hyalella azteca* with fluoride.

Summary of Toxicity Test Conditions	
1. Test Species and Age:	*Hyalella azteca,* 14 days old, GLEC culture
2. Test Type and Duration:	Static, 96 hours
3. Test Dates:	September 17- September 21, 2009
4. Test Temperature (°C):	22 ± 1
5. Light Quality:	Ambient Laboratory, 10-20 µE/m²/s
6. Photoperiod:	16 h light, 8 h darkness
7. Feeding Regime:	None
8. Size of Test Vessel:	150 mL beaker
9. Volume of Test Solutions:	100 mL
10. No. of Test Organisms per Test Vessel:	5
11. No. of Test Vessels per Treatment:	4
12. Total No. of Test Organisms per Treatment:	20
13. Target or Nominal Test Concentrations (mg F/L):	100, 60, 36, 21.6, 12.9, and 7.8
14. Analytical Test Concentrations (average of samples collected at test initiation and termination-mg F/L):	89.4, 58.9, 32.7, 22.8, 14.3, and 8.5
15. Renewal of Test Solutions:	None
16. Dilution and Primary Control Water:	USEPA MHRW
17. Test Material:	Sodium Fluoride: Sigma Aldrich, 99+% ACS Reagent Cas. No. 7681-49-4, Batch # 06810JJ
18. Secondary Control Water:	None
19. Aeration:	None
20. Endpoints Measured:	Mortality (LC_{50})

Table 101. Test results for 96-hour toxicity test on *Hyalella azteca* with fluoride.

Results of a *Hyalella azteca* 96-Hour Static Acute Toxicity Test

Conducted 09/17/09 - 09/21/09 Using: Fluoride (Sodium Fluoride Sigma Aldrich Cas. No. 7681-49-4)

	Cumulative Percent Affected [a]				**LC_{50} Values* (mg/L)**			
Nominal (Measured) Concentrations	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
Primary Control/ Dilution Water	0 (0)	0 (0)	0 (0)	0 (0)	25.1	16.0	13.8	13.4
7.8 (8.5) mg/L	0 (0)	0 (0)	0 (0)	0 (0)	**96-Hour LC_{50}* = 13.4 mg/L**			
12.9 (14.3) mg/L	0 (0)	45 (45)	70 (70)	70 (70)	**LC_{50} 95% Confidence Limits**			
21.6 (22.8) mg/L	20 (20)	80 (80)	90 (90)	95 (95)	**24-Hr**	**48-Hr**	**72-Hr**	**96-Hr**
36 (32.7) mg/L	100 (100)	100 (100)	100 (100)	100 (100)	LL 23.3 UL 27.1	13.9 18.3	12.0 15.6	11.8 15.2
60 (58.9) mg/L	100 (100)	100 (100)	100 (100)	100 (100)				
100 (89.4) mg/L	100 (100)	100 (100)	100 (100)	100 (100)	LL = Lower Limit UL = Upper Limit NR = Confidence Intervals are not reliable			
					Method(s) Used to Determine LC_{50} Confidence Limit Values: Probit and Spearman-Karber			

[a] Cumulative percent affected is the total percentage of test organisms observed dead, immobile, exhibiting loss of equilibrium or other defined endpoints.

* All LC_{50} values are determined based on measured concentrations.

Table 102. Analytical chemistry data for 96-hour toxicity test on *Hyalella azteca* with fluoride.

Nominal (and Measured) Test Concentrations		Fluoride[a] (mg/L)	Temperature (°C)	pH (s.u.)	D.O. (mg/L)	Conductivity (mmhos)	Alkalinity (mg/L)	Hardness (mg/L)	Ammonia (mg/L)
Dilution water/Control	Day 0	ND	22.0	7.84	8.0	300	54	80	ND
	Day 1		21.9	7.79	7.5				
	Day 2		22.1	7.79	7.1				
	Day 3		22.0	7.84	7.9				
	Day 4	ND	22.2	7.81	8.0	325	68	88	
		ND							
		ND							
7.8 (8.5) mg/L	Day 0	8.4	22.0	7.84	8.0	368			
	Day 1		22.0	7.78	7.5				
	Day 2		22.1	7.83	7.3				
	Day 3		22.0	7.91	7.7				
	Day 4	8.6	22.2	7.87	8.0	363			
		8.5							
12.9 (14.3) mg/L	Day 0	14.1	22.0	7.85	8.0	393			
	Day 1		22.0	7.85	7.5				
	Day 2		22.1	7.88	7.4				
	Day 3		21.9	7.97	8.1				
	Day 4	14.5	22.2	7.89	8.0	377			
		14.3							
21.6 (22.8) mg/L	Day 0	22.0	22.0	7.85	8.0	432			
	Day 1		21.9	7.90	7.5				
	Day 2		22.0	7.89	7.3				
	Day 3		21.8	8.02	8.0				
	Day 4	23.5	21.9	7.97	8.0	422	66	100	
		22.8							
36 (32.7) mg/L	Day 0	33.4	22.0	7.88	8.0	502			
	Day 1	32.0	21.9	7.91	7.5				
	Day 2								
	Day 3								
	Day 4								
		32.7							
60 (58.9) mg/L	Day 0	58.1	22.0	7.88	8.0	622			
	Day 1	59.6	21.9	7.92	7.5				
	Day 2								
	Day 3								
	Day 4								
		58.9							
100 (89.4) mg/L	Day 0	91.0	22.0	7.89	8.0	781	72	68	ND
	Day 1	87.7	21.9	7.99	7.5		70	68	
	Day 2								
	Day 3								
	Day 4								
		89.4							

[a] Fluoride Analysis EPA 300.0

ND Not Detect; below detection limit

Attachment 7

Facilities with NPDES Permit Limits Based on the Incorrect Chronic Standard for Zinc

Attachment 7

Facilities with NPDES Permit Limits Based on the Incorrect Chronic Standard for Zinc

NPDES ID	Permit Name	Parameter Description	Limit Value	Limit Unit
IL0034631	ALCOA EXTRUSIONS INC	Zinc, total (as Zn)	.061	Milligrams per Liter
IL0021130	BLOOMINGDALE, VILLAGE OF	Zinc, total (as Zn)	.042	Milligrams per Liter
IL0032735	BOLINGBROOK, VILLAGE OF	Zinc, total (as Zn)	.084	Milligrams per Liter
IL0021083	CASEYVILLE TOWNSHIP EAST STP	Zinc, total (as Zn)	.038	Milligrams per Liter
IL0027979	CENTRALIA, CITY OF	Zinc, total (as Zn)	.028	Milligrams per Liter
IL0028321	DECATUR SD MAIN STP	Zinc, total (as Zn)	.075	Milligrams per Liter
IL0028517	DUQUOIN, CITY OF	Zinc, total (as Zn)	.054	Milligrams per Liter
IL0028622	EFFINGHAM, CITY OF	Zinc, total (as Zn)	.028	Milligrams per Liter
IL0034479	HANOVER PARK, VILLAGE OF	Zinc, total (as Zn)	.044	Milligrams per Liter
IL0029173	HIGHLAND, CITY OF	Zinc, total (as Zn)	.025	Milligrams per Liter
IL0026280	ITASCA, VILLAGE OF	Zinc, total (as Zn)	.045	Milligrams per Liter
IL0022519	JOLIET, CITY OF	Zinc, total (as Zn)	.073	Milligrams per Liter
IL0022055	LAKE COUNTY PUBLIC WORKS DEPA	Zinc, total (as Zn)	.053	Milligrams per Liter
IL0004073	MARATHON PETROLEUM COMPANY,	Zinc, total (as Zn)	.055	Milligrams per Liter
IL0029874	METROPOLIS, CITY OF	Zinc, total (as Zn)	.026	Milligrams per Liter
IL0036218	MONMOUTH, CITY OF	Zinc, total (as Zn)	.043	Milligrams per Liter
IL0078786	NL Properties, LLC	Zinc, total (as Zn)	.135	Milligrams per Liter
IL0035297	NUCOR STEEL INC-BOURBONNAIS	Zinc, total (as Zn)	.052	Milligrams per Liter
IL0021636	OFALLON, CITY OF	Zinc, total (as Zn)	.0379	Milligrams per Liter
IL0036382	ROCK ISLAND SW STP	Zinc, total (as Zn)	.048	Milligrams per Liter
IL0048721	ROSELLE, VILLAGE OF	Zinc, total (as Zn)	.04	Milligrams per Liter
IL0026859	SCOTT AIR FORCE BASE	Zinc, total (as Zn)	.044	Milligrams per Liter
IL0000329	US STEEL CORP GRANITE CITY WKS	Zinc, total (as Zn)	.17	Milligrams per Liter
IL0079073	VILLAGAE OF ITASCA	Zinc, total (as Zn)	0.45	Milligrams per Liter

Attachment 8

Agency Errata Sheet Numbers 1, 2 and 3 from R02-11

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:

WATER QUALITY AMENDMENTS TO)
35 Ill. Adm. Code 302.208(e)-(g), 302.504(a),) R02-11
302.575(d), 303.444, 309.141(h); and) (Rulemaking - Water)
PROPOSED 35 Ill. Adm. Code 301.267,)
301.313, 301.413, 304.120, and 309.157)

AGENCY'S ERRATA SHEET

THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY submits this ERRATA SHEET for the above-entitled matter to the Illinois Pollution Control Board and the participants on the Service List. The revisions suggested below are based on the Agency's ongoing review of the proposal.

Section 304.120 Deoxygenating Wastes

***

g) Compliance with the BOD$_5$ numerical standard in Part 304 ~~Section 304.120 for Publicly Owned Treatment Works, Publicly Regulated Treatment Works or other domestic sewage treatment works~~ will be determined by the analysis of 5 day carbonaceous biochemical oxygen demand (CBOD$_5$) (STORET number 80082). Effluent from the treatment works subject to the requirements of Section 304.120(a) shall not exceed 25 mg/L CBOD$_5$.

(Source: Amended at 13 Ill. Reg. 7754, effective May 4, 1989, amended in __________ at __________ Ill. Reg. ________________, effective ________________, 2002).

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:

WATER QUALITY AMENDMENTS TO)
35 Ill. Adm. Code 302.208(e)-(g), 302.504(a),) R02-11
302.575(d), 303.444, 309.141(h); and) (Rulemaking - Water)
PROPOSED 35 Ill. Adm. Code 301.267,)
301.313, 301.413, 304.120, and 309.157)

AGENCY'S ERRATA SHEET NUMBER 2

THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY submits this ERRATA SHEET NUMBER 2 for the above-entitled matter to the Illinois Pollution Control Board and the participants on the Service List. The revisions suggested below (double underlined) are based on the information gathered at the January 29, 2002 hearing and are in addition to the revisions suggested in the Agency's ERRATA SHEET.

Section 302.504 Chemical Constituents

The following concentrations of chemical constituents must not be exceeded, except as provided in Sections 302.102 and 302.530:

a) The following standards must be met in all waters of the Lake Michigan Basin. Acute aquatic life standards (AS) must not be exceeded at any time except for those waters for which the Agency has approved a zone of initial dilution (ZID) pursuant to Sections 302.102 and 302.530. Chronic aquatic life standards (CS) and human health standards (HHS) must not be exceeded outside of waters in which mixing is allowed pursuant to Section 302.102 and 302.530 by the arithmetic average of at least four consecutive samples collected over a period of at least four days. The samples used to demonstrate compliance with the CS or HHS must be collected in a manner which assures an average representation of the sampling period.

Constituent	STORET Number	Unit	AS	CS	HHS

Constituent	STORET Number	Unit	AS	CS	HHS
***					
Cadmium (dissolved)	01025	µg/L	exp[A +Bln(H)] X {1.138672-[(lnH)(0.041838)]}* A=-3.6867, and B=1.128	exp[A +Bln(H)] X {1.101672-[(lnH)(0.041838)]}* A = -2.715, and B = 0.7852	NA

***

(Source: Amended at 21 Ill. Reg. 1356, effective December 24, 1997, amended in __________ at __________ Ill. Reg. ________________, effective ________________, 2002)

***

Section 302.575 Procedures for Deriving Tier I Water Quality Criteria and Values in the Lake Michigan Basin to Protect Wildlife

***

d) Calculation of TSV. The TSV, measured in milligrams per liter (mg/L), is calculated according to the equation:

$$TSV = \{ [TD \times Wt] / [UF_a \times UF_s \times UF_l] \} \; / \; \{ W + \Sigma[F_{TLi} \times BAF_{WLTLi}] \}$$

Where:

TSV = target species value in milligrams of substance per liter (mg/L).
TD = test dose that is toxic to the test species, either NOAEL or LOAEL.
UF_a = the uncertainty factor for extrapolating toxicity data across species (unitless). A species-specific UF_a shall be selected and applied to each target species, consistent with the equation
UF_s = the uncertainty factor for extrapolating from subchronic to chronic exposures (unitless)
UF_l = the uncertainty factor for extrapolation from LOAEL to NOAEL (unitless)
Wt = average weight in kilograms (kg) of the target species
W = average daily volume of water in liters consumed per day (L/d) by the target species
F_{TLi} = average daily amount of food consumed by the target species in kilograms (kg/d) for trophic level i
BAF_{WLTLi} = aquatic life bioaccumulation factor with units of liter per kilogram

(L/kg), as derived in Section 302.570 <u>for trophic leveli</u>

(Source: Added at 21 Ill. Reg. 1356, effective December 24, 1997, amended in __________ at __________ Ill. Reg. _______________, effective ________________, 2002)

Respectfully Submitted

ILLINOIS ENVIRONMENTALPROTECTION AGENCY

By:________________________________

Sanjay K Sofat
Assistant Counsel
Division of Legal Counsel

DATED: February 20, 2002

Illinois Environmental Protection Agency
1021 North Grand Avenue East
P.O. Box 19276
Springfield, Illinois 62794-9276
(217) 782-5544

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:

WATER QUALITY AMENDMENTS TO 35 Ill. Adm. Code 302.208(e)-(g), 302.504(a), 302.575(d), 303.444, 309.141(h); and PROPOSED 35 Ill. Adm. Code 301.267, 301.313, 301.413, 304.120, and 309.157	)))))	R02-11 (Rulemaking - Water)

AGENCY'S ERRATA SHEET NUMBER 3

THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY submits this ERRATA SHEET NUMBER 3 for the above-entitled matter to the Illinois Pollution Control Board and the participants on the Service List. The revisions suggested below are based on the Agency's ongoing review of the proposal and the information gathered at the January 29, 2002 hearing. These revisions are in addition to the revisions suggested in the Agency's ERRATA SHEET and ERRATA SHEET NUMBER 2.

Section 302.208 Numeric Standards for Chemical Constituents

***

b) The chronic standard (CS) for the chemical constituents listed in subsection (e) shall not be exceeded by the arithmetic average of at least four consecutive samples collected over any period of at least four days, except as provided in subsection (d). The samples used to demonstrate attainment ~~compliance~~ or lack of attainment ~~compliance~~ with a CS must be collected in a manner that ~~which~~ assures an average representative of the sampling period. For the metals that have water quality based standards dependent upon hardness, the chronic water quality standard will be calculated according to subsection (e) using the hardness of the water body at the time the metals sample was collected. To calculate attainment status of chronic metals standards, the concentration of the metal in each sample is divided by the calculated water quality standard for the sample to determine a quotient. The water quality standard is attained if the mean of the sample quotients is less than or equal to one for the duration of the averaging period.

***

e) Numeric Water Quality Standards for the Protection of Aquatic Organisms

Constituent	Storet Number	AS (µg/L) (~~ug/L~~)	CS (µg/L) (~~ug/L~~)
Cyanide (weak acid dissociable)	00718	49 ~~22~~	11 ~~9.9~~ ~~5.2~~
Zinc (dissolved)	01090	exp[A+Bln(H)] X 0.978* where A= 0.9035 ~~0.8875~~, and B=0.8473	exp[A+Bln(H)] X 0.986* where A=- 0.8165 ~~0.8227~~, and B=0.8473

where: µg/L ~~ug/L~~ = microgram per liter,

exp[x] = base natural ~~neutral~~ logarithms raised to the x- power, and

ln(H) = natural logarithm of Hardness (STORET 00900).

* = conversion factor multiplier for dissolved metals

(Source: Amended at 20 Ill. Reg.7682, effective May 24, 1996; amended in ___________ at
___________ Ill. Reg. ________________, effective ________________, 2002)

***

Section 304.120 Deoxygenating Wastes

***

g) Compliance with the BOD_5 numerical standard in Part 304 ~~Section 304.120 for Publicly Owned Treatment Works, Publicly Regulated Treatment Works or other domestic~~

~~sewage treatment works~~ will be determined by the analysis of 5 day carbonaceous biochemical oxygen demand (CBOD$_5$) (STORET number 80082), unless federal regulations require treatment works treating industrial wastes to comply with more stringent requirements determined by the analysis of 5 day biochemical oxygen demand (BOD$_5$). Effluent from the treatment works subject to the requirements of Section 304.120(a) shall not exceed 25 mg/L CBOD$_5$.

(Source: Amended at 13 Ill. Reg. 7754, effective May 4, 1989, amended in __________ at __________ Ill. Reg. _______________, effective ________________, 2002).

Respectfully Submitted

ILLINOIS ENVIRONMENTALPROTECTION AGENCY

By:________________________________

Sanjay K Sofat
Assistant Counsel
Division of Legal Counsel

DATED: March 6, 2002

Illinois Environmental Protection Agency
1021 North Grand Avenue East
P.O. Box 19276
Springfield, Illinois 62794-9276
(217) 782-5544