

## MINUTES FROM WHOLE EFFLUENT TOXICITY (WET) SUBCOMMITTEE

Subcommittee to The NELAC Institute's (TNI's) Proficiency Testing Board

Date: June 5, 2008

The meeting was called the order at 11:30 AM EST by RaeAnn Haynes, the elected Chair for this Subcommittee.

The Subcommittee members present were (in addition to the Chair) Faust Parker, Jim Pletl, and Shawn Kassner (for Jeff Lowry), Lori Pillsbury (ODEQ), and Chris Rucinski. RaeAnn Haynes served to take minutes for this meeting.

Minutes from the 5/15 meeting were approved. R. Haynes will send all approved minutes to TNI for posting.

### **Action Items:**

The committee reviewed the old action items. R. Haynes has not contacted the Regional Lab group with the questions about their support of changes to the WET PT program. An additional question was added.

R. Haynes did contact the third provider of WET PT's and was assured that the data will be sent soon. It has not yet been received, however. The subcommittee my have to move forward with the data received to date.

R Haynes did ask the PT Board to consider asking EPA DMRQA specialist Patrick Yellin to the August meeting to participate in a special meeting about the future role of TNI in the DMRQA program. However, the topic did not get on the future agenda for the PT Board or raised to the TNI Board. After a further discussion at the subcommittee level, C. Kircher will add this topic to the future PT Board agenda.

### **Data Review:**

Dr. C. Kircher lead the subcommittee through his analysis of the large spreadsheet of data put together by J. Lowry. A summary of the analysis is attached to the minutes.

The ACTION ITEMS from this meeting are as follows:

- R. Haynes will contact the EPA Regional Lab group about how to speak to them on a teleconference with a preview of the 8 questions from the subcommittee.

The next teleconference for this Subcommittee will be Thursday, June 19th at 11:30 AM EST (or equivalent Local Time).

## Attachment A

### CARL KIRCHER'S ANALYSIS OF BIG EXCEL SPREADSHEET OF WHOLE EFFLUENT TOXICITY PT DATA

The following is my "data reduction" and analysis of the WET PT data submitted by Jeff Lowry. The reduced data and numbers below resulted from my combining the PT results for the same concentrations (or similar concentrations) of reference toxicants where statistically indistinguishable PT results are observed. The tabulation does not pay attention to the actual numbers of PT studies for a given ref. toxicant concentration or range. The PT analysis also does not pay attention to the actual number of participants in a given PT study, UNLESS a study with obvious outlier acceptance range values relative to the other studies had few actual participants (e.g., 5 participants).

Pimephales promelas	LC50	20 C	MHSF	Acute 48-hr
Ref. Tox. concentration		Mean	+/-	2 Std. Dev.
1.8-2.0 g/L KCl		(29.7 to 42.9 %)	+/-	2 (6.5 to 15.4 %)
0.0088 g/L ZnSO4.7H2O		(41.9 to 59.3 %)	+/-	2 (21.4 to 28.4 %)
0.40 g/L Phenol		(20.1 to 21.6 %)	+/-	2 (12.2 to 16.5 %)
0.32 g/L (NH4)2HPO4		36.4 %	+/-	2 (11.9 %)
0.40 g/L (NH4)2HPO4		(25.6 to 29.0 %)	+/-	2 (9.3 to 9.9 %)
0.48 g/L (NH4)2HPO4		26.4 %	+/-	2 (6.9 %)

Pimephales promelas	LC50	25 C	MHSF	Acute 48-hr
Ref. Tox. concentration		Mean	+/-	2 Std. Dev.
1.8-2.0 g/L KCl		(27.2 to 49.3 %)	+/-	2 (9.0 to 13.5 %)
0.0044 g/L ZnSO4.7H2O		(43.7 to 50.4 %)	+/-	2 (10.3 to 22.1 %)
0.20 g/L Phenol		(20.8 to 24.0 %)	+/-	2 (10.3 to 14.3 %)
0.32 g/L (NH4)2HPO4		37.1 %	+/-	2 (11.6 %)
0.40 g/L (NH4)2HPO4		(27.8 to 29.2 %)	+/-	2 (8.8 to 9.4 %)
0.48 g/L (NH4)2HPO4		28.3 %	+/-	2 (8.3 %)

Pimephales promelas	LC50	25 C	20% DMW	Acute 48-hr
Ref. Tox. concentration		Mean	+/-	2 Std. Dev.
1.8-2.0 g/L KCl		(56.2 to 59.4 %)	+/-	2 (11.3 to 15.2 %)
0.0088 g/L ZnSO4.7H2O		(26.9 to 35.6 %)	+/-	2 (8.9 to 19.2 %)
0.20 g/L Phenol		(17.3 to 22.4 %)	+/-	2 (10.6 to 14.7 %)
0.25 g/L Phenol		21.8 %	+/-	2 (16.7 %)
0.24 g/L (NH4)2HPO4		41.7 %	+/-	2 (15.0 %)
0.30 g/L (NH4)2HPO4		(29.7 to 34.5 %)	+/-	2 (6.1 to 13.5 %)
0.36 g/L (NH4)2HPO4		32.2 %	+/-	2 (5.3 %)
0.40 g/L (NH4)2HPO4		36.1 %	+/-	2 (6.0 %)

#### CCK Observations (Pimephales Acute):

As temperature increased from 20 C to 25 C, mean was not affected when KCl & (NH4)2HPO4 were reference toxicants, but mean decreased when Zn & Phenol were reference toxicants.

As MHSF was changed to 20% DMW at 25 C, the mean increased when KCl was ref. tox., decreased when (NH4)2HPO4 was ref. tox., but was unaffected when Phenol was ref. tox. (cannot tell for Zn).

Pimephales promelas	NOEC Survival	MHSF	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
	1.6-2.0 g/L KCl	25 %	12.5 – 50 %
	0.0022 g/L ZnSO4.7H2O	25 %	12.5 – 50 %
	0.050 g/L Phenol	25 %	12.5 – 50 %
%)	0.15-0.18 g/L (NH4)2HPO4	25 – 50 %	(12.5 to 25) – (50 to 100

Pimephales promelas	NOEC Survival	20% DMW	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
	1.6-3.0 g/L KCl	25 %	12.5 – 50 %
%)	0.0044 g/L ZnSO4.7H2O	25 – 50 %	(12.5 to 25) – (50 to 100
	0.050 g/L Phenol	25 %	12.5 – 50 %
	0.15 g/L (NH4)2HPO4	25 %	12.5 – 50 %

Pimephales promelas	NOEC(ON) Growth	MHSF	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
	1.6-2.0 g/L KCl	25 %	12.5 – 50 %
	0.0022 g/L ZnSO4.7H2O	25 %	12.5 – 50 %
	0.050 g/L Phenol	25 %	12.5 – 50 %
	0.15-0.18 g/L (NH4)2HPO4	25 %	12.5 – 50 %

Pimephales promelas	NOEC(ON) Growth	20% DMW	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
	1.6 g/L KCl	50 %	25 – 100 %
	1.8-3.0 g/L KCl	25 %	12.5 – 50 %
%)	0.0044 g/L ZnSO4.7H2O	25 – 50 %	(12.5 to 25) – (50 to 100
	0.050 g/L Phenol	25 %	12.5 – 50 %
	0.15 g/L (NH4)2HPO4	12.5 %	6.25 – 25 %

Pimephales promelas	NOEC(SN) Growth	MHSF	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
	1.6-2.0 g/L KCl	25 %	12.5 – 50 %
	0.0022 g/L ZnSO4.7H2O	25 %	12.5 – 50 %
	0.050 g/L Phenol	25 %	12.5 – 50 %

	0.15-0.18 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	25 %		12.5 – 50 %
Pimephales promelas	NOEC(SN) Growth	20% DMW		Chronic 7-day
Limit	Ref. Tox. concentration	Median		Lower limit – Upper
	1.6-1.8 g/L KCl	50 %		25 – 100 %
	2.0-3.0 g/L KCl	25 %		12.5 – 50 %
%)	0.0044 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	25 – 50 %		(12.5 to 25) – (50 to 100
	0.050 g/L Phenol	25 %		12.5 – 50 %
	0.15 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	12.5 %		6.25 – 25 %
Pimephales promelas	IC <sub>25</sub> (ON) Growth	MHSF		Chronic 7-day
	Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
	1.6 g/L KCl	39.7 %	+/-	2 (12.7 %)
	1.8 g/L KCl	34.9 %	+/-	2 (8.3 %)
	2.0 g/L KCl	(29.0 to 29.9 %)	+/-	2 (3.2 to 4.0 %)
	0.0022 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(36.8 to 38.9 %)	+/-	2 (13.8 to 17.6 %)
	0.050 g/L Phenol	(32.2 to 32.9 %)	+/-	2 (12.0 to 14.0 %)
	0.15-0.18 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(39.5 to 40.9 %)	+/-	2 (13.2 to 14.8 %)
Pimephales promelas	IC <sub>25</sub> (ON) Growth	20% DMW		Chronic 7-day
	Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
	1.6 g/L KCl	49.9 %	+/-	2 (12.1 %)
	1.8-2.0 g/L KCl	(35.7 to 44.7 %)	+/-	2 (8.7 to 16.1 %)
	2.4 g/L KCl	33.7 %	+/-	2 (8.1 %)
	3.0 g/L KCl	26.2 %	+/-	2 (6.5 %)
	0.0044 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(42.4 to 49.7 %)	+/-	2 (13.1 to 15.3 %)
	0.050 g/L Phenol	(28.7 to 30.4 %)	+/-	2 (5.6 to 13.5 %)
	0.15 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	28.3 %	+/-	2 (14.7 %)
Pimephales promelas	IC <sub>25</sub> (SN) Growth	MHSF		Chronic 7-day
	Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
	1.6-1.8 g/L KCl	(52.1 to 56.3 %)	+/-	2 (13.4 to 13.4 %)
	2.0 g/L KCl	(31.1 to 41.7 %)	+/-	2 (6.0 to 18.2 %)
	0.0022 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(37.7 to 43.1 %)	+/-	2 (14.4 to 18.3 %)
	0.050 g/L Phenol	(37.8 to 41.1 %)	+/-	2 (14.2 to 16.5 %)
	0.15-0.18 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(43.2 to 45.0 %)	+/-	2 (13.8 to 15.0 %)
Pimephales promelas	IC <sub>25</sub> (SN) Growth	20% DMW		Chronic 7-day
	Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
	1.6-2.0 g/L KCl	(35.3 to 59.7 %)	+/-	2 (4.3 to 18.1 %)
	2.4-3.0 g/L KCl	(26.7 to 32.9 %)	+/-	2 (8.6 to 11.6 %)
	0.0044 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(37.9 to 66.0 %)	+/-	2 (12.4 to 32.5 %)
	0.050 g/L Phenol	(37.3 to 40.3 %)	+/-	2 (7.4 to 19.0 %)
	0.15 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	30.4 %	+/-	2 (16.6 %)

CCK Observations (Pimephales Chronic):

Overall, in comparing the PT acceptance limits between the ON results and the SN results, no impacts were observed. There may be some impacts observed for the 20% DMW NOEC endpoint for (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> as ref. tox., but the differences may be reflected by the nature of how ON is defined in relation to how SN is defined.

The switch from MHSF to 20% DMW appears to have no influence on the PT acceptance criteria for the NOEC Survival, NOEC(ON) Growth, and NOEC(SN) Growth. However, since the reported values for NOEC can only be <6.25%, 6.25%, 12.5%, 25%, 50%, 100%, and >100%, discernable trends may not be discerned.

For IC<sub>25</sub>(ON) Growth, the switch from MHSF to 20% DMW increases the mean % when KCl is ref. tox. and decreases the mean % when Phenol and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> are ref. toxicants.

For IC<sub>25</sub>(SN) Growth, the switch from MHSF to 20% DMW increases the mean % when Zn is ref. tox. and decreases the mean % when (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> is ref. tox.

Ceriodaphnia dubia	LC50	20 C	MHSF	Acute 48-hr
	Ref. Tox. concentration	Mean	+/- 2	Std. Dev.
	0.9-1.2 g/L KCl	(17.0 to 30.9 %)	+/- 2	(1.2 to 18.3 %)
	0.0022 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(27.5 to 28.6 %)	+/- 2	(13.1 to 14.9 %)
	0.12 g/L Phenol	(18.1 to 20.3 %)	+/- 2	(8.0 to 8.6 %)
	0.30 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(34.1 to 46.1 %)	+/- 2	(19.4 to 20.2 %)
Ceriodaphnia dubia	LC50	25 C	MHSF	Acute 48-hr
	Ref. Tox. concentration	Mean	+/- 2	Std. Dev.
	0.9-1.4 g/L KCl	(36.3 to 49.7 %)	+/- 2	(11.6 to 19.0 %)
	0.0022 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(27.8 to 37.5 %)	+/- 2	(11.6 to 16.4 %)
	0.015 g/L Phenol	(32.2 to 42.2 %)	+/- 2	(18.0 to 21.0 %)
	0.20 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(44.8 to 52.2 %)	+/- 2	(19.4 to 20.8 %)
Ceriodaphnia dubia	LC50	25 C	20% DMW	Acute 48-hr
	Ref. Tox. concentration	Mean	+/- 2	Std. Dev.
	0.5-0.6 g/L KCl	(39.5 to 42.5 %)	+/- 2	(14.3 to 17.0 %)
	0.8-1.0 g/L KCl	(17.1 to 27.3 %)	+/- 2	(2.9 to 10.6 %)
	1.2-1.4 g/L KCl	(16.1 to 18.3 %)	+/- 2	(3.8 to 8.5 %)
	0.0022 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(22.5 to 43.2 %)	+/- 2	(9.5 to 15.2 %)
	0.060 g/L Phenol	(25.8 to 27.0 %)	+/- 2	(6.7 to 12.0 %)
	0.20 g/L (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	(43.0 to 50.7 %)	+/- 2	(22.4 to 24.2 %)

CCK Observations (Ceriodaphnia Acute):

As temperature increased from 20 C to 25 C, mean was affected when KCl was the reference toxicant (mean increased). No discernable effects were observed for the other reference toxicants.

As MHSF was changed to 20% DMW at 25 C, the mean decreased when KCl was ref. tox., but no discernable effects were observed for the other reference toxicants.

Ceriodaphnia dubia	NOEC Survival	MHSF	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper

	0.7-0.8 g/L KCl	50 %	25 – 100 %
	1.0-1.4 g/L KCl	25 %	12.5 – 50 %
	0.0015-0.0022 g/L ZnSO4.7H2O	25 %	12.5 – 50 %
%)	0.010 g/L Phenol	25 – 50 %	(12.5 to 25) – (50 to 100
%)	0.20 g/L (NH4)2HPO4	25 – 50 %	(12.5 to 25) – (50 to 100

Ceriodaphnia dubia	NOEC Survival	20% DMW	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
	0.6-0.8 g/L KCl	25 %	12.5 – 50 %
50 %)	1.0-1.4 g/L KCl	12.5 – 25 %	(6.25 to 12.5) – (25 to
	0.0015 g/L ZnSO4.7H2O	50 %	25 – 100 %
50 %)	0.0022 g/L ZnSO4.7H2O	12.5 – 25 %	(6.25 to 12.5) – (25 to
>100 %)	0.010 g/L Phenol	50 – 100 %	(25 to 50) – (100 to
	0.20 g/L (NH4)2HPO4	50 %	25 – 100 %

Ceriodaphnia dubia	NOEC Reproduction	MHSF	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
	0.7-1.4 g/L KCl	25 %	12.5 – 50 %
	0.0015-0.0022 g/L ZnSO4.7H2O	12.5 %	6.25 – 25 %
	0.010 g/L Phenol	25 %	12.5 – 50 %
50 %)	0.20 g/L (NH4)2HPO4	12.5 – 25 %	(6.25 to 12.5) – (25 to

Ceriodaphnia dubia	NOEC Reproduction	20% DMW	Chronic 7-day
Limit	Ref. Tox. concentration	Median	Lower limit – Upper
50 %)	0.6-0.8 g/L KCl	12.5 – 25 %	(6.25 to 12.5) – (25 to
	1.0-1.4 g/L KCl	12.5 %	6.25 – 25 %
	0.0015 g/L ZnSO4.7H2O	25%	12.5 – 50 %
to 25 %)	0.0022 g/L ZnSO4.7H2O	6.25 – 12.5 %	(<6.25 to 6.25) – (12.5
	0.010 g/L Phenol	25 %	12.5 – 50 %
50 %)	0.20 g/L (NH4)2HPO4	12.5 – 25 %	(6.25 to 12.5) – (12.5 to

Ceriodaphnia dubia	IC25 Reproduction	MHSF	Chronic 7-day
	Ref. Tox. concentration	Mean	+/- 2 Std. Dev.
	0.7-0.8 g/L KCl	(42.0 to 42.2 %)	+/- 2 (15.3 to 17.7 %)
	1.0 g/L KCl	(27.2 to 31.5 %)	+/- 2 (7.2 to 11.7 %)
	1.2-1.4 g/L KCl	(26.0 to 26.4 %)	+/- 2 (5.7 to 7.1 %)

0.0015 g/L ZnSO4.7H2O	25.9 %	+/-	2 (13.8 %)
0.0022 g/L ZnSO4.7H2O	(15.7 to 17.6 %)	+/-	2 (7.6 to 8.6 %)
0.010 g/L Phenol	(26.4 to 29.6 %)	+/-	2 (13.6 to 15.3 %)
0.20 g/L (NH4)2HPO4	(23.5 to 23.7 %)	+/-	2 (13.4 to 18.2 %)
<b>Ceriodaphnia dubia</b>	<b>IC25 Reproduction</b>	<b>20% DMW</b>	<b>Chronic 7-day</b>
Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
0.6-0.8 g/L KCl	(21.2 to 28.7 %)	+/-	2 (3.7 to 8.5 %)
1.0-1.4 g/L KCl	(14.1 to 18.0 %)	+/-	2 (2.0 to 7.7 %)
0.0015 g/L ZnSO4.7H2O	33.0 %	+/-	2 (13.1 %)
0.0022 g/L ZnSO4.7H2O	(12.0 to 14.7 %)	+/-	2 (5.1 to 7.8 %)
0.010 g/L Phenol	(34.1 to 41.2 %)	+/-	2 (17.9 to 20.9 %)
0.20 g/L (NH4)2HPO4	(21.7 to 24.3 %)	+/-	2 (13.0 to 14.0 %)

**CCK Observations (Ceriodaphnia Chronic):**

The switch from MHSF to 20% DMW appears to have no influence on the PT acceptance criteria for the NOEC Survival when the ref. tox. is Zn. However, the median increases for Phenol as the ref. tox. but decreases when KCl and (NH4)2HPO4 are the ref. toxicants.

For IC25 Reproduction, the switch from MHSF to 20% DMW increases the mean % when Phenol is ref. tox. and decreases the mean % when KCl is the ref. toxicant.

For NOEC Reproduction, the switch from MHSF to 20% DMW decreases the mean % when KCl is ref. tox.

<b>Daphnia magna</b>	<b>LC50</b>	<b>20 C</b>	<b>MHSF</b>	<b>Acute 48-hr</b>
Ref. Tox. concentration	Mean	+/-	2	Std. Dev.
1.0-1.2 g/L KCl	(59.4 to 69.9 %)	+/-	2 (7.2 to 18.8 %)	
1.4-1.8 g/L KCl	(45.9 to 50.3 %)	+/-	2 (11.9 to 16.2 %)	
2.0 g/L KCl	35.0 %	+/-	2 (2.7 %)	
0.0088 g/L ZnSO4.7H2O	(26.6 to 30.1 %)	+/-	2 (11.0 to 14.5 %)	
0.040 g/L Phenol	(26.0 to 28.5 %)	+/-	2 (12.2 to 13.5 %)	
0.40 g/L (NH4)2HPO4	(50.4 to 56.4 %)	+/-	2 (14.9 to 22.3 %)	

<b>Daphnia pulex</b>	<b>LC50</b>	<b>20 C</b>	<b>MHSF</b>	<b>Acute 48-hr</b>
Ref. Tox. concentration	Mean	+/-	2	Std. Dev.
1.0 g/L KCl	(56.6 to 70.4 %)	+/-	2 (13.0 to 30.8 %)	
1.2-1.6 g/L KCl	(49.6 to 52.3 %)	+/-	2 (14.5 to 19.9 %)	
0.0088 g/L ZnSO4.7H2O	(35.4 to 43.8 %)	+/-	2 (15.7 to 22.8 %)	
0.080 g/L Phenol	(30.7 to 33.1 %)	+/-	2 (13.7 to 17.1 %)	
0.40 g/L (NH4)2HPO4	(42.6 to 44.1 %)	+/-	2 (17.6 to 18.6 %)	

<b>Daphnia pulex</b>	<b>LC50</b>	<b>25 C</b>	<b>MHSF</b>	<b>Acute 48-hr</b>
Ref. Tox. concentration	Mean	+/-	2	Std. Dev.
1.0-1.4 g/L KCl	(51.0 to 60.2 %)	+/-	2 (14.6 to 21.2 %)	
1.6 g/L KCl	39.4 %	+/-	2 (11.1 %)	
0.0088 g/L ZnSO4.7H2O	(26.5 to 29.1 %)	+/-	2 (11.7 to 14.8 %)	
0.040 g/L Phenol	(32.0 to 37.5 %)	+/-	2 (20.1 to 25.0 %)	
0.40 g/L (NH4)2HPO4	(39.8 to 42.7 %)	+/-	2 (15.5 to 22.2 %)	

CCK Observations (Daphnia pulex):

Increasing the test temperature from 20 C to 25 C decreases the mean for all the reference toxicants listed.

CCK Observations (across the board for Freshwater Toxicity):

It will not be possible to combine test species in order to make common acceptance criteria for Freshwater Acute or for Freshwater Chronic Toxicity. For example, under the same test conditions the Mean LC50 is lower for Daphnia species than for Pimephales promelas when Phenol is the reference toxicant, but the Mean LC50 is higher for Daphnia species than for Pimephales promelas when Ammonium Phosphate is the reference toxicant.

As another example, the Medians for NOEC Survival (chronic) appear to be the same for Pimephales promelas and for Ceriodaphnia dubia when the Zn and Ammonium Phosphate reference toxicant concentrations are the same, but higher concentrations of KCl and Phenol as reference toxicants are required for Pimephales, in order to produce the same Median for NOEC Survival as for Ceriodaphnia.

Mysidopsis bahia Acute 48-hr	LC50	20 C	40-fathoms seawater		
Ref. Tox. concentration		Mean	+/-	2	Std. Dev.
0.5 g/L KCl		(68.7 to 78.2 %)	+/-	2	(15.5 to 16.6 %)
1.2-1.4 g/L KCl		(43.0 to 55.6 %)	+/-	2	(12.7 to 19.5 %)
1.5-1.6 g/L KCl		(33.2 to 36.7 %)	+/-	2	(4.5 to 5.7 %)
0.0176 g/L ZnSO4.7H2O		(37.1 to 43.7 %)	+/-	2	(15.8 to 22.6 %)
0.080 g/L Phenol		(29.2 to 31.9 %)	+/-	2	(12.1 to 12.4 %)
0.0005 g/L CuSO4		(36.0 to 40.8 %)	+/-	2	(15.4 to 20.5 %)

  

Mysidopsis bahia 7-day	NOEC Survival	40-fathoms seawater		Chronic
Limit	Ref. Tox. concentration	Median	Lower limit – Upper	
	0.5 g/L KCl	50 %	25 – 100 %	
	1.1-1.4 g/L KCl	25 %	12.5 – 50 %	
	0.00264 g/L ZnSO4.7H2O	50 %	25 – 100 %	
	0.00025 g/L CuSO4	25 %	12.5 – 50 %	
	0.0005 g/L CuSO4	12.5 % (to 100%!?)	6.25 – 25 %	

  

Mysidopsis bahia 7-day	NOEC(ON) Growth	40-fathoms seawater		Chronic
Limit	Ref. Tox. concentration	Median	Lower limit – Upper	
	0.5 g/L KCl	100 %	50 – >100 %	
	1.1-1.4 g/L KCl	25 – 50 %	(12.5 to 25) – (50 to 100) %	
	0.00264 g/L ZnSO4.7H2O	25 %	12.5 – 50 %	
	0.00025 g/L CuSO4	100 %	50 – >100 %	

  

Mysidopsis bahia 7-day	NOEC(SN) Growth	40-fathoms seawater		Chronic
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Limit	Ref. Tox. concentration	Median	Lower limit – Upper
%	0.5 g/L KCl	50 %	25 – 100 %
	1.1-1.4 g/L KCl	25 – 50 %	(12.5 to 25) – (50 to 100)
%)	0.00264 g/L ZnSO4.7H2O	25 – 50 %	(12.5 to 25) – (50 to 100)
>100 %)	0.0066 g/L ZnSO4.7H2O	12.5 %	6.25 – 25 %
	0.00025 g/L CuSO4	25 – 100 %	(6.25 to 50) – (25 to
	0.0005 g/L CuSO4	12.5 %	6.25 – 25 %

Mysidopsis bahia 7-day	IC25(ON) Growth	40-fathoms seawater	Chronic
Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
0.5 g/L KCl	51.6 %	+/-	2 (17.2 %)
1.1-1.4 g/L KCl	(36.2 to 45.6 %)	+/-	2 (9.1 to 21.6 %)
0.00264 g/L ZnSO4.7H2O	(39.2 to 50.8 %)	+/-	2 (12.9 to 18.8 %)
0.0066 g/L ZnSO4.7H2O	33.3 %	+/-	2 (23.2 %)
0.00025 g/L CuSO4	70.5 %	+/-	2 (30.6 %)

Mysidopsis bahia 7-day	IC25(SN) Growth	40-fathoms seawater	Chronic
Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
0.5 g/L KCl	(62.3 to 65.3 %)	+/-	2 (17.8 to 20.1 %)
1.1-1.4 g/L KCl	(36.6 to 47.1 %)	+/-	2 (8.8 to 20.0 %)
0.00264 g/L ZnSO4.7H2O	(48.0 to 72.9 %)	+/-	2 (21.9 to 27.3 %)
0.0066 g/L ZnSO4.7H2O	18.0 %	+/-	2 (9.8 %)
0.00025 g/L CuSO4	(39.1 to 68.0 %)	+/-	2 (18.1 to 32.0 %)
0.0005 g/L CuSO4	21.9 %	+/-	2 (11.4 %)

Menidia beryllina Acute 48-hr	LC50	20 C	40-fathoms seawater
Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
1.0 g/L KCl	(81.1 to 91.9 %)	+/-	2 (8.5 to 19.5 %)
0.0353 g/L ZnSO4.7H2O	(37.8 to 68.7 %)	+/-	2 (14.2 to 34.1 %)
0.050 g/L Phenol	30.8 %	+/-	2 (5.4 %)
0.060 g/L Phenol	(21.5 to 26.4 %)	+/-	2 (3.9 to 7.2 %)
0.0005 g/L CuSO4	(17.3 to 21.6 %)	+/-	2 (9.0 to 11.0 %)

Cyprinodon variegatus Acute 48-hr	LC50	20 C	40-fathoms seawater
Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
4.8 g/L KCl	44.9 %	+/-	2 (14.5 %)
5.4 g/L KCl	34.9 %	+/-	2 (1.5 %)
6.0-7.2 g/L KCl	(25.0 to 30.3 %)	+/-	2 (5.2 to 9.0 %)
0.0110 g/L ZnSO4.7H2O	(74.7 to 100 %)	+/-	2 (19.1 to 49 %)

	0.110 g/L ZnSO4.7H2O	(38.2 to 42.2 %)	+/-	2 (21.3 to 24.5 %)
	0.080 g/L Phenol	80.8 %	+/-	2 (27.3 %)
	0.80 g/L Phenol	13.1 %	+/-	2 (6.6 %)
Cyprinodon variegatus	NOEC Survival		40-fathoms seawater	Chronic
7-day				
Limit	Ref. Tox. concentration	Median	Lower limit – Upper	
	2.4 g/L KCl	50 %	25 – 100 %	
	3.0-3.6 g/L KCl	25 %	12.5 – 50 %	
%)	0.00662 g/L ZnSO4.7H2O	25 – 50 %	(12.5 to 25) – (50 to 100	
	0.0265 g/L ZnSO4.7H2O	6.25 %	<6.25 – 12.5 %	
	0.00005 g/L CuSO4	12.5 %	6.25 – 25 %	
Cyprinodon variegatus	NOEC(ON) Growth		40-fathoms seawater	Chronic
7-day				
Limit	Ref. Tox. concentration	Median	Lower limit – Upper	
	2.4 g/L KCl	50 %	25 – 100 %	
	3.0-3.6 g/L KCl	25 %	12.5 – 50 %	
%)	0.00662 g/L ZnSO4.7H2O	25 – 50 %	(12.5 to 25) – (50 to 100	
	0.0265 g/L ZnSO4.7H2O	6.25 %	<6.25 – 12.5 %	
Cyprinodon variegatus	NOEC(SN) Growth		40-fathoms seawater	Chronic
7-day				
Limit	Ref. Tox. concentration	Median	Lower limit – Upper	
	2.4 g/L KCl	50 %	25 – 100 %	
	3.0-3.6 g/L KCl	25 – 50 %	(12.5 to 25) – (50 to 100	
%)	0.00662 g/L ZnSO4.7H2O	25 – 100(1x) %	(12.5 to 50) – (50 to	
>100 %)	0.0265 g/L ZnSO4.7H2O	6.25 %	<6.25 – 12.5 %	
	0.00005 g/L CuSO4	12.5 %	6.25 – 25 %	
Cyprinodon variegatus	IC25(ON) Growth		40-fathoms seawater	Chronic
7-day				
	Ref. Tox. concentration	Mean	+/-	2 Std. Dev.
	2.4 g/L KCl	58.0 %	+/-	2 (4.4 %)
	3.0 g/L KCl	(39.7 to 44.4 %)	+/-	2 (7.2 to 15.0 %)
	3.6 g/L KCl	32.4 %	+/-	2 (8.0 %)
	0.00662 g/L ZnSO4.7H2O	(37.1 to 50.5 %)	+/-	2 (13.0 to 25.5 %)
	0.0265 g/L ZnSO4.7H2O	9.8 %	+/-	2 (3.7 %)
Cyprinodon variegatus	IC25(SN) Growth		40-fathoms seawater	Chronic
7-day				

Ref. Tox. concentration	Mean	+/-	2	Std. Dev.
2.4 g/L KCl	60.8 %	+/-	2 (1.7 %)	
3.0-3.6 g/L KCl	(35.5 to 54.1 %)	+/-	2 (4.8 to 16.4 %)	
0.00662 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	(18.1 to 100 %)	+/-	2 (5.0 to 25.0 %)	
0.0265 g/L ZnSO <sub>4</sub> .7H <sub>2</sub> O	30.7 %	+/-	2 (19.1 %)	
0.00005 g/L CuSO <sub>4</sub>	20.8 %	+/-	2 (11.8 %)	

CCK Observations (Saltwater Acute and Chronic):

There is not enough data to look for trends or to make comparisons. We would be comparing apples and oranges otherwise.

CCK Conclusions:

I think there is not enough PT data to determine acceptance criteria based on actual laboratory performance rather than a consensus mean or consensus median. The existing data is best characterized with reference toxicants that produce effluent percentages only in the 25-50% range for the various test endpoints. Part of the reason for this shortcoming is that the Toxicity measurements are based on 5 dilutions, with much more laboratory measurements in the 0-50% effluent range than in the 50-100% range. The computer programs associated with calculating Toxicity results may be subject to inherent assumptions that, although not as important in determining a detrimental impact to a lake, stream, or estuary, may become critically important in determining PT acceptance criteria.

I believe we need a few accredited Toxicity laboratories that would be willing to submit their control chart data for their test species, test conditions, endpoints, and reference toxicants. If it turns out, for example, that all laboratories have about the same LC50 and 2-sigma variance for the same test species, conditions, endpoints, and reference toxicant, then that data can be used to formulate a PT sample such that an Assigned Value for Effluent %, with standard deviation, can be reliably known and verified.