FINAL REPORT

Chironomus dilutus and *Hyalella azteca*
10-day Whole Sediment Toxicity Testing Results
Kalamazoo River Sediment Sampling Line 6B Oil Spill
Marshall, Michigan

for:

Enbridge Energy
Limited Partnership (Enbridge)
1601 Pratt Avenue
Marshall, MI 49068

Prepared by:

Great Lakes Environmental Center

739 Hastings Street
Traverse City, Michigan 49686
Phone: 231.941.2230
Fax: 231.941.2240

Contact:

G.M. DeGraeve
231.941.2230
mick@glec.com

June 10, 2012

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Marshall, Michigan

Great Lakes Environmental Center, Inc. (GLEC) has completed our analysis of the *Chironomus dilutus* and *Hyalella azteca* 10-day survival and growth whole sediment toxicity tests. These tests were performed with thirty sediment samples that were collected by AECOM personnel during February 2012 from the Enbridge Energy, Limited Partnership (Enbridge) site, Kalamazoo River, Line 6B Spill, Marshall, Michigan.

The sample identification numbers, survival, and growth test results for the thirty sediment samples and a laboratory control are summarized and provided in the following tables:

- Table 1: 10-Day *Chironomus dilutus* (*C. dilutus*) Average Percent Survival
- Table 2: 10-Day *C. dilutus* Average Growth and Biomass Estimates (expressed as average ash-free-dry-weight (AFDW))
- Table 3: 10-Day *Hyalella azteca* (*H. azteca*) Average Percent Survival
- Table 4: 10-Day *H. azteca* Average Growth and Biomass Estimates

Water quality data for the overlying water for each sediment sample tested are summarized in Table 5 for the *C. dilutus* tests and Table 6 for the *H. azteca* tests. Summaries of the statistically significant differences (p=0.05) between the laboratory controls and all thirty sediment samples, and between six pooled upstream reference sites from the Kalamazoo River are provided in Table 7 for the *C. dilutus* tests, and in Table 8 for the *H. azteca* tests.

METHODS

Thirty sediment samples were analyzed at our Traverse City, Michigan laboratory following GLEC's written Standard Operating Procedures (SOPs), which are based on the procedures outlined in U.S. EPA Method EPA/600/R-99/064 *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*, Second Edition; and American Society for Testing and Materials (ASTM) 1706-95B, *Standard Test Methods for Measuring the Toxicity of Sediment Associated Contaminants with Freshwater Invertebrates* (ASTM 2000).

Thirty-six sediment samples were collected and shipped overnight to GLEC by AECOM personnel. The samples were received at GLEC, where they were assigned unique GLEC laboratory identification numbers and stored at 0-6°C until test initiation (see table below). Of these thirty-six samples, thirty (7 reference sediment samples, three samples from the Battle Creek River and 20 investigative sediment samples) sediment samples were used for whole sediment toxicity testing. The remaining six sediment samples were sample duplicates that were submitted to an analytical laboratory for bulk sediment chemistry analysis.

Sample I.D.	Sample Description	GLEC Lab. ID Number	Date Sampled	Date Received
SETC 0000 C210S 022412 D004	Talmadge Creek Reference	9232	February 24, 2012	February 25, 2012
SEKR 0000 R024S 022012 D004	Kalamazoo River Reference	9203	February 20, 2012	February 21, 2012
SEKR 0000 C023S 022212 D004	Kalamazoo River Reference	9216	February 22, 2012	February 23, 2012
SEKR 0000 R022S 022212 D004	Kalamazoo River Reference	9222	February 22, 2012	February 23, 2012
SEKR 0000 L020S 022312 D004	Kalamazoo River Reference	9225	February 23, 2012	February 24, 2012
SEKR 0000 C019S 022312 D004	Kalamazoo River Reference	9230	February 23, 2012	February 24, 2012
SEKR 0000 L021S 022312 D004	Kalamazoo River Reference	9231	February 23, 2012	February 24, 2012
SEBC 0000 L010S 022412 D004	Battle Creek	9233	February 24, 2012	February 25, 2012
SEBC 0000 R011S 022412 D004	Battle Creek	9236	February 24, 2012	February 25, 2012
SEBC 0000 L012S 022412 D004	Battle Creek	9238	February 24, 2012	February 25, 2012
SEKR 1061 L005S 022012 D004	Site Sample	9204	February 20, 2012	February 21, 2012
SEKR 0565 R003S 022012 D004	Site Sample	9205	February 20, 2012	February 21, 2012
SEKR 1132 L006S 022112 D004	Site Sample	9206	February 21, 2012	February 22, 2012
SEKR 3644 R025S 022112 D004	Site Sample	9207	February 21, 2012	February 22, 2012
SEKR 0510 C001S 022112 D004	Site Sample	9208	February 21, 2012	February 22, 2012
SEKR 0335 R001S 022112 D004	Site Sample	9209	February 21, 2012	February 22, 2012
SEKR 0886 R004S 022112 D004	Site Sample	9212	February 21, 2012	February 22, 2012

Sample I.D.	Sample Description	GLEC Lab. ID Number	Date Sampled	Date Received
SEKR 3700 L027S 022212 D004	Site Sample	9213	February 22, 2012	February 23, 2012
SEKR 3673 L026S 022212 D004	Site Sample	9215	February 22, 2012	February 23, 2012
SEKR 1495 L008S 022212 D004	Site Sample	9218	February 22, 2012	February 23, 2012
SEKR 1522 R009S 022212 D004	Site Sample	9220	February 22, 2012	February 23, 2012
SEKR 1477 R007S 022212 D004	Site Sample	9221	February 22, 2012	February 23, 2012
SEKR 3736 L028S 022212 D004	Site Sample	9223	February 22, 2012	February 23, 2012
SEKR 2816 R016S 022312 D004	Site Sample	9224	February 23, 2012	February 24, 2012
SEKR 2621 R015S 022312 D004	Site Sample	9226	February 23, 2012	February 24, 2012
SEKR 3301 R017S 022312 D004	Site Sample	9227	February 23, 2012	February 24, 2012
SEKR 1934 L013S 022312 D004	Site Sample	9228	February 23, 2012	February 24, 2012
SEKR 2131 R014S 022312 D004	Site Sample	9229	February 23, 2012	February 24, 2012
SEKR 3510 R018S 022412 D004	Site Sample	9234	February 24, 2012	February 25, 2012
SEKR 3771 C029S 022412 D004	Site Sample	9235	February 24, 2012	February 25, 2012
Sediment Samples Submitted For Bulk Chemistry Analyses				
SEKR 1132 L006D 022112 D004	Duplicate Site Sample	9210	February 21, 2012	February 22, 2012
SEKR 0510 C001D 022112 D004	Duplicate Site Sample	9211	February 21, 2012	February 22, 2012
SEKR 1522 R009D 022212 D004	Duplicate Site Sample	9214	February 22, 2012	February 23, 2012
SEKR 0000 R022D 022212 D004	Duplicate Site Sample	9217	February 22, 2012	February 23, 2012
SEKR 3673 L026D 022212 D004	Duplicate Site Sample	9219	February 22, 2012	February 23, 2012
SEKR 3510 R018D 022412 D004	Duplicate Site Sample	9237	February 24, 2012	February 25, 2012

The 10-day *C. dilutus* toxicity tests were initiated on March 2, 2012, and the *H. azteca* toxicity tests were initiated on March 6, 2012. For each test species there were twenty investigative sediment samples, three samples from the Battle Creek River, and 7 reference sediment samples (Talmadge Creek and Kalamazoo River), a GLEC laboratory control sediment and one water only exposure.

Summary of Test Procedures: 10-Day *Chironomus dilutus* and *Hyalella azteca* Whole Sediment Toxicity Tests

Second to third instar *C. dilutus* (11-12 days old at test initiation; provided by a commercial supplier: Aquatic Bio Systems) and *H. azteca* (12-13 days old, cultured in-house) were used to initiate the 10-day whole sediment toxicity tests and water only exposure. *C. dilutus* and *H. azteca* were continuously exposed for 10 days to each of the collected sediment samples, and to a laboratory control sediment. In the water only exposures, test organisms were exposed to the overlying water that was used for the sediment exposures. There were eight replicate beakers for each sediment sample, for the overlying water only and for the GLEC laboratory control sediment; each replicate contained 10 test organisms. The GLEC laboratory control sediment is a reference sediment that is collected from the Boardman River, a local river in a primarily forested watershed in the Pere Marquette State Forest.

The *C. dilutus* and *H. azteca* were exposed in 470 mL glass test chambers, each containing 100 mL of whole sediment and 175 mL of overlying water. Prior to adding the whole sediments to each test chamber, all sediment samples were thoroughly homogenized. At toxicity test initiation the homogenized sediment was added to each replicate test chamber using a pre-cleaned stainless steel spoon. After the addition of the sediment to the test chambers, the overlying water was added immediately. Test organisms were added to each replicate test chamber the following day.

Overlying water was intermittently supplied to each test chamber at least twice daily (once every 12-hours) via a static-renewal water delivery system. The overlying water for all sediments and the water only control consisted of de-chlorinated municipal (Traverse City, Michigan) tap (Lake Michigan sourced) water, with an average hardness of 129 mg/L and an average alkalinity of 98 mg/L. Temperature, dissolved oxygen, pH, and specific conductance of the overlying water was measured daily prior to use.

The *C. dilutus* were fed 1.5 mL of Tetrafin® goldfish food slurry (4 mg/mL dry solids) once daily. The *H. azteca* were fed 1.0 mL of a mixture of yeast, trout food, and cerophyl (YTC; 1800 (1700-1900) mg/L solids) once daily.

The test chambers were placed in a temperature controlled water bath under the specified conditions of 23 ± 1°C; photoperiod 16 hours light: eight hours dark; and light intensity of 100-1000 lux.

Temperature and dissolved oxygen (DO) concentrations of the overlying water in the test chambers were measured daily in two alternating replicates for each test sediment, and the results were recorded on the laboratory bench data sheets. If the DO dropped below 2.5 mg/L, the number of daily overlying water renewals was increased (up to 4 times per day) for all treatments until the DO recovered to greater than 3.0 mg/L. Once the DO increased to above 3.0 mg/L, additional water renewals were suspended, unless the DO values dropped back below 2.5 mg/L, at which time the additional water renewals were re-initiated. Alkalinity, hardness, pH, and total ammonia (as N) were measured in the overlying water on test days 0 and 10 for both the *C. dilutus* (Table 5) and *H. azteca* (Table 6) tests. These results were recorded on the laboratory bench data sheets.

Observations of organism behavior and any anomalies observed in the test chambers were made daily for each test chamber and recorded on the laboratory bench data sheets.

In several sediment samples, resident species were present in the collected sediments that may have affected either *H. azteca* survival (see SEKR 0335 R001S 022112 D004, replicate 6) or *C. dilutus* survival (see SEKR 0000 C019S 022312 D0004, replicate 7). The effect of indigenous organisms is apparent when the comparison of the percent survival in other replicates from the same sediment sample indicates that the resident organisms were preying upon the laboratory test organisms. The presence of indigenous organisms in a replicate of a sediment sample is noted on Tables 1 – 4 (see footnote d); these replicates were excluded from the statistical analysis.

The number of *C. dilutus* surviving in each replicate test chamber was recorded at test termination (10 days); a summary of the percent survival at test termination is provided in Tables 1, 2, and 7. The average ash free dry weight [AFDW in milligrams (mg)] of the surviving organisms for each *C. dilutus* replicate, and the biomass [AFDW (mg) of the surviving organisms divided by the initial number of organisms] was also determined at test termination. The results are summarized in Table 2.

The number of surviving *H. azteca* in each replicate chamber was recorded at test termination (10 days); and the survival data are summarized in Tables 3, 4, and 8. The average dry weight [in milligrams (mg)] of the surviving organisms for each *H. azteca* replicate, and the biomass [dry weight (mg) of the surviving organisms divided by the initial number of organisms] was also determined at test termination. The data are summarized in Table 4.

A statistical procedure, using the program TOXCALC (version 5.0.32) and following statistical guidelines provided in U.S. EPA Method 600/R-99/064 and ASTM Method 1706-95B (2000), was used to compare the 10-day survival and growth data from the investigative sediment samples to survival and growth data from the designated controls and reference samples. Prior to analysis, all percent survival data were transformed using an arc sine-square root transformation. All transformed data were then tested using either the homoscedastic or heteroscedastic t-tests, which are used for comparing a single treatment to a single control.

The homoscedastic t-test assumes the data are normally distributed (Shapiro-Wilk Test or Kolmogorov D Test) and the variances are equal (F-test). If the variances are not equal, the data are analyzed using the heteroscedastic t-test. If the data are not normally distributed, then the data are analyzed using a nonparametric t-test (e.g., Steel's Many-One Rank Test or Wilcoxon Rank Sum Test with Bonferroni's Adjustment).

Growth data were initially evaluated for normal distribution and homogeneity of variances. In those cases where the data were not normally distributed or homogenous, the data were analyzed using either the heteroscedastic t-test or the nonparametric test. In addition to growth being evaluated as average dry weight of the surviving organisms, growth was also analyzed as biomass (average dry weight of surviving organisms divided by the number of initial organisms).

The survival and growth data for *C. dilutus* and *H. azteca* for each investigative sample were compared statistically (p= 0.05) to GLEC's control sediment and to two reference sediments. The control and reference sediment samples included:

1. The Talmadge Creek Upstream reference sediment sample; SETC 0000 C210S 022412 D004 (GLC Number: 9232). This sample was collected Upstream of the Line 6B spill.

2. GLEC's laboratory control sediment sample (GLC Number: CS105) that is collected from the Boardman River, a local river in a primarily forested watershed in the Pere Marquette State Forest.

3. Pooled results of the six Kalamazoo River samples collected immediately Upstream of the confluence of Talmadge Creek and the Kalamazoo River.

    a. GLC Number: 9203 SEKR 0000 R024S 022012 D004
    b. GLC Number: 9216 SEKR 0000 C023S 022212 D004
    c. GLC Number: 9222 SEKR 0000 R022S 022212 D004
    d. GLC Number: 9225 SEKR 0000 L020S 022312 D004
    e. GLC Number: 9230 SEKR 0000 C019S 022312 D004
    f. GLC Number: 9231 SEKR 0000 L021S 022312 D004

Organisms exposed to the GLEC laboratory control sediment and the "water only" control achieved acceptable survival and growth, as specified in the U.S. EPA /600/R-99/064. In this instance, GLEC's laboratory control sediment and water only control results were used confirm test acceptably and the health of the test organisms.

RESULTS

10-Day *Chironomus dilutus*

The organisms exposed to the laboratory control sediment and to water only control exceeded the minimum survival (70 percent) and growth (0.48 mg AFDW at test termination) criteria for acceptable controls for the *C. dilutus* tests (Tables 1 and 2). The acceptability requirements for survival and growth for the *C. dilutus* test can be found in U.S. EPA Method 600/R-99/064, Table 12.1.

The overlying water quality measurements (Table 5) were also within the acceptable limits of the (with the noted exceptions below) U.S. EPA testing protocol (i.e., daily mean temperatures were 23°C ± 1 °C; except as described later in this section, dissolved oxygen (DO) was maintained above 2.5 mg/L in the overlying water; and there were no variations greater than 50% in overlying water hardness, alkalinity, or total ammonia measurements within each test type). Consequently, the *C. dilutus* whole sediment toxicity tests were conducted following the standard protocols and are valid assessments of sediment toxicity, with the following exceptions to the protocol.

Noted Exceptions to the Protocol
On March 8, 2012 the DO measured in at least one of two replicates of the following sediment samples fell below the acceptable dissolved oxygen limit of 2.5 mg/L:

- GLEC laboratory control (2.3 mg/L),
- SEKR 0000 R024S 022012 D004; GLC Number: 9203 (2.1mg/L),
- SEKR 3644 R025S 022112 D004; GLC Number: 9207 (2.1 mg/L),
- SEKR 0000 C023S 022212 D004; GLC Number: 9216 (1.4 mg/L),
- SEKR 0000 R022S 022212 D004; GLC Number: 9222 (2.0 mg/L and 2.2 mg/L),
- SEKR 2621 R015S 022312 D004; GLC Number: 9226 (2.4 mg/L).

In response to the drop in DO on March 8, 2012, a third overlying water renewal was initiated in all of the sediment sample replicates. In the subsequent measurement on March 9, 2012, the DO remained below 2.5 mg/L in the following investigative samples in at least one of the two replicates where measurements were taken:

- SEKR 0000 R024S 022012 D004; GLC Number: 9203 (1.7 and 2.1 mg/L),
- SEKR 1061 L005S 022012 D004; GLC Number: 9204 (2.0 mg/L),
- SEKR 0000 C023S 022212 D004; GLC Number: 9216 (2.3 mg/L).

In response, a fourth overlying water renewal was initiated on March 9, 0212 in all of the test sediment replicates. This additional renewal was continued through test termination. The DO remained above 2.5 mg/L with the additional water renewals.

The brief drop in DO was unlikely to have affected the test results (see EPA /600/R-99/064 Manual, Section 12.3.6.2.2).

All test chambers were observed daily to assess organism behavior, and no unusual observations were noted with the test organisms in these sediment samples. However, in investigative sediment sample SEKR 1061 L005S 022012 D004 (GLC Number: 9204) foreign midges (not the test organism *C. dilutus*), were observed on test days 5 through 7, which could help explain the observed brief reduction in dissolved oxygen.

GLEC

TABLE 1. **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**

(page 1 of 5) ***Chironomus dilutus*** **10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]							
Replicate Number	**GLEC Laboratory Control** **CS105**	**SETC 0000 C210S 022412 D004** **Upstream Talmadge Creek GLC#: 9232**	**SEKR 0000 R024S 022012 D004** **Upstream Kalamazoo River GLC#: 9203**	**SEKR 0000 C023S 022212 D004** **Upstream Kalamazoo River GLC#: 9216**	**SEKR 0000 R022S 022212 D004** **Upstream Kalamazoo River GLC#: 9222**	**SEKR 0000 L020S 022312 D004** **Upstream Kalamazoo River GLC#: 9225**	**SEKR 0000 C019S 022312 D004** **Upstream Kalamazoo River GLC#: 9230**	**SEKR 0000 L021S 022312 D004** **Upstream Kalamazoo River GLC#: 9231**
1	10	10	10	5	8	8	5	10
2	10	10	10	5	10	10	10	10
3	10	10	10	6	8	10	6	7
4	10	9	10	9	10	10	10	10
5	10	9	10	6	9	10	9	10
6	10	7	9	7	10	10	10	10
7	10	9	10	9	9	10	1[d]	10
8	9	10	10	8	10	10	10	10
10-Day Percent Survival	**98.8**	**92.5**	**98.8**	**68.8**[a, b]	**92.5**[b]	**97.5**	**85.7**	**96.3**

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from six Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

[d] Not used in statistical analysis due to foreign organism present

TABLE 1. cont... (page 2 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**
***Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	SEBC 0000 L010S 022412 D004 Upstream Battle Creek GLC#: 9233	SEBC 0000 R011S 022412 D004 Upstream Battle Creek GLC#: 9236	SEBC 0000 L012S 022412 D004 Upstream Battle Creek GLC#: 9238	SEKR 1061 L005S 022012 D004 GLC#: 9204	SEKR 0565 R003S 022012 D004 GLC#: 9205	SEKR 1132 L006S 022112 D004 GLC#: 9206
1	10	4	7	10	10	10
2	10	4	6	10	9	9
3	10	1	7	9	10	10
4	10	0	8	10	10	10
5	9	3	5	10	8	7
6	10	2	6	10	6	8
7	10	4	10	10	8	10
8	10	2	4	6	9	10
10-Day Percent Survival	**98.8**	**25.0** [a, b, c]	**66.3** [a, b, c]	**93.8**	**87.5** [b]	**92.5**

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

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TABLE 1. cont… (page 3 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**
***Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	SEKR 3644 R025S 022112 D004 GLC#: 9207	SEKR 0510 C001S 022112 D004 GLC#: 9208	SEKR 0335 R001S 022112 D004 GLC#: 9209	SEKR 0886 R004S 022112 D004 GLC#: 9212	SEKR 3700 L027S 022212 D004 GLC#: 9213	SEKR 3673 L026S 022212 D004 GLC#: 9215
1	8	9	5	10	9	9
2	9	10	5	7	7	9
3	8	10	9	7	10	10
4	9	7	5	4	10	10
5	10	10	8	7	10	9
6	7	10	6	9	10	9
7	10	8	10	6	10	9
8	9	9	9	5	10	10
10-Day Percent Survival	87.5 [b]	91.3	71.3 [a, b, c]	68.8 [a, b, c]	95.0	93.8 [b]

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

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TABLE 1. cont... (page 4 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**
***Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	**SEKR 1495 L008S 022212 D004 GLC#: 9218**	**SEKR 1522 R009S 022212 D004 GLC#: 9220**	**SEKR 1477 R007S 022212 D004 GLC#: 9221**	**SEKR 3736 L028S 022212 D004 GLC#: 9223**	**SEKR 2816 R016S 022312 D004 GLC#: 9224**	**SEKR 2621 R015S 022312 D004 GLC#: 9226**
1	6	10	10	8	9	10
2	4	7	10	8	8	10
3	6	10	10	10	8	8
4	7	10	10	10	10	10
5	8	10	10	9	10	8
6	7	10	7	8	8	9
7	7	10	10	10	10	9
8	8	10	8	9	9	8
10-Day Percent Survival	**66.3** [a, b, c]	**96.3**	**93.8**	**90.0** [b]	**90.0** [b]	**90.0** [b]

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 1. cont… (page 5 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**
***Chironomus dilutus* 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	SEKR 3301 R017S 022312 D004 GLC#: 9227	SEKR 1934 L013S 022312 D004 GLC#: 9228	SEKR 2131 R014S 022312 D004 GLC#: 9229	SEKR 3510 R018S 022412 D004 GLC#: 9234	SEKR 3771 C029S 022412 D004 GLC#: 9235	GLEC Secondary Control Water Only
1	10	8	7	10	10	10
2	10	4	10	10	8	10
3	10	5	7	8	10	10
4	9	6	8	8	10	10
5	10	7	10	10	7	10
6	10	6	8	10	10	10
7	10	7	10	10	10	10
8	10	5	10	10	10	10
10-Day Percent Survival	**98.8**	**60.0** [a, b, c]	**87.5** [b]	**95.0**	**93.8**	**100.0**

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

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TABLE 2.

(page 1 of 5)

Comparison of Average[1] Ash-Free-Dry Weight (AFDW) (mg), Biomass[2] (AFDW) (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Chironomus dilutus 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	GLEC Laboratory Control CS105		SETC 0000 C210S 022412 D004 Upstream Talmadge Creek GLC#: 9232		SEKR 0000 R024S 022012 D004 Upstream Kalamazoo River GLC#: 9203		SEKR 0000 C023S 022212 D004 Upstream Kalamazoo River GLC#: 9216		SEKR 0000 R022S 022212 D004 Upstream Kalamazoo River GLC#: 9222		SEKR 0000 L020S 022312 D004 Upstream Kalamazoo River GLC#: 9225		SEKR 0000 C019S 022312 D004 Upstream Kalamazoo River GLC#: 9230	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	1.23000	1.23000	0.58800	0.58800	1.12400	1.12400	0.91000	0.45500	1.37125	1.09700	1.29375	1.03500	0.87000	0.43500
2	1.28500	1.28500	0.81400	0.81400	1.49200	1.49200	1.43200	0.71600	1.08300	1.08300	1.25300	1.25300	0.66900	0.66900
3	1.28600	1.28600	0.73900	0.73900	1.51800	1.51800	1.14333	0.68600	1.27625	1.02100	0.83900	0.83900	0.72167	0.43300
4	0.83500	0.83500	0.95111	0.85600	1.39600	1.39600	1.38778	1.24900	1.02700	1.02700	0.87500	0.87500	0.84400	0.84400
5	1.16700	1.16700	0.60333	0.54300	1.66000	1.66000	1.28000	0.76800	1.24667	1.12200	1.14800	1.14800	0.58778	0.52900
6	1.15500	1.15500	0.88286	0.61800	1.27333	1.14600	1.05857	0.74100	1.00300	1.00300	0.91000	0.91000	0.94400	0.94400
7	0.97600	0.97600	1.13444	1.02100	1.42400	1.42400	1.06111	0.95500	1.10111	0.99100	0.95600	0.95600	[d]Not Used	[d]Not Used
8	1.13778	1.02400	0.59600	0.59600	1.27700	1.27700	0.96000	0.76800	0.92100	0.92100	1.07000	1.07000	0.74800	0.74800
Average[1] Ash-Free-Dry Weight (mg)	1.13397		[b, c] 0.78859		1.39554		1.15410		1.12866		1.04309		[b] 0.76921	
Average Biomass[2] (AFDW) Weight (mg)		1.11975		[b, c] 0.72188		1.37963		[b] 0.79225		1.03313		1.01075		[b] 0.65743
10-Day Percent Survival	98.8		92.5		98.8		[a, b] 68.8		[b] 92.5		97.5		85.7	

Note: Average Ash-Free-Dry Weight (AFDW) of *Chironomus dilutus* at test initiation = 0.21811mg

[1] Average Ash-Free-Dry-Weight (AFDW) is the total ash-free-dry weight of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

[d] Not used in statistical analysis due to foreign organism present

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TABLE 2.
cont…
(page 2 of 5)

Comparison of Average[1] Ash-Free-Dry Weight (AFDW) (mg), Biomass[2] (AFDW) (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Chironomus dilutus 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 0000 L021S 022312 D004 Upstream Kalamazoo River GLC#: 9231		SEBC 0000 L010S 022412 D004 Upstream Battle Creek GLC#: 9233		SEBC 0000 R011S 022412 D004 Upstream Battle Creek GLC#: 9236		SEBC 0000 L012S 022412 D004 Upstream Battle Creek GLC#: 9238		SEKR 1061 L005S 022012 D004 GLC#: 9204		SEKR 0565 R003S 022012 D004 GLC#: 9205		SEKR 1132 L006S 022112 D004 GLC#: 9206	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[1] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	1.00200	1.00200	0.75700	0.75700	1.40750	0.56300	0.76286	0.53400	0.70700	0.70700	0.79800	0.79800	0.77500	0.77500
2	1.09600	1.09600	0.69900	0.69900	1.12500	0.45000	1.04000	0.62400	0.62300	0.62300	0.82778	0.74500	0.92667	0.83400
3	0.91429	0.64000	0.86000	0.86000	0.85000	0.08500	0.92429	0.64700	0.53250	0.47333	0.66200	0.66200	0.97700	0.97700
4	1.02100	1.02100	0.92800	0.92800	0[d]	0.00000	0.58375	0.46700	0.57500	0.57500	0.63200	0.63200	1.04400	1.04400
5	0.89100	0.89100	0.60111	0.54100	1.20000	0.36000	0.82200	0.41100	0.93100	0.93100	0.99125	0.79300	1.11857	0.78300
6	1.15400	1.15400	0.72600	0.72600	1.15000	0.23000	0.92667	0.55600	0.86300	0.86300	0.80167	0.48100	1.17250	0.93800
7	1.10600	1.10600	0.83000	0.83000	1.18250	0.47300	0.86500	0.86500	0.72500	0.72500	0.94375	0.75500	0.85000	0.85000
8	0.98900	0.98900	0.90600	0.90600	1.53500	0.30700	1.39500	0.55800	1.03167	0.61900	1.00778	0.90700	0.82000	0.82000
Average[1] Ash-Free-Dry Weight (mg)	1.02166		b, c 0.78839		1.20714		b, c 0.91494		b, c 0.74852		b, c 0.83303		b, c 0.96047	
Average Biomass[2] (AFDW) Weight (mg)		0.98738		b, c 0.78088		a, b, c 0.30850		a, b, c 0.58275		b, c 0.68954		b, c 0.72162		b, c 0.87763
10-Day Percent Survival	96.3		98.8		25.0 [a, b, c]		66.3 [a, b, c]		93.8		87.5 [b]		92.5	

Note: Average Ash-Free-Dry Weight (AFDW) of *Chironomus dilutus* at test initiation = 0.21811mg

[1] Average Ash-Free-Dry-Weight (AFDW) is the total ash-free-dry weight of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p \leq 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p \leq 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p \leq 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

[d] Not used in statistical analysis program did not recognize 0.0000

TABLE 2.
cont…
(page 3 of 5)

Comparison of Average[1] Ash-Free-Dry Weight (AFDW) (mg), Biomass[2] (AFDW) (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Chironomus dilutus 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 3644 R025S 022112 D004 GLC#: 9207		SEKR 0510 C001S 022112 D004 GLC#: 9208		SEKR 0335 R001S 022112 D004 GLC#: 9209		SEKR 0886 R004S 022112 D004 GLC#: 9212		SEKR 3700 L027S 022212 D004 GLC#: 9213		SEKR 3673 L026S 022212 D004 GLC#: 9215		SEKR 1495 L008S 022212 D004 GLC#: 9218	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	1.26625	1.01300	0.83889	0.75500	1.36600	0.68300	0.77800	0.77800	1.37222	1.23500	0.99556	0.89600	0.92000	0.55200
2	0.94556	0.85100	0.86500	0.86500	1.03800	0.51900	0.85714	0.60000	1.47286	1.03100	1.04000	0.93600	0.86250	0.34500
3	1.14250	0.91400	0.90000	0.90000	0.90667	0.81600	0.90571	0.63400	0.92900	0.92900	0.98700	0.98700	0.80500	0.48300
4	1.22000	1.09800	0.86000	0.60200	1.08400	0.54200	0.80750	0.32300	1.02300	1.02300	0.88000	0.88000	0.78857	0.55200
5	0.91400	0.91400	0.84400	0.84400	0.93250	0.74600	0.87286	0.61100	0.99100	0.99100	1.19556	1.07600	0.68625	0.54900
6	1.34714	0.94300	0.95000	0.95000	0.94500	0.56700	0.66000	0.59400	0.94900	0.94900	1.02778	0.92500	0.68143	0.47700
7	1.18200	1.18200	0.71375	0.57100	0.95900	0.95900	0.65500	0.39300	1.13100	1.13100	0.92778	0.83500	0.95429	0.66800
8	1.05667	0.95100	0.88111	0.79300	0.82444	0.74200	1.09600	0.54800	0.98700	0.98700	0.85100	0.85100	0.87750	0.70200
Average[1] Ash-Free-Dry Weight (mg)	1.13426		[b, c] 0.85659		1.00695		[b, c] 0.82903		1.10688		[b, c] 0.98808		[b, c] 0.82194	
Average Biomass[2] (AFDW) Weight (mg)		[b] 0.98325		[b, c] 0.78500		[b, c] 0.69675		[a, b, c] 0.56012		1.03450		[b] 0.92325		[a, b, c] 0.54100
10-Day Percent Survival	87.5 [b]		91.3		71.3 [a, b, c]		68.8 [a, b, c]		95.0		93.8 [b]		66.3 [a, b, c]	

Note: Average Ash-Free-Dry Weight (AFDW) of *Chironomus dilutus* at test initiation = 0.21811mg

[1] Average Ash-Free-Dry-Weight (AFDW) is the total ash-free-dry weight of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 2.
cont…
(page 4 of 5)

Comparison of Average[1] Ash-Free-Dry Weight (AFDW) (mg), Biomass[2] (AFDW) (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Chironomus dilutus 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 1522 R009S 022212 D004 GLC# 9220		SEKR 1477 R007S 022212 D004 GLC#: 9221		SEKR 3736 L028S 022212 D004 GLC#: 9223		SEKR 2816 R016S 022312 D004 GLC#: 9224		SEKR 2621 R015S 022312 D004 GLC#: 9226		SEKR 3301 R017S 022312 D004 GLC# 9227		SEKR 1934 L013S 022312 D004 GLC#: 9228	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	1.03500	1.03500	0.97300	0.97300	0.91875	0.73500	1.19778	1.07800	1.13300	1.13300	1.10700	1.10700	0.46000	0.36800
2	0.99286	0.69500	0.92000	0.92000	0.91250	0.73000	1.42750	1.14200	1.27400	1.27400	0.94000	0.94000	0.96500	0.38600
3	0.70200	0.70200	0.61900	0.61900	0.71900	0.71900	1.22125	0.97700	1.08500	0.86800	0.89300	0.89300	0.92400	0.46200
4	0.72900	0.72900	0.96600	0.96600	0.68400	0.68400	1.34400	1.34400	0.77100	0.77100	0.95000	0.85500	0.67667	0.40600
5	1.09600	1.09600	0.91400	0.91400	1.00667	0.90600	1.23100	1.23100	1.00875	0.80700	0.86200	0.86200	0.69000	0.48300
6	1.10700	1.10700	0.78000	0.54600	1.01625	0.81300	1.35250	1.08200	0.84667	0.76200	1.11800	1.11800	0.80000	0.48000
7	0.90200	0.90200	0.94700	0.94700	0.91300	0.91300	1.15600	1.15600	0.91333	0.82200	1.00300	1.00300	0.77286	0.54100
8	0.89300	0.89300	0.98125	0.78500	0.92222	0.83000	1.27444	1.14700	0.89375	0.71500	0.97500	0.97500	0.61000	0.30500
Average[1] Ash-Free-Dry Weight (mg)	b, c 0.93211		b, c 0.88753		b, c 0.88655		1.27556		b 0.99069		b, c 0.98100		b, c 0.73732	
Average Biomass[2] (AFDW) Weight (mg)		b 0.89488		b, c 0.83375		b, c 0.79125		1.14463		b 0.89400		b 0.96913		a, b, c 0.42888
10-Day Percent Survival	96.3		93.8		90.0 [b]		90.0 [b]		90.0 [b]		98.8		60.0 [a, b, c]	

Note: Average Ash-Free-Dry Weight (AFDW) of *Chironomus dilutus* at test initiation = 0.21811mg

[1] Average Ash-Free-Dry-Weight (AFDW) is the total ash-free-dry weight of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 2.
cont...
(page 5 of 5)

Comparison of Average[1] Ash-Free-Dry Weight (AFDW) (mg), Biomass[2] (AFDW) (mg) and Percent Survival Between Reference Sediments and Investigative Sediment Samples;
Chironomus dilutus 10-Day Whole Sediment Toxicity Tests Conducted: March 02-12, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 2131 R014S 022312 D004 GLC#: 9229		SEKR 3510 R018S 022412 D004 GLC# 9234		SEKR 3771 C029S 022412 D004 GLC#: 9235		GLEC Secondary Control Water Only	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	0.93857	0.65700	1.23800	1.23800	0.98700	0.98700	0.71500	0.71500
2	0.86900	0.86900	1.00300	1.00300	0.89750	0.71800	0.69600	0.69600
3	0.88571	0.62000	1.01375	0.81100	0.73000	0.73000	0.59400	0.59400
4	0.85125	0.68100	1.29625	1.03700	0.80700	0.80700	0.61200	0.61200
5	0.84100	0.84100	0.96000	0.96000	1.00714	0.70500	0.66300	0.66300
6	0.85625	0.68500	1.24500	1.24500	0.63900	0.63900	0.68500	0.68500
7	0.79500	0.79500	1.25300	1.25300	0.85100	0.85100	0.56400	0.56400
8	0.69300	0.69300	1.26600	1.26600	0.93800	0.93800	0.65200	0.65200
Average[1] Ash-Free-Dry Weight (mg)	b, c 0.84122		1.15938		b, c 0.85708		0.64762	
Average Biomass[2] (AFDW) Weight (mg)		b, c 0.73013		1.10163		b, c 0.79687		0.64762
10-Day Percent Survival	87.5 b		95.0		93.8		100.0	

Note: Average Ash-Free-Dry Weight (AFDW) of*Chironomus dilutus* at test initiation = 0.21811mg

[1] Average Ash-Free-Dry-Weight (AFDW) is the total ash-free-dry weight of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

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TABLE 3. **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples; *Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012; Line 6B Spill, Marshall, Michigan; Enbridge Energy, Limited Partnership**

	Number Test Organisms Surviving per Replicate[r]							
Replicate Number	GLEC Laboratory Control CS105	SETC 0000 C210S 022412 D004 Upstream Talmadge Creek GLC#: 9232	SEKR 0000 R024S 022012 D004 Upstream Kalamazoo River GLC#: 9203	SEKR 0000 C023S 022212 D004 Upstream Kalamazoo River GLC#: 9216	SEKR 0000 R022S 022212 D004 Upstream Kalamazoo River GLC#: 9222	SEKR 0000 L020S 022312 D004 Upstream Kalamazoo River GLC#: 9225	SEKR 0000 C019S 022312 D004 Upstream Kalamazoo River GLC#: 9230	SEKR 0000 L021S 022312 D004 Upstream Kalamazoo River GLC#: 9231
1	10	9	10	7	9	10	8	8
2	10	9	9	7	10	9	10	10
3	8	9	10	7	10	10	8	10
4	10	10	6	8	8	9	9	9
5	10	10	9	10	10	9	9	10
6	10	9	10	9	10	9	8	10
7	9	10	7	10	10	9	9	7
8	10	10	8	5	10	10	9	8
10-Day Percent Survival	96.3	95.0	86.3	78.8 [a, b]	96.3	93.8	87.5 [a, b]	90.0

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

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TABLE 3. cont… (page 2 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**
***Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	SEBC 0000 L010S 022412 D004 Upstream Battle Creek GLC#: 9233	SEBC 0000 R011S 022412 D004 Upstream Battle Creek GLC#: 9236	SEBC 0000 L012S 022412 D004 Upstream Battle Creek GLC#: 9238	SEKR 1061 L005S 022012 D004 GLC#: 9204	SEKR 0565 R003S 022012 D004 GLC#: 9205	SEKR 1132 L006S 022112 D004 GLC#: 9206
1	10	10	7	9	8	10
2	10	9	6	9	7	8
3	9	10	9	7	7	9
4	9	9	9	9	8	10
5	10	5	9	8	8	10
6	10	6	10	10	9	10
7	10	9	9	10	9	10
8	7	7	9	7	6	10
10-Day Percent Survival	93.8	a, b 81.3	a, b 85.0	a, b 86.3	a, b, c 77.5	96.3

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 3. cont... (page 3 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**
***Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	SEKR 3644 R025S 022112 D004 GLC#: 9207	SEKR 0510 C001S 022112 D004 GLC#: 9208	SEKR 0335 R001S 022112 D004 GLC#: 9209	SEKR 0886 R004S 022112 D004 GLC#: 9212	SEKR 3700 L027S 022212 D004 GLC#: 9213	SEKR 3673 L026S 022212 D004 GLC#: 9215
1	6	8	9	8	10	10
2	10	9	9	9	10	10
3	10	8	9	10	9	8
4	8	6	10	10	8	9
5	7	10	8	10	9	8
6	10	9	2[d]	7	8	10
7	10	10	9	7	9	9
8	10	10	10	8	10	9
10-Day Percent Survival	88.8	87.5	91.4	86.3[b]	91.3	91.3

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

[d] Not used in statistical analysis due to foreign organism present

TABLE 3. cont… (page 4 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples;**
***Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	SEKR 1495 L008S 022212 D004 GLC#: 9218	SEKR 1522 R009S 022212 D004 GLC#: 9220	SEKR 1477 R007S 022212 D004 GLC#: 9221	SEKR 3736 L028S 022212 D004 GLC#: 9223	SEKR 2816 R016S 022312 D004 GLC#: 9224	SEKR 2621 R015S 022312 D004 GLC#: 9226
1	9	10	10	10	10	8
2	10	8	8	10	9	10
3	10	10	10	9	8	10
4	10	10	8	9	8	10
5	9	10	10	10	9	8
6	9	10	10	10	10	10
7	8	9	9	10	8	10
8	10	10	9	9	9	10
10-Day Percent Survival	**93.8**	**96.3**	**92.5**	**96.3**	**88.8** [b]	**95.0**

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 3. cont… (page 5 of 5) **Comparison of the Number of Surviving Organisms per Replicate and Percent Survival Between Reference Sediment and Investigative Sediment Samples; *Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012; Line 6B Spill, Marshall, Michigan; Enbridge Energy, Limited Partnership**

	Number Test Organisms Surviving per Replicate[r]					
Replicate Number	SEKR 3301 R017S 022312 D004 GLC#: 9227	SEKR 1934 L013S 022312 D004 GLC#: 9228	SEKR 2131 R014S 022312 D004 GLC#: 9229	SEKR 3510 R018S 022412 D004 GLC#: 9234	SEKR 3771 C029S 022412 D004 GLC#: 9235	GLEC Secondary Control Water Only
1	10	9	9	10	10	10
2	8	9	10	10	10	10
3	8	10	10	7	10	10
4	10	8	10	10	9	10
5	10	10	10	10	10	10
6	10	6	7	9	9	9
7	10	10	10	10	9	8
8	10	7	10	10	10	10
10-Day Percent Survival	**95.0**	**86.3**	**95.0**	**95.0**	**96.3**	**96.3**

[r] Replicates initiated with 10 organisms each

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 4.

(page 1 of 5)

Comparison of Average[1] Dry Weight (mg), Biomass[2] (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Hyalella azteca 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	GLEC Laboratory Control CS105		SETC 0000 C210S 022412 D004 Upstream Talmadge Creek GLC#: 9232		SEKR 0000 R024S 022012 D004 Upstream Kalamazoo River GLC#: 9203		SEKR 0000 C023S 022212 D004 Upstream Kalamazoo River GLC#: 9216		SEKR 0000 R022S 022212 D004 Upstream Kalamazoo River GLC#: 9222		SEKR 0000 L020S 022312 D004 Upstream Kalamazoo River GLC#: 9225		SEKR 0000 C019S 022312 D004 Upstream Kalamazoo River GLC#: 9230	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	0.13800	0.13800	0.08556	0.07700	0.16000	0.16000	0.13286	0.09300	0.14778	0.13300	0.13500	0.13500	0.18750	0.15000
2	0.17900	0.17900	0.13778	0.12400	0.16333	0.14700	0.16286	0.11400	0.13300	0.13300	0.13333	0.12000	0.14300	0.14300
3	0.16250	0.13000	0.09667	0.08700	0.16300	0.16300	0.14857	0.10400	0.18500	0.18500	0.10400	0.10400	0.10500	0.08400
4	0.16500	0.16500	0.09900	0.09900	0.25833	0.15500	0.14625	0.11700	0.12250	0.09800	0.14222	0.12800	0.19000	0.17100
5	0.16500	0.16500	0.14500	0.14500	0.13667	0.12300	0.09100	0.09100	0.15800	0.15800	0.12778	0.11500	0.16111	0.14500
6	0.19400	0.19400	0.10333	0.09300	0.17300	0.17300	0.12889	0.11600	0.14300	0.14300	0.09667	0.08700	0.15625	0.12500
7	0.21000	0.18900	0.10000	0.10000	0.15571	0.10900	0.10500	0.10500	0.14300	0.14300	0.10556	0.09500	0.20667	0.18600
8	0.19700	0.19700	0.13600	0.13600	0.22875	0.18300	0.22600	0.11300	0.15600	0.15600	0.09800	0.09800	0.13778	0.12400
Average[1] Dry Weight (mg)	0.17631		[b, c] 0.11292		0.17985		[b] 0.14268		[b] 0.14854		[b] 0.11782		0.16091	
Average Biomass[2] Weight (mg)		0.16963		[b, c] 0.10763		0.15163		[b] 0.10663		[b] 0.14363		[b] 0.11025		[b] 0.14100
10-Day Percent Survival	96.3		95.0		86.3		78.8 [a, b]		96.3		93.8		87.5 [a, b]	

Note: Average dry weight of *H. azteca* at test initiation = 0.02725mg

[1] Average Dry Weight is the total dry weight of surviving organisms

[2] Biomass weight is the total Dry Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 4.
cont…
(page 2 of 5)

Comparison of Average[1] Dry Weight (mg), Biomass[2] (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Hyalella azteca 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 0000 L021S 022312 D004 Upstream Kalamazoo River GLC#: 9231		SEBC 0000 L010S 022412 D004 Upstream Battle Creek GLC#: 9233		SEBC 0000 R011S 022412 D004 Upstream Battle Creek GLC#: 9236		SEBC 0000 L012S 022412 D004 Upstream Battle Creek GLC#: 9238		SEKR 1061 L005S 022012 D004 GLC#: 9204		SEKR 0565 R003S 022012 D004 GLC#: 9205		SEKR 1132 L006S 022112 D004 GLC#: 9206	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	0.17250	0.13800	0.10600	0.10600	0.15100	0.15100	0.15429	0.10800	0.10000	0.09000	0.12750	0.10200	0.14800	0.14800
2	0.08700	0.08700	0.15200	0.15200	0.16333	0.14700	0.11500	0.06900	0.12111	0.10900	0.13571	0.09500	0.14875	0.11900
3	0.11200	0.11200	0.13222	0.11900	0.14000	0.14000	0.11889	0.10700	0.16143	0.11300	0.14143	0.09900	0.11333	0.10200
4	0.12667	0.11400	0.12889	0.11600	0.08667	0.07800	0.17778	0.16000	0.11889	0.10700	0.14375	0.11500	0.11000	0.11000
5	0.13500	0.13500	0.14600	0.14600	0.20800	0.10400	0.13222	0.11900	0.13625	0.10900	0.08750	0.07000	0.11600	0.11600
6	0.11800	0.11800	0.13200	0.13200	0.25000	0.15000	0.14700	0.14700	0.10000	0.10000	0.15778	0.14200	0.14900	0.14900
7	0.10429	0.07300	0.09100	0.09100	0.16333	0.14700	0.15556	0.14000	0.11800	0.11800	0.11111	0.10000	0.16200	0.16200
8	0.13250	0.10600	0.16286	0.11400	0.19286	0.13500	0.19333	0.17400	0.09000	0.06300	0.15167	0.09100	0.13500	0.13500
Average[1] Dry Weight (mg)	b 0.12350		b 0.13137		0.16940		b 0.14926		b, c 0.11821		b 0.13206		b 0.13526	
Average Biomass[2] Weight (mg)		b 0.11038		b 0.12200		b 0.13150		b 0.12800		b, c 0.10113		b, c 0.10175		b 0.13013
10-Day Percent Survival	90.0		93.8		81.3 [a, b]		85.0 [a, b]		86.3 [a, b]		77.5 [a, b, c]		96.3	

Note: Average dry weight of *H. azteca* at test initiation = 0.02725mg

[1] Average Dry Weight is the total dry weight of surviving organisms

[2] Biomass weight is the total Dry Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 4.
cont…
(page 3 of 5)

Comparison of Average[1] Dry Weight (mg), Biomass[2] (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Hyalella azteca 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 3644 R025S 022112 D004 GLC#: 9207		SEKR 0510 C001S 022112 D004 GLC#: 9208		SEKR 0335 R001S 022112 D004 GLC#: 9209		SEKR 0886 R004S 022112 D004 GLC#: 9212		SEKR 3700 L027S 022212 D004 GLC#: 9213		SEKR 3673 L026S 022212 D004 GLC#: 9215		SEKR 1495 L008S 022212 D004 GLC#: 9218	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	0.14667	0.08800	0.10625	0.08500	0.11111	0.10000	0.08250	0.06600	0.10700	0.10700	0.14300	0.14300	0.09667	0.08700
2	0.09800	0.09800	0.10556	0.09500	0.11667	0.10500	0.12667	0.11400	0.14000	0.14000	0.16000	0.16000	0.07300	0.07300
3	0.12600	0.12600	0.13500	0.10800	0.10778	0.09700	0.10800	0.10800	0.13333	0.12000	0.14875	0.11900	0.09500	0.09500
4	0.11625	0.09300	0.14000	0.08400	0.12900	0.12900	0.11400	0.11400	0.17000	0.13600	0.13889	0.12500	0.11200	0.11200
5	0.14143	0.09900	0.13200	0.13200	0.15000	0.12000	0.11800	0.11800	0.16667	0.15000	0.18625	0.14900	0.14778	0.13300
6	0.09500	0.09500	0.11667	0.10500	[d]Not used	[d]Not used	0.11571	0.08100	0.11750	0.09400	0.14100	0.14100	0.13333	0.12000
7	0.09200	0.09200	0.12400	0.12400	0.12889	0.11600	0.13143	0.09200	0.17444	0.15700	0.12778	0.11500	0.13000	0.10400
8	0.11100	0.11100	0.12900	0.12900	0.16500	0.16500	0.13125	0.10500	0.18200	0.18200	0.12667	0.11400	0.09800	0.09800
Average[1] Dry Weight (mg)	[b, c] 0.11579		[b, c] 0.12356		[b] 0.12978		[b, c] 0.11595		[b] 0.14887		[b] 0.14654		[b, c] 0.11072	
Average Biomass[2] Weight (mg)		[b, c] 0.10025		[b, c] 0.10775		[b] 0.11886		[b, c] 0.09975		[b] 0.13575		[b] 0.13325		[b, c] 0.10275
10-Day Percent Survival	88.8		87.5		91.4		86.3 [b]		91.3		91.3		93.8	

Note: Average dry weight of *H. azteca* at test initiation = 0.02725mg

[1] Average Dry Weight is the total dry weight of surviving organisms

[2] Biomass weight is the total Dry Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

[d] Not used for statistical analysis due to foreign organism.

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TABLE 4.
cont…
(page 4 of 5)

Comparison of Average[1] Dry Weight (mg), Biomass[2] (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
Hyalella azteca 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 1522 R009S 022212 D004 GLC# 9220		SEKR 1477 R007S 022212 D004 GLC#: 9221		SEKR 3736 L028S 022212 D004 GLC#: 9223		SEKR 2816 R016S 022312 D004 GLC#: 9224		SEKR 2621 R015S 022312 D004 GLC#: 9226		SEKR 3301 R017S 022312 D004 GLC# 9227		SEKR 1934 L013S 022312 D004 GLC#: 9228	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	0.12900	0.12900	0.10400	0.10400	0.08200	0.08200	0.10600	0.10600	0.10625	0.08500	0.14500	0.14500	0.11778	0.10600
2	0.09250	0.07400	0.15625	0.12500	0.11100	0.11100	0.17667	0.15900	0.10500	0.10500	0.14875	0.11900	0.08889	0.08000
3	0.12600	0.12600	0.14700	0.14700	0.07333	0.06600	0.10125	0.08100	0.09500	0.09500	0.14375	0.11500	0.13800	0.13800
4	0.10700	0.10700	0.13625	0.10900	0.11333	0.10200	0.16625	0.13300	0.11000	0.11000	0.13900	0.13900	0.09375	0.07500
5	0.10900	0.10900	0.15200	0.15200	0.09000	0.09000	0.12333	0.11100	0.11750	0.09400	0.13800	0.13800	0.16200	0.16200
6	0.10900	0.10900	0.15500	0.15500	0.07600	0.07600	0.17500	0.17500	0.09500	0.09500	0.14900	0.14900	0.08833	0.05300
7	0.16667	0.15000	0.10333	0.09300	0.10100	0.10100	0.14750	0.11800	0.10400	0.10400	0.11400	0.11400	0.10600	0.10600
8	0.14500	0.14500	0.15667	0.14100	0.11111	0.10000	0.14000	0.12600	0.11600	0.11600	0.15100	0.15100	0.12142	0.08500
Average[1] Dry Weight (mg)	b, c 0.12302		b 0.13881		a, b, c 0.09472		b 0.14200		b, c 0.10609		b 0.14106		b, c 0.11452	
Average Biomass[2] Weight (mg)		b 0.11863		b 0.12825		b, c 0.09100		b 0.12613		b, c 0.10050		b 0.13375		b, c 0.10063
10-Day Percent Survival	96.3		92.5		96.3		88.8 [b]		95.0		95.0		86.3	

Note: Average dry weight of *H. azteca* at test initiation = 0.02725mg

[1] Average Dry Weight is the total dry weight of surviving organisms

[2] Biomass weight is the total Dry Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)

TABLE 4.
cont…
(page 5 of 5)

Comparison of Average[1] Dry Weight (mg), Biomass[2] (mg) and Percent Survival Between Reference Sediment and Investigative Sediment Samples;
***Hyalella azteca* 10-Day Whole Sediment Toxicity Tests Conducted: March 06-16, 2012;**
Line 6B Spill, Marshall, Michigan;
Enbridge Energy, Limited Partnership

REPLICATE #	SEKR 2131 R014S 022312 D004 GLC#: 9229		SEKR 3510 R018S 022412 D004 GLC# 9234		SEKR 3771 C029S 022412 D004 GLC#: 9235		GLEC Secondary Control Water Only	
	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)	Average[1] Weight (mg)	Biomass[2] Weight (mg)
1	0.11778	0.10600	0.21900	0.21900	0.11500	0.11500	0.08600	0.08600
2	0.14000	0.14000	0.13100	0.13100	0.12300	0.12300	0.13200	0.13200
3	0.11900	0.11900	0.13143	0.09200	0.10500	0.10500	0.12600	0.12600
4	0.18700	0.18700	0.22600	0.22600	0.14556	0.13100	0.09000	0.09000
5	0.13000	0.13000	0.19300	0.19300	0.17900	0.17900	0.10500	0.10500
6	0.18857	0.13200	0.08778	0.07900	0.14667	0.13200	0.15889	0.14300
7	0.14200	0.14200	0.14100	0.14100	0.11444	0.10300	0.13375	0.10700
8	0.11700	0.11700	0.07900	0.07900	0.10400	0.10400	0.11300	0.11300
Average[1] Dry Weight (mg)	[b] 0.14267		0.15103		[b] 0.12908		0.11808	
Average Biomass[2] Weight (mg)		[b] 0.13413		0.14500		[b] 0.12400		0.11275
10-Day Percent Survival	95.0		95.0		96.3		96.3	

Note: Average dry weight of *H. azteca* at test initiation = 0.02725mg

[1] Average Dry Weight is the total dry weight of surviving organisms

[2] Biomass weight is the total Dry Weight of surviving organisms divided by the initial number of organisms

[a] Significantly different ($p < 0.05$) from Upstream Talmadge Creek sediment sample SETC 0000 C210S 022412 D004 (GLC Number: 9232)

[b] Significantly different ($p < 0.05$) from GLEC Laboratory Control

[c] Significantly different ($p < 0.05$) from 6 Upstream Kalamazoo River sediment samples (SEKR 0000 R024S 022012 D004, SEKR 0000 C023S 022212 D004, SEKR 0000 R022S 022212 D004, SEKR 0000 L020S 022312 D004, SEKR 0000 C019S 022312 D004, and SEKR 0000 L021S 022312 D004)