ANCHOR QEA

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April 12, 2011

Sean Sheldrake, Project Coordinator
U.S. Environmental Protection Agency, Region 10
1200 Sixth Avenue, M/S ECL-111
Seattle, Washington 98101

Re: Scope of Work for Benthic Toxicity Retesting –
Gasco Project Area Identification Report Data Gaps Sampling

Project Number: 000029-02.28

Dear Sean:

As discussed during a meeting with the U.S. Environmental Protection Agency (EPA) on April 8, 2011, Anchor QEA, LLC, has prepared this letter on behalf of NW Natural to describe the proposed scope of work for completion of surface sediment sampling at the Gasco Sediments Site Area of Interest in order to retest midge bioassays. This sampling will facilitate retesting of benthic toxicity recently completed as part the data gaps sampling activities identified in the EPA-approved *Final Project Area Identification Report – Gasco Sediments Cleanup Action* (Final AIR; Anchor QEA 2010[1]).

INTRODUCTION AND BIOASSAY RESULTS SUMMARY

Surface sediment sampling was conducted in October 2010 as part of the EPA-approved Final AIR (Anchor QEA 2010). The sampling included the completion of co-located chemical testing and freshwater bioassays at 20 stations within the Gasco Sediments Site Area of Interest and three upriver reference samples. The bioassay testing was performed using the 28-day freshwater amphipod (*Hyalella azteca*) and the 10-day freshwater midge (*Chironomus dilutus*, formerly *C. tentans*) for survival and growth endpoints. Analysis of the bioassay data by Anchor QEA and the biological laboratory indicated that the midge bioassay results should not

[1] All references cited in this letter are provided in the memorandum included as Attachment 1.

be used to make regulatory decisions, as summarized in this letter and further documented in Attachment 1.

Anchor QEA conducted a detailed analysis of the bioassay data using the EPA-recommended Reference Envelope Approach (REA) for the Portland Harbor Remedial Investigation/Feasibility Study (RI/FS; Windward 2009). This analysis indicates that the midge bioassay results cannot be used to make regulatory decisions based on two specific lines of evidence, which are:

1. The high variability observed in replicates for the midge survival endpoint for individual test sediments
2. The midge test results do not meet the performance standard established for reference sediments for this bioassay test

The midge test results show survival rates that are below the performance standard of 70 percent for all three reference sediments. Chemical analyses of the reference sediments did not contain any detected constituents above the Regional Sediment Evaluation Team (RSET) Interim Freshwater Screening Level (SL) 1 benchmarks and should not have resulted in adverse impacts to bioassay endpoints. Alternatively, the amphipod test results for the three reference sediments show high survival and the results meet the reference sediment performance standard established for this bioassay test.

In addition, the variability within replicate treatments for all the midge bioassay tests (test and reference sediments) was very high. For example, for the midge survival endpoint, the range and average replicate standard deviation of the test samples is much higher than those observed for the amphipod tests. Similarly, for many of the test sediments, individual replicate test responses of 90 to 100 percent survival and 0 to 10 percent survival were shown for the same test sediment. This lack of agreement of test response within individual test sediment replicates indicates that the appropriate classification of the test sediment is uncertain due to within test sediment replicate variability. The same high rate of variability and low survival were found in the midge test results for the three upriver reference sediments analyzed for this testing program, as previously noted.

Based on these standard bioassay data evaluations, the biological testing laboratory (see Attachment 1) and Anchor QEA conclude that the high variability observed in the midge survival bioassay test results indicate that there is considerable uncertainty in interpreting the midge survival bioassay data for any purpose and that these data should not be used for

regulatory decision making using the Portland Harbor REA interpretive thresholds. In contrast, the biological testing laboratory (see Attachment 1) and Anchor QEA conclude that the amphipod bioassay results met all quality assurance/quality control (QA/QC) requirements (including reference sediment performance standards) and are of sufficient quality for regulatory decision making.

PROPOSED ADDENDUM TO AIR DATA GAPS SAMPLING SCOPE OF WORK

Due to the issues identified for the midge bioassay test results, NW Natural proposes to recollect and retest surface sediments from the 20 stations and 3 upstream reference stations previously occupied during the Final AIR data gaps sampling. The proposed retest locations are shown on Figure 1 and the target coordinates are summarized on Table 1. At each location, surface sediments will be collected from 0 to 30 centimeters below mudline and submitted for co-located chemical and biological toxicity testing. Sediment chemical analysis will be performed for the conventional and chemical analytes identified in the EPA-approved *Final Data Gaps QAPP – Project Area Identification Report* (Appendix A of the Final AIR; Anchor QEA 2010). The biological toxicity testing will be completed using the 10-day freshwater midge (*Chironomus dilutus*) for the survival and growth bioassay. Retesting is not proposed for the 28-day freshwater amphipod (*Hyalella azteca*) survival and growth bioassay because the initial testing results met all the QA/QC requirements and are of sufficient quality for regulatory decision making. The chemical and biological testing sampling and analysis procedures will be completed in accordance with the EPA-approved *Final Data Gaps QAPP – Project Area Identification Report*.

The proposed retesting and additional surface sediment sampling is scheduled to occur the week of April 18, pending EPA approval. Please let NW Natural know if this proposed scope of work amendment is approved and if work can proceed. Please contact me if you have any questions.

Sincerely,

Ryan Barth, P.E.
Anchor QEA, LLC

LIST OF ATTACHMENTS

Table 1	Proposed Target Sampling Station Geographical Coordinates
Figure 1	Proposed Retest and Additional Surface Sediment Grab Sample Locations
Attachment 1	NW Natural Gasco Sediment Cleanup Action: Bioassay Interpretation Summary and Recommendations

Cc:

Audie Huber, Umatilla Tribe
Brian Cunninghame, Warm Springs Tribe
Erin Madden, Nez Perce Tribe
Sheila Fleming, Ridolfi
David Allen, Stratus Consulting
Jennifer Peers, Status Consulting
Jeff Baker, Grande Ronde Tribe
Tom Downey, Siletz Tribe
Rob Neely, NOAA
Jeremy Buck, USFW
Greg Smith, USFW
Jim Anderson, DEQ
Matt McClincy, DEQ
Mike Poulsen, DEQ
Jennifer Peterson, DEQ
Rick Kepler, ODFW
Cyril Young, DSL
Lori Cora, EPA
Dana Davoli, EPA
Rene Fuentes, EPA
Chip Humphrey, EPA
Kristine Koch, EPA
Nancy Munn, NOAA-NMFS
Preston Sleeger, USDOI
John Edwards, Anchor QEA
Tom Schadt, Anchor QEA
Carl Stivers, Anchor QEA
John Verduin, Anchor QEA

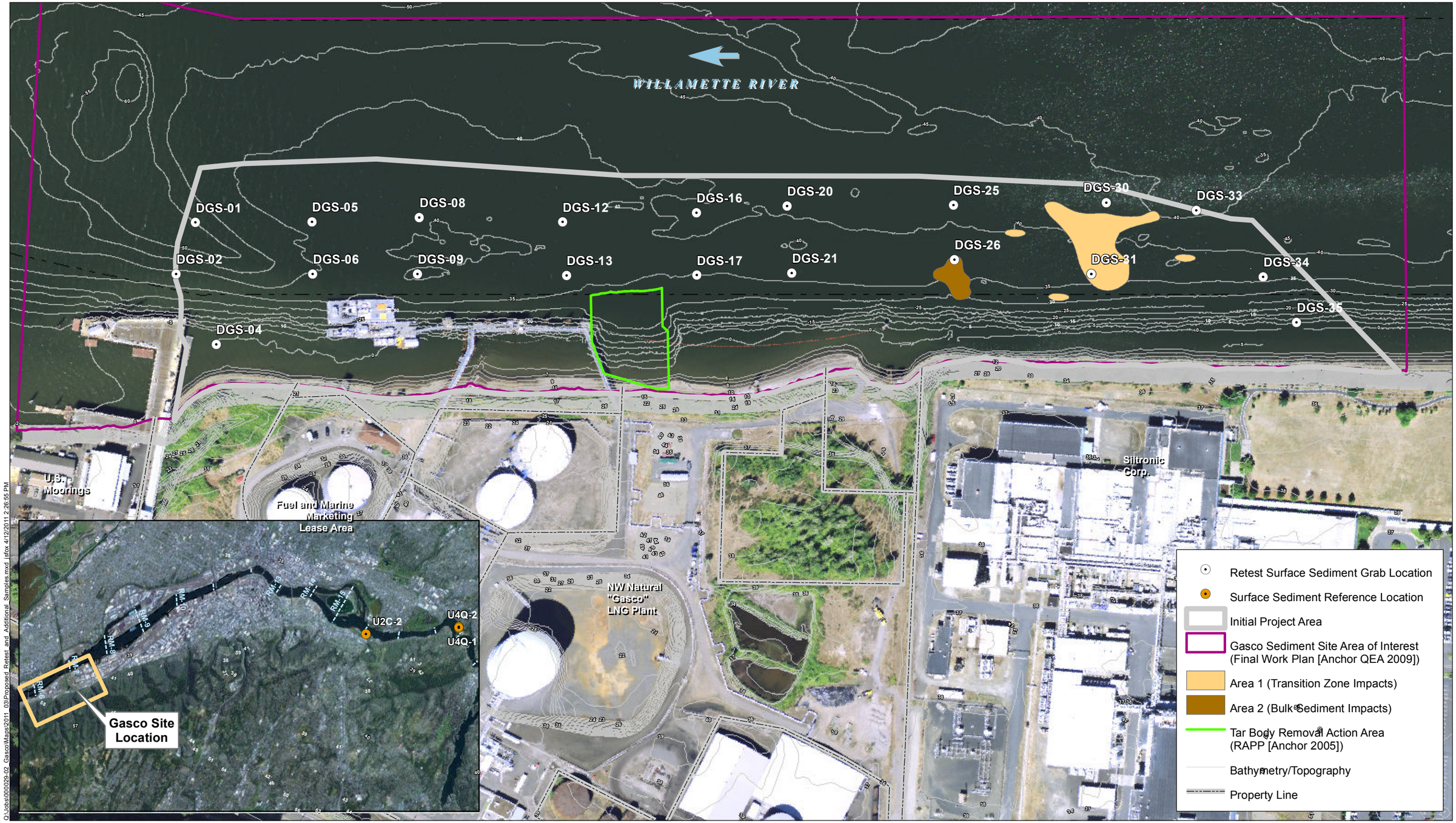
Table 1
Proposed Target Sampling Station Geographical Coordinates

Sample ID	X Coordinates[1]	Y Coordinates[1]	Testing Parameters
DGS-01	7623280.676	706385.046	bioassay, bulk sediment
DGS-02	7623225.013	706287.450	bioassay, bulk sediment
DGS-04	7623216.875	706087.897	bioassay, bulk sediment
DGS-05	7623539.542	706230.992	bioassay, bulk sediment
DGS-06	7623486.276	706131.115	bioassay, bulk sediment
DGS-08	7623732.978	706111.786	bioassay, bulk sediment
DGS-09	7623679.379	706015.087	bioassay, bulk sediment
DGS-12	7624008.788	705948.315	bioassay, bulk sediment
DGS-13	7623953.539	705850.355	bioassay, bulk sediment
DGS-16	7624275.993	705822.232	bioassay, bulk sediment
DGS-17	7624198.360	705700.471	bioassay, bulk sediment
DGS-20	7624444.752	705721.260	bioassay, bulk sediment
DGS-21	7624375.505	705596.812	bioassay, bulk sediment
DGS-25	7624752.537	705538.876	bioassay, bulk sediment
DGS-26	7624660.608	705425.505	bioassay, bulk sediment
DGS-30	7625039.539	705365.388	bioassay, bulk sediment
DGS-31	7624931.923	705262.482	bioassay, bulk sediment
DGS-33	7625204.076	705266.943	bioassay, bulk sediment
DGS-34	7625255.557	705069.017	bioassay, bulk sediment
DGS-35	7625263.042	704939.315	bioassay, bulk sediment

Notes:

1 Coordinates are provided in northing and easting in Oregon HARN State Plane North, International Feet

2 Chemical analyses and/or biological toxicity testing may be triggered at these stations if the adjacent proposed retest stations show bioassay toxicity using the EPA-recommended REA for the Portland Harbor RI/FS (Windward 2009)



NOTE:
1. Area 1 and Area 2 zones of impact are those shown in Figure 1 of the Statement of Work.
2. Arrow indicates direction of flow of river.
3. Horizontal datum is NAD83 HARN Oregon State Plane North, Intl. Feet.
4. Vertical datum is NAVD88.
5. Bathymetry surveyed by Lower Willamette Group in 2009.
6. Topography surveyed by Spencer B. Gross, Inc. 2006.
7. Aerial imagery from July 2007.

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Feet
0 100 200 300 400

Figure 1
Proposed Midge Retest
Surface Sediment Grab Sample Locations
AIR Data Gaps Investigation Sampling
Gasco Sediments Cleanup Action

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MEMORANDUM

To:	Sean Sheldrake, EPA Region 10	**Date:**	March 21, 2011
From:	Taku Fuji, Anchor QEA, LLC	**Project:**	000029-02.28
Cc:	Bob Wyatt, NW Natural Patty Dost, Pearl Legal Group PC Carl Stivers, Ryan Barth, Kim Slinski, John Edwards, and Ben Hung, Anchor QEA, LLC		
Re:	NW Natural Gasco Sediment Cleanup Action: Bioassay Interpretation Summary and Recommendations		

INTRODUCTION AND BIOASSAY RESULTS SUMMARY

In accordance with the U.S. Environmental Protection Agency (EPA)-approved *Final Area Identification Report – Gasco Sediments Cleanup Action* (Anchor QEA 2010), freshwater bioassays were conducted on 20 sediment samples within the Gasco Sediments Site Area of Interest and three upriver reference samples. The following two sediment toxicity tests were conducted on each of the 20 surface sediment samples and the three reference sediments:

1. 28-day freshwater amphipod (*Hyalella azteca)* survival and growth bioassay
2. 10-day freshwater midge (*Chironomus dilutus*, formerly *C. tentans*) survival and growth bioassay

The interpretation of the bioassay results is based on the current EPA-recommended Reference Envelope Approach (REA) for the Portland Harbor Remedial Investigation/Feasibility Study (RI/FS). To implement the REA, the Lower Willamette Group (LWG) and EPA agreed on a set of upriver bioassay data that could be used to characterize background conditions in the Lower Willamette River. Using the reference sample data set, Reference Envelope Values (REVs) were calculated for each reference sample that met the biological and chemical criteria for inclusion in the REA.

Following the REA, the REVs were then used to establish four effects thresholds or "levels," to define the potential for toxicity for each test sediment. The effects levels were based on

methods developed for the Calcasieu Superfund Site Baseline Ecological Risk Assessment and are defined in the *Draft Benthic Toxicity Reanalysis Technical Memorandum* (Windward 2009). The bioassays results from the NW Natural Gasco Sediments Cleanup Action Data Gaps Project were evaluated against the REVs using the procedures documented in the *Draft Benthic Toxicity Reanalysis Technical Memorandum*.

The effects levels for bioassay interpretation are summarized as follows:

- **Level 0 (non-toxic) —** Mean response is not significantly different from the negative control mean, or mean negative-control-adjusted response is greater than or equal to the REV
- **Level 1 (non-toxic) —** Mean response is significantly different from the negative control mean, and REV is greater than the mean negative-control-adjusted response, which is greater than to equal to 0.9 times the REV
- **Level 2 (uncertain) –** Mean response is significantly different from the negative control mean and 0.9 times the REV is greater than the mean negative-control-adjusted response, which is greater than or equal to 0.8 times the REV
- **Level 3 (toxic) –** Mean response is significantly different from the negative control mean and 0.8 times the REV is greater than the mean negative-control-adjusted response

A quality assurance/quality control (QA/QC) review of the sediment bioassays was conducted evaluating:

- Laboratory holding times for bioassay samples
- Bioassay performance in negative control tests
- Bioassay performance in positive control tests;
- Bioassay test conditions

All bioassays tests conducted for this project met appropriate QA/QC criteria established for these tests.

BIOASSAY RESULTS SUMMARY

The results and interpretation of the sediment bioassays are presented on Tables 1 and 2. Anchor QEA, LLC's more detailed analysis of the bioassay data indicates that the midge bioassay results cannot be used to make regulatory decisions based on two specific lines of evidence, which are:

1. The high variability observed in replicates for the midge survival endpoint for individual test sediments

2. The midge test results do not meet the performance standard established for reference sediments for this bioassay test

The reference sediment samples collected as part of this sampling program (REF-U2C-2, REF-U4Q-1, and REF-U4Q-2) did not contain any detected constituents above the Regional Sediment Evaluation Team (RSET) Interim Freshwater Screening Level (SL) 1 benchmarks and should not have resulted in adverse impacts to bioassay endpoints. The reference sediment performance standard established for the survival endpoint for both bioassays is that reference sediment survival must be greater than 70 percent (SEF 2009).

The midge test results (Table 2) show survival rates that are below the performance standard of 70 percent for all three reference sediments. The amphipod test results for the three reference sediments (Table 1) show high survival and the results meet the reference sediment performance standard established for this bioassay test.

In addition, the variability within replicate treatments for all the midge bioassay tests (test and reference sediments) was very high. For example, for the midge survival endpoint, while the negative control replicates for *C. dilutes* percent survival had a standard deviation of 8.3 percent, the replicate standard deviation of the test samples ranged from 17.3 to 37.8 percent with an average of 27.5 percent. In comparison, the average standard deviation for percent survival of *H. azteca* test samples was 9.2 percent.

Figure 1 shows the summary statistics for each test and reference sediment of the midge survival bioassay (showing mean and standard deviations), and Figure 2 shows the individual midge replicate results for each test and reference sediment. Similarly, Figure 3 shows the summary statistics for each test and reference sediment of the amphipod survival bioassay (showing mean and standard deviations) and Figure 4 shows the individual replicate results for each amphipod test and reference sediment.

Figures 1 and 2 show the high variability observed among the individual replicate results for the midge survival endpoint. For many of the test sediments (Figure 2), individual replicate test responses of 90 to 100 percent survival and 0 to 10 percent survival were shown for the same test sediment. This lack of agreement of test response within individual test sediment replicates indicates that the appropriate classification of the test sediment is uncertain due to within test sediment replicate variability. The same high rate of variability and low survival were found in

the midge test results for the three upriver reference sediments analyzed for this testing program, as previously noted.

Attachment 1 to this memo presents a letter from Northwestern Aquatics Sciences, the laboratory that conducted the sediment bioassay tests, describing their evaluation of the amphipod and midge bioassay results that arrives at the same conclusions as Anchor QEA regarding the midge survival bioassay results.

CONCLUSIONS

Based on these standard bioassay data evaluations, Anchor QEA concludes that the high variability observed in the midge survival bioassay test results indicate that there is considerable uncertainty in interpreting the midge survival bioassay data for any purpose and that these data should not be used for regulatory decision making using the Portland Harbor REA interpretive thresholds.

In contrast, Anchor QEA concludes that the amphipod bioassay results met all QA/QC requirements (including reference sediment performance standards) and are of sufficient quality for regulatory decision making.

RECOMMENDATIONS

Based on the evaluation of bioassay results, NW Natural and Anchor QEA would like to meet with EPA to discuss possible retesting of the midge bioassays.

REFERENCES

Anchor QEA, LLC, 2010. Final Project Area Identification Report – Gasco Sediments Cleanup Action. Prepared for the U.S. Environmental Protection Agency. Seattle, WA. July 2010.

Windward, 2009. Portland Harbor RI/FS: Benthic Toxicity Reanalysis Technical Memorandum. Draft. Prepared for the Lower Willamette Group. Seattle, WA. November 13, 2009.

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Figure 2 Summary of Individual Survival Replicate Data for the Midge Bioassay

Figure 3 Statistical Summary of Individual Survival Replicates for the *Hyalella* Bioassay (Mean Survival ± SD)

Figure 4 Summary of Individual Survival Replicate Data for the *Hyalella* Bioassay

Figure 5 Proposed Re-testing and Additional Chemical and Bioassay Sampling Stations

List of Attachments

Attachment 1 Letter to Dr. Fuji Re: Anchor QEA Gasco Project from Northwestern Aquatic Sciences, March 3, 2011

Table 1

Summary of Sediment Bioassay Results: *Hyalella azteca* 28-day Amphipod Sediment Toxicity Test

Sediment Sample Identification	Survival, REV = 88.1%			Growth, REV = 73.6%		
	Percent Survival (Mean ± SD)	Negative-control Adjusted Response (%)	Effect Level	Individual Dry Weight (mg) (Mean ± SD)	Negative-control Adjusted Response (%)	Effect Level
Test Samples						
DGS-01SG-101014	88.8 ± 11.3	93.4	0	0.21 ± 0.01	101.1	0
DGS-02SG-101014	93.8 ± 7.4	98.7	0	0.15 ± 0.01	71.7	1
DGS-04SG-101013	91.3 ± 11.3	96.1	0	0.18 ± 0.12	90.0	0
DGS-05SG-101014	91.3 ± 8.3	96.1	0	0.21 ± 0.06	101.5	0
DGS-06SG-101014	92.5 ± 7.1	97.4	0	0.17 ± 0.02	84.3	0
DGS-08SG-101013	88.8 ± 9.9	93.4	0	0.20 ± 0.05	95.7	0
DGS-09SG-101013	90.0 ± 14.1	94.7	0	0.16 ± 0.02	77.9	0
DGS-12SG-101013	92.5 ± 10.4	97.4	0	0.14 ± 0.01	70.2	1
DGS-13SG-101013	97.5 ± 4.6	102.6	0	0.16 ± 0.01	77.0	0
DGS-16SG-101013	93.8 ± 7.4	98.7	0	0.15 ± 0.02	71.3	1
DGS-17SG-101013	95.0 ± 10.7	100.0	0	0.17 ± 0.03	82.0	0
DGS-20SG-101013	96.3 ± 5.2	101.3	0	0.17 ± 0.01	81.7	0
DGS-21SG-101013	96.3 ± 7.4	101.3	0	0.14 ± 0.02	68.3	1
DGS-25SG-101012	90.0 ± 17.7	94.7	0	0.15 ± 0.03	75.2	0
DGS-26SG-101013	97.5 ± 4.6	102.6	0	0.15 ± 0.01	71.9	1
DGS-30SG-101012	87.5 ± 11.6	92.1	0	0.15 ± 0.02	75.5	0
DGS-31SG-101012	95.0 ± 7.6	100.0	0	0.15 ± 0.03	71.5	1
DGS-33SG-101012	97.5 ± 4.6	102.6	0	0.14 ± 0.01	69.9	1
DGS-34SG-101012	88.8 ± 8.3	93.4	0	0.15 ± 0.02	74.9	0
DGS-35SG-101012	82.5 ± 14.9	86.8	1	0.19 ± 0.06	91.0	0
Reference and Control						
U2C-2-101014	87.5 ± 10.4	--	--	0.17 ± 0.02	--	--
U4Q-1-101014	92.5 ± 10.4	--	--	0.17 ± 0.04	--	--
U4Q-2-101014	93.8 ± 7.4	--	--	0.15 ± 0.02	--	--
Negative Control	95.0 ± 7.6	--	--	0.20 ± 0.02	--	--

Notes:

mg	milligrams
REV	Reference Envelope Values
SD	standard deviation
--	not applicable

Table 2

Summary of Sediment Bioassay Results: *Chironomus dilutus* 10-day Midge Sediment Toxicity Test

Sediment Sample Identification	Survival, REV = 93.9%			Growth, REV = 91.0%		
	Percent Survival (Mean ± SD)	Negative-Control Adjusted Response (%)	Effect Level	Average ash-free dry weight (mg)/midge (Mean ± SD)	Negative-Control Adjusted Response (%)	Effect Level
Test Samples						
DGS-01SG-101014	70.0 ± 23.9	76.7	2	0.79 ± 0.30	103.0	0
DGS-02SG-101014	68.8 ± 17.3	75.3	2	0.71 ± 0.21	92.4	0
DGS-04SG-101013	37.5 ± 34.1	41.1	3	0.34 ± 0.32	44.8	3
DGS-05SG-101014	40.0 ± 32.1	43.8	3	0.75 ± 0.39	97.4	0
DGS-06SG-101014	63.8 ± 20.0	69.9	3	0.87 ± 0.27	113.3	0
DGS-08SG-101013	45.0 ± 29.3	49.3	3	0.54 ± 0.30	70.0	3
DGS-09SG-101013	66.3 ± 27.2	72.6	3	0.64 ± 0.29	83.9	1
DGS-12SG-101013	56.3 ± 27.7	61.6	3	0.46 ± 0.24	59.4	3
DGS-13SG-101013	38.8 ± 28.5	42.5	3	0.72 ± 0.32	94.1	0
DGS-16SG-101013	61.3 ± 26.4	67.1	3	0.72 ± 0.30	94.0	0
DGS-17SG-101013	53.8 ± 32.5	58.9	3	0.64 ± 0.31	83.3	1
DGS-20SG-101013	40.0 ± 29.3	43.8	3	0.37 ± 0.36	48.0	3
DGS-21SG-101013	52.5 ± 29.2	57.5	3	0.80 ± 0.48	104.0	0
DGS-25SG-101012	61.3 ± 23.0	67.1	3	0.67 ± 0.31	87.4	1
DGS-26SG-101013	33.8 ± 37.8	37.0	3	0.28 ± 0.24	36.4	3
DGS-30SG-101012	58.8 ± 21.0	64.4	3	0.65 ± 0.29	84.8	1
DGS-31SG-101012	35.0 ± 26.2	38.4	3	0.37 ± 0.26	48.0	3
DGS-33SG-101012	55.0 ± 34.2	60.3	3	0.66 ± 0.36	85.8	1
DGS-34SG-101012	53.8 ± 29.7	58.9	3	0.66 ± 0.27	86.3	1
DGS-35SG-101012	20.0 ± 21.4	21.9	3	0.21 ± 0.20	27.6	3
Reference and Control						
U2C-2-101014	68.8 ± 31.4	--	--	0.88 ± 0.17	--	--
U4Q-1-101014	65.0 ± 19.3	--	--	0.88 ± 0.16	--	--
U4Q-2-101014	66.3 ± 23.3	--	--	0.94 ± 0.28	--	--
Negative Control	91.3 ± 8.3	--	--	0.77 ± 0.16	--	--

Notes:

mg	milligrams
REV	Reference Envelope Values
SD	standard deviation
--	not applicable

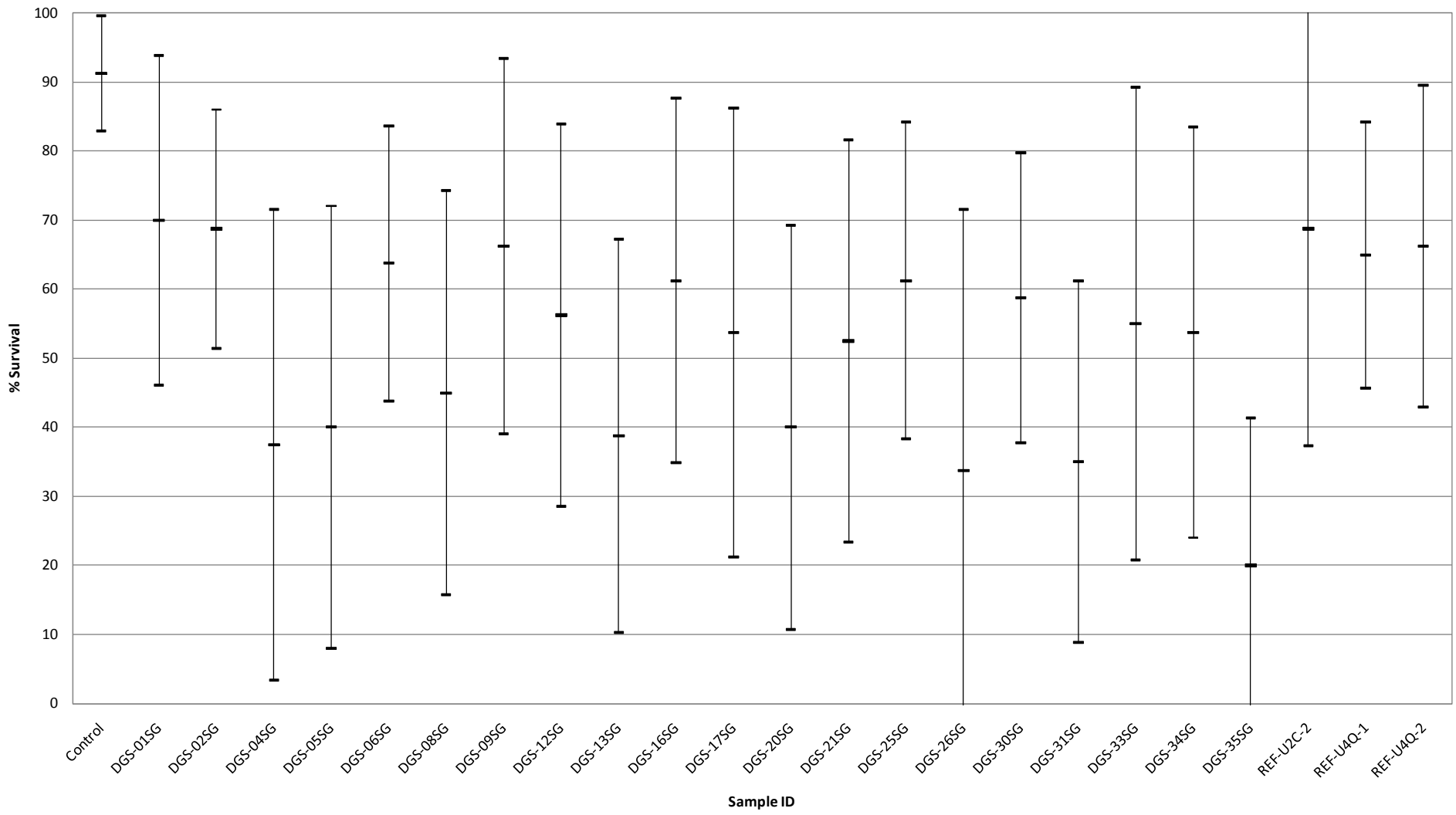

Figure 1

Statistical Summary of Individual Survival Replicates for the Midge Bioassay (Mean Survival ± SD)

Bioassay Interpretation Summary and Recommendations

NW Natural Gasco Sediment Cleanup Action

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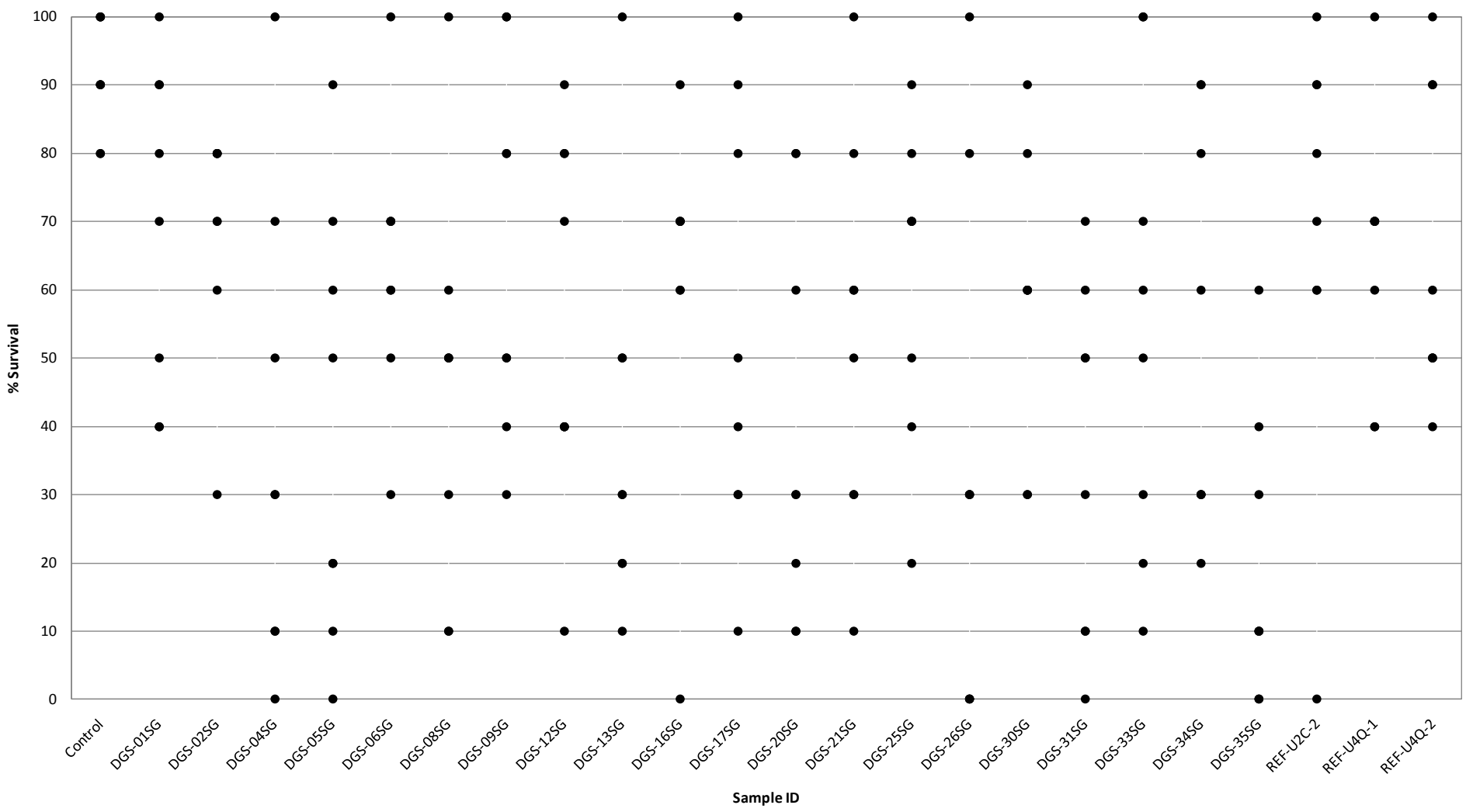

Figure 2
Summary of Individual Survival Replicate Data for the Midge Bioassay
Bioassay Interpretation Summary and Recommendations
NW Natural Gasco Sediment Cleanup Action

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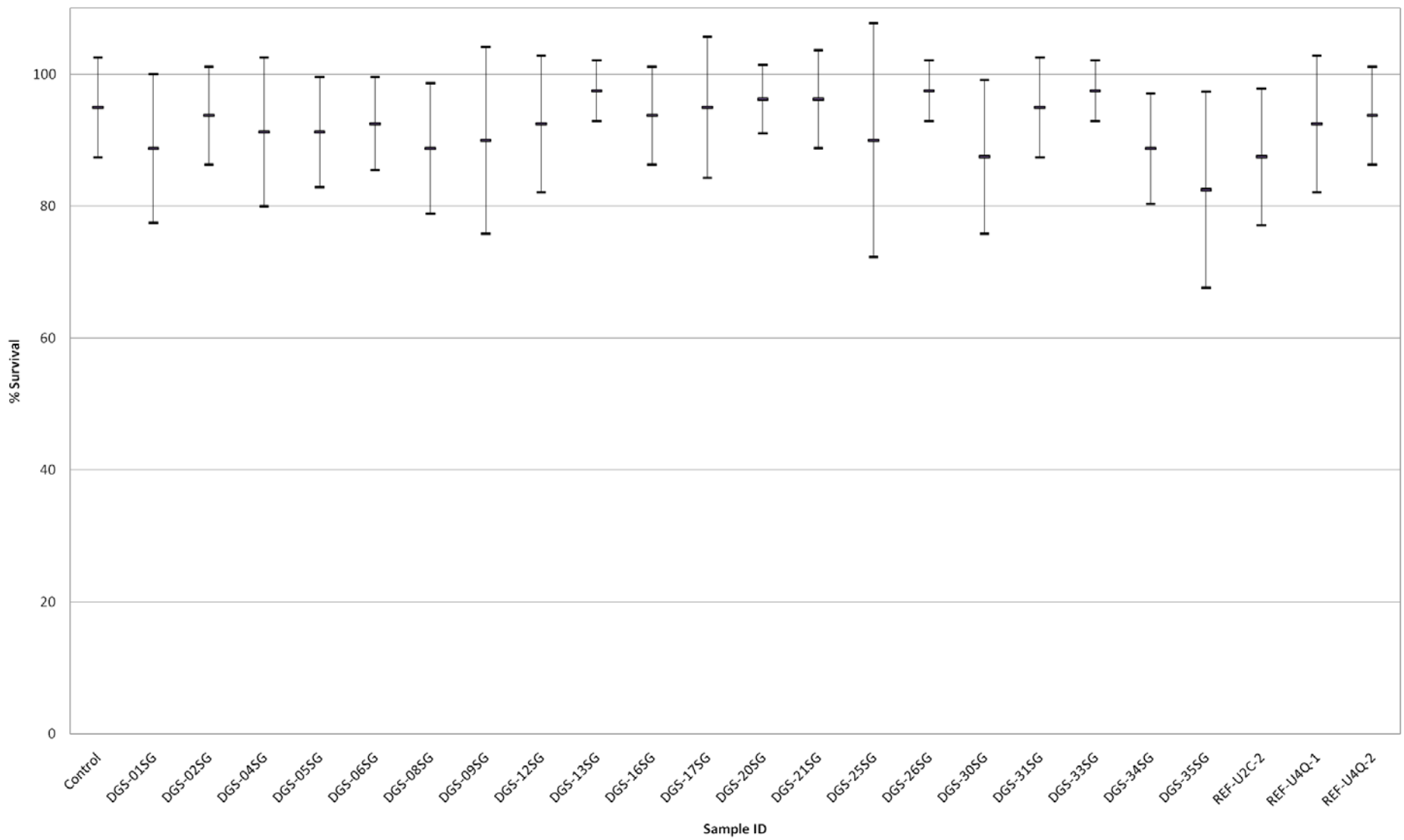

Figure 3

Statistical Summary of Individual Survival Replicates for the *Hyalella* Bioassay (Mean Survival ± SD)

Bioassay Interpretation Summary and Recommendations

NW Natural Gasco Sediment Cleanup Action

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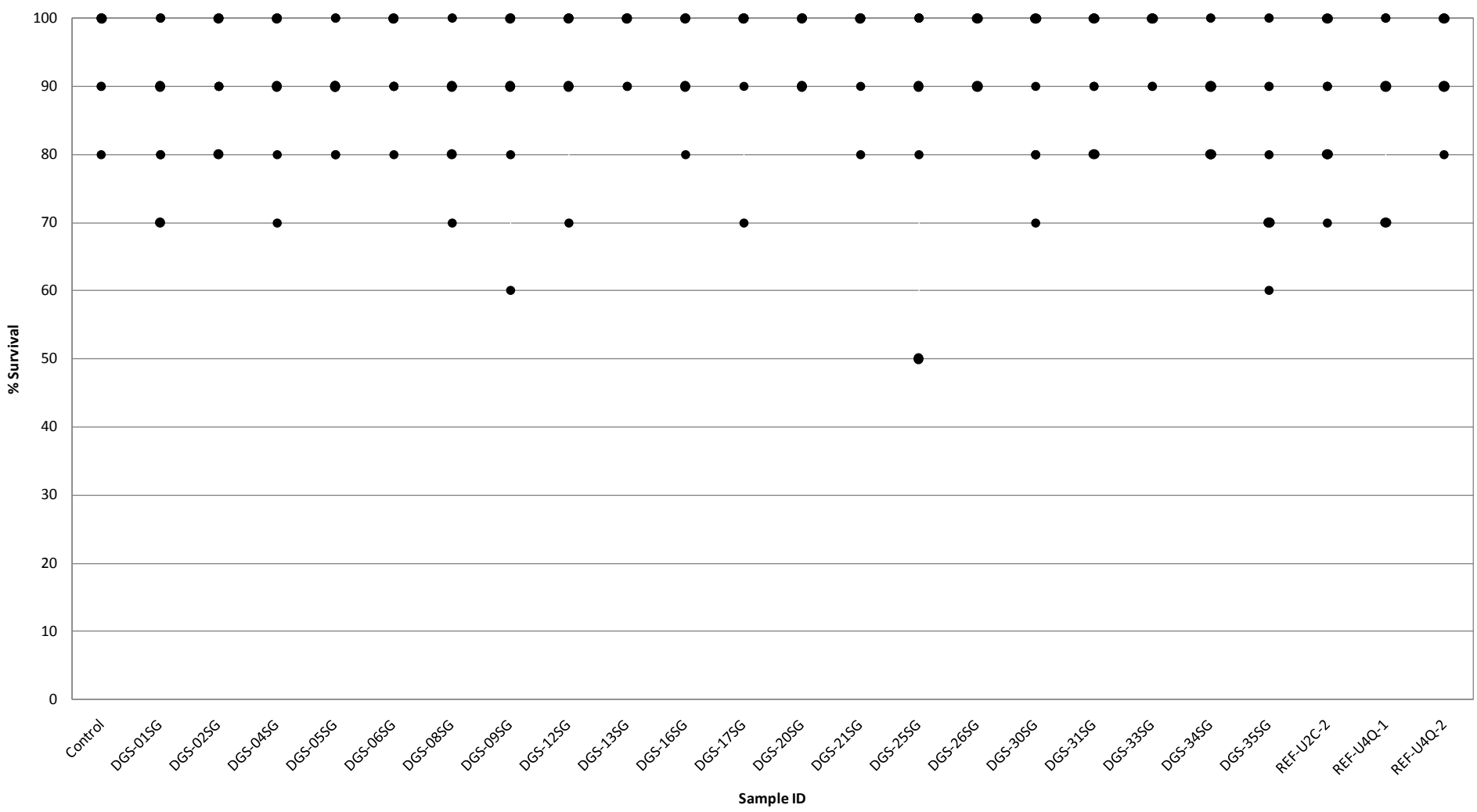

Figure 4
Summary of Individual Survival Replicate Data for the *Hyalella* Bioassay
Bioassay Interpretation Summary and Recommendations
NW Natural Gasco Sediment Cleanup Action

ANCHOR QEA

NORTHWESTERN AQUATIC SCIENCES
A Division of NAS Associates, Inc
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March 3, 2011

Taku Fuji, Ph.D.
Anchor QEA, LLC
6650 SW Redwood Lane
Suite 333
Portland, OR 97224

Dear Dr. Fuji:

Northwestern Aquatic Sciences (NAS) has reviewed the sediment bioassay test results for the 10-day midge (*Chironomus dilutus*) and the 28-day amphipod (*Hyallela azteca*) tests that were conducted for Anchor QEA as part of the NW Natural Gasco Sediment Cleanup Action Data Gaps Project. These tests, No. 814-1 for the amphipod and No. 814-2 for the midge, were completed for twenty test sediments and three reference sediments received by NAS on October 15, 2010. The sediment bioassay reports for these two tests were provided to Anchor QEA on December 6, 2010.

As presented in the 28-day Amphipod, *Hyallela azteca* , sediment bioassay report (No. 814-1), the negative control sediment bioassay response met the survival and weight acceptability criteria specified for this test protocol and the reference sediments test responses met the Sediment Evaluation Framework for the Pacific Northwest (SEF, 2009) reference sediment performance standards for both the survival and growth endpoints. The reference toxicant (positive control) result was within laboratory control limits and is acceptable. In addition, the variability observed for the survival endpoint within individual test replicates for these tests were within the normal range of standard deviations for this test (standard deviations on the percent mortality reported ranged from 4.6% to 17.7% with a mean of 9.2%).

As presented in the 10-day Midge, *Chironomus dilutes*, sediment bioassay report (No. 814-2), the negative control sediment bioassay response met survival and weight acceptability criteria specified for this test protocol. The reference sediment test responses did not meet the SEF reference sediment performance standard for survival but

the performance standard was met for the growth endpoint. The reference toxicant (positive control) result was within laboratory control limits and is acceptable.

NAS' review of the mortality endpoint results for the midge bioassay indicated that there was an unusually high level of variability observed within individual replicates for test and reference sediments. The standard deviations on the percent mortality reported for individual replicates for sediment being tested ranged from 17.3% to 37.8% with a mean of 27.5%. These reported standard deviations are higher than normally observed for this sediment bioassay. For example, the mean standard deviation for mortality between replicates of the negative control sediment for the previous forty-four 10-day midge tests conducted by NAS was 8.7%.

The source of the high variability observed for the mortality endpoint in the midge test is uncertain. Based on the results of the review of the negative control and reference toxicant test results, as well as the water quality observations recorded during the sediment bioassay, the source of the high variability does not appear to be related to laboratory conditions. The high variability and lack of agreement within individual test sediment replicates presents challenges for the appropriate classification and/or interpretation of the midge sediment bioassay results.

Please feel free to contact us with any additional questions or concerns regarding these sediment bioassay tests or results.

Sincerely,

Richard S. Caldwell

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Laboratory Director

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