MORRISON KNUDSEN CORPORATION
MK-FERGUSON GROUP
2420 MALL DRIVE
CORPORATE SQUARE ONE, SUITE 211
N. CHARLESTON, SC 29406

Transmittal Letter

Project Name: SOUTHDIV ERAC CONTRACT N62467-93-D-1106	Transmittal No. 94-4324-2431 / Work Order No. 4324
	Date: July 16, 1998
To: Mr. Robert J. Meddick, Contracting Officer Department of the Navy Southern Division Naval Facilities Engineering Command 2155 Eagle Drive Post Office Box 190010 North Charleston, South Carolina 29419-9010	**REFERENCES**
	Item: DO 0009 SOW 007 NSWC Crane
	Equipment No.
	Purchase Order No.
	Vendor Order No.

TRANSMITTED HEREWITH	FOR STATUS NOTED
For: ☐ Review and comment ☐ Approval ☐ Record ☐ Information	Reviewed A Reviewed as Noted - Resubmit B Not Reviewed - Resubmit C For Information D

QUANTITY	ITEM	TITLE/DESCRIPTION

DO 0009 SOW 007 NSWC Crane

Appendix G Toxicity Testing and Leaching Procedures of the Quality Assurance Project Plan for Full Scale Operations at the Bioremediation Facility, NSWC Crane, Crane, Indiana dated March 12, 1998 has been revised and is hereby forwarded for review and approval.

Text Changes dated 7/14/98

By: for

Name: R.S. Newman

Title: Program Manager
SOUTHDIV RAC II

DO 0009 SOW 007 NSWC Crane
Final, Toxicity Test Procedure (Appendix G of the QAPP)
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Quality Assurance Project Plan
For Full-Scale Operations at the Bioremediation Facility
NSWC Crane, Crane, Indiana

APPENDIX G

Instructions for Replacement Pages

A. **Main Text**: Remove pages G-1 through G-9, dated 05/13/98 and discard. Replace new pages G-1 through G-11, dated 07/14/98.

B. **Attachment A:** Remove cover page, dated 05/13/98; and discard. Replace with new cover page, dated 07/14/98.

1. Remove pages A-1 and A-2 and replace with the attached new pages A-1 and A-2.

2. Append attached pages A-3a through A-3f after page A-3.

3. Remove page A-8 and replace with the attached new page A-8.

4. Remove page A-9 and discard. Replace with attached page A-9 noted as "THIS PAGE IS LEFT BLANK INTENTIONALLY"

5. Remove page A-13 and replace with the attached new page A-13.

6. Remove pages A-15 and A-16 replace with the attached new pages A-15 and A-16.

7. Append attached pages A-33a and A-33b after page A-33.

8. Remove pages A-41 through A-49 and replace with the attached new pages A-41 through A-49a.

NSWC CRANE
CRANE, INDIANA

COMMENT-RESOLUTION

EPA REGION V COMMENTS ON APPENDIX G OF QUALITY ASSURANCE PROJECT PLAN FOR THE BIOREMEDIATION FACILITY

A. GENERAL COMMENTS

Comment 1: The Plan needs to be specific to the Navy's project. The SOPs have generic language with options and it is not clear what specifically will be required for the Navy's project.

Response 1: Comment noted. The plan and standard operating procedures have been revised to specifically include the specifications for this project.

Comment 2: The Objectives should be tabelized and written clearly with the goals defined.

Response 2: This section has been re-written to include a table to clearly define the goals for this aspect of the project.

Comment 3: References to other portions of the approved Work Plan or the Quality Assurance Project Plan (QAPP) must be precise. In several areas references are made to sections that don't exist in the Appendix.

Response 3: Comment noted. All references have been verified. In addition, references now contain page numbers and locations to aid in the location of the referenced material.

Comment 4: Are all the sections of a QAPP represented or referenced to?

Response 4: All sections that are referred to in this appendix are present in the approved Quality Assurance Project Plan (QAPP), Rev. 2 dated March 12, 1998 or the Full-Scale Operational Plan, Rev. 2 dated March 12, 1998. Tables, diagrams, and specific text instructions have not been re-created in this appendix, rather they have been explicitly referenced, in order to prevent multiple copies of the same material within several sections of the QAPP.

B. SPECIFIC COMMENTS

Comment 1: Page G-3, Section 1.0 Introduction

a. The second paragraph refers to Appendix G for sampling descriptions but they are not described within that appendix.
b. The Introduction should tie the Appendix to the main text of the approved Work Plan.

Response 1:
a. Sampling information pertaining to sample locations and sample frequency is contained in Appendix G of the Operational Plan as cited. The wording of this statement has been changed to specifically state, "sampling locations and frequency" rather than "sampling information".
b. Comment noted. Text has been revised.

Comment 2: Page G-4, Section 2.0 Test Methods

a. Note that the preservation is only for the finished compost. There needs to be an explanation on how the soil will be prepared to bring the temperature up to a temperature comfortable for the test species used for certain test methods.
b. Explain in the introduction or a piece on how the whole sampling ties in, how one compost pile will be constructed for a control.
c. Explain that the pile used for testing will be representative for the Solid Waste Management Unit (SWMU) and the worst-case scenario.
d. There should be included a justification why only 5 samples from a full-scale pile shall be representative of the potential volume of soil per SWMU. If it is not a sufficient amount, then more samples should be taken either in that pile or from a second pile. Remember, the limited sampling needs to have a clear objective of representativeness.

Response 2:
a. Method specifications such as temperature stabilization and method conditions are specified in the standard operating procedures of the laboratory. The general test specifications have also been added to Section 2.0.
b. Comment noted. Text has been revised.
c. Comment noted. Text has been revised.
d. To be consistent with the approved Appendix G of the Operational Plan, the five samples were selected. These five samples will be representative of one windrow. However, the windrow selected will not be representative of all the soil in the SWMU. The windrow will represent the worst case scenario of the contaminated soil from the first windrow activities for that SWMU.

Comment 3: Page G-5, Section 3.0 Remedial Goals

a. Is this section meant to be just goals or goals and objectives?
b. Tabelizing this information would make it clearer for the reviewer.
c. "Objectives" are different than "Action Levels."
d. What are the goals for compliance? Pass/Fail, toxic effects, etc.
e. How will levels be determined for the Worm and Microtox tests? What is it based on? How many live or die? The concentration of contaminants in the worm?
f. What exactly is the control windrow? Describe it. Doesn't the control also provide a basis for determining if the amendment ratios are also toxic or leachable?
g. Exactly how will the control and sample data be compared? Statistics, direct comparison? Explain, include the details, and formulas.
h. Define significant mortality.
i. Include the objectives of the pathogen test, don't just reference the regulation.
j. Table 1-4C is not included.
k. The TCLP levels should be included in the goal table.
l. Leachate concentrations should be compared to a goal table. Include and explain its use.

Response 3:
a. This section has been revised to contain both goals and objectives for the toxicity analysis.
b. Comment noted. The table has been provided in subsequent revisions to this appendix.
c. Comment noted.
d. The goals for compliance have been provided in Table G-2 of this appendix.
e. The microtox test will be evaluated on a decrease in the illumination of the organism and the earthworm test will be evaluated on the basis of mortality. The levels that will be used to compare data from the various samples will be the EC-

50 and the LC-50 respectively.

f. Comment noted. Text has been revised.

g. The LC-50 data from the control pile will be compared to the LC-50 data of the windrows. The goal is to have a higher average LC-50 from the windrows than the LC-50 from the control pile. This will demonstrate that the treated soil from the SWMU is no more toxic than similar uncontaminated soil which has gone through the treatment procedure.

h. This text has been revised comparing the LC-50 data of the control pile and the LC-50 data of the windrow. Significant mortality will be determined in comparison of the windrow data to the control pile data. Acceptable ranges have been provided in Table G-2.

i. Comment noted. Table G-2 and the text have been revised.

j. Table 1-4C is located in Section 1, Page 10 of 44 of the approved QAPP. It is not located in this appendix.

k. TCLP levels are shown in Table 1-4C for all compounds of concern for this project. However, they have also been incorporated into the goal table for this appendix.

l. Comment noted. Table G-2 of this appendix has been revised to incorporate TCLP levels.

Comment 4: Section 4.0, Quality Control Procedures

a. Define the control compost windrow. Size, how it is comparable, where it is located, how long it was treated, was it treated the same as the full-scale test pile, handling, etc.

b. Explain where the worms are supplied from.

c. Identify borrow locations for each SWMU.

d. What happens if background levels are toxic? Will only one control be made, or one per SWMU testing?

e. QC decontamination is referred to Appendix G, which this is, and is not included. Do you mean another appendix?

f. Section 4.6 of this QAPP in Appendix G is not included.

g. This plan should act like an independent amendment to the approved QAPP and Work Plan.

h. Be consistent with terms, control versus artificial soil.

i. Define LC-50.

j. What is the reference toxicant?

k. How is the sensitivity evaluated? By an SOP?

l. What are the Quality controls for the pH meter and any other field parameter equipment, etc. This should be in the QAPP.

m. The computer program or its statistical references must be checked to make sure the Agency's agree with their use.

n. Explain how calculations will be made.

Response 4:

a. Comment noted. Text has been revised.

b. The worms, *Eisenia Foetida* will be supplied from a commercial source that can verify the species. For this project, Carolina Biological Supply Company will be utilized. Text in Section 4.2 has been revised to include the source.

c. Borrow locations will be selected based on the Navy recommendations of site characteristics and similar soil characterization. Borrow soil will be tested for various contaminants of concern prior to establishment of the window in order to confirm non-contaminated source soil. In the completion report for each SWMU, information as to the location and source of each borrow soil will be provided.

d. One control windrow will be made for each SWMU. Potentially this control windrow, which represents background soil material, could be toxic. In which case another plan will be developed for disposing of this material.

e. The appendix reference is Appendix G of the Operational Plan. The appendix

submitted for this review is Appendix G of the QAPP. The Operational plan appendix contains the specific site information needed to perform equipment decontamination in Section 5.0. The reference has been clarified.

f. This reference has been clarified.

g. The plan has been revised to act as an amendment to the approved QAPP, however references to tables and specifications continue to be present in this appendix rather than reproduce various sections over again. References to the QAPP have been revised to include specific page and section numbers.

h. Comment noted. Text has been revised.

i. *Comment noted. Text has been revised.*

j. The reference toxicant to be used in this analysis is potassium chloride (KCl).

k. Sensitivity will be evaluated by comparing the LC-50 of the KCl contaminated soil with previous laboratory control limits as well as published recommended limits for this species of earthworm.

l. The text has been revised to contain quality control requirements of equipment utilized in association with this methodology.

m. Comment noted. Printouts of the computer calculations will accompany all data summary reports.

n. Raw data showing calculations will be provided with all data summary reports in order to show all calculations made during analysis.

Comment 5: SOP/SED/201, Earthworm Test

a. What are the external sources for the worms. Worm age?

b. What are the test conditions the worms must be acclimated to?

c. *Is the Artificial soil the compost, the control, or both?*

d. Are the samples individually homogenized, or grouped, or what?

e. What is the mechanical manipulation?

f. The pass/fail procedure needs to be defined for this project.

g. SOP/GEN/303 referenced is not included.

h. In Sections F and G, explain "when appropriate" and "by appropriate means."

i. Are both acute and bioaccumulation effects addressed?

j. Define artificial soil in Section I.

k. What is the exact volume of compost to be tested?

l. What is the reference toxicant test.

m. What does "or as specified in the workplan" mean in Section O. Be specific to this site.

n. *Include the computer program analysis.*

o. How do you establish pass or fail? Compared to what?

Response 5:

a. The specification of the worm size, supplier, age, and weight has been included in the text.

b. The test condition specifications including temperature, worm addition instructions, and worm denseness have been added to subsequent revisions of this appendix.

c. The artificial soil is prepared in the laboratory as a blank and also to act as a dilutant to the compost material. The soil consists of a prepared homogenous mixture of 10% sphagnum peat, 20% colloidal kaolinite clay, and 70% grade 70 silica sand. This artificial soil will be used as a blank to verify that no more than 10% of the worms died at the end of the 14-day toxicity exposure period. In addition the artificial soil will be used as a dilutant to the compost material during the analysis to determine the LC-50 (the concentration of compost that will result in 50% mortality)

d. The five samples collected on site will be maintained separately in 1-gallon steel cans. *Upon receipt each compost sample will be individually homogenized and* analyzed. Appropriate portions of compost will be mixed with artificial soil to create a range of five varying concentrations of compost/artificial soil mixture. The

expected concentrations for these mixtures will be 6.25%, 12.5%, 25%, 50%, and 100%. Each concentration of compost/artificial soil will be analyzed with three separate replicates (i.e., one sample compost collected will have five concentrations created of the compost and three replicates of each concentration or 15 sample chambers). The text has been revised to clearly state the sample preparation techniques.

e. Mechanical manipulation discussed in the laboratory SOP is matrix specific. For the compost material utilized in this project this manipulation will be performed by using a blender.

f. The mortality of the earthworms will be utilized to determine pass/fail criteria. From the mortality data, the LC50 value will be calculated to evaluate the concentration of compost which results in 50% mortality of the earthworms. Mortality criteria has been added to the text.

g. SOP/GEN/303 has now been attached for reference and review.

h. "When appropriate" and "by appropriate means" refer to the weighing of the earthworms to study bioaccumulation effects, which are not being studied in this project. Specific weight characterization of the earthworms is not relevant to this study. Worms will be between 300 and 500 mg at the beginning of this project and will not be re-weighed at any time during the duration of this analysis.

i. Only acute effects will be addressed in this 14-day test. Bioaccumulation analysis would require the analysis of the organisms surviving after 28 days to determine the levels of explosives that are present in the organisms. However, because the explosive contaminants have already degraded in the treated compost material (verified by laboratory analysis on Day End), the bioaccumulation analysis would not provide additional information in this study.

j. The artificial soil is described above in Response 5.c. In addition, the artificial soil is defined by the laboratory in their SOP Section 2.C.

k. The artificial soil and compost mixture contained in each analysis vessel will be 200 g of material. In the five dilutions concentrations of 6.25%, 12.5%, 25%, 50%, and 100% will be made or 12.5 g, 25 g, 50 g, 100g, and 200g of compost respectively in each of the analysis vessels.

l. The reference toxicant test is performed by exposing earthworms to varying concentrations of KCl. Similar to the test performed with the compost, the LC-50 is determined. This value refers to the concentration of KCl which results in 50% mortality. It will be compared to other LC-50 values obtained by the laboratory using the same type of organism and also to published recommended limits for this species of earthworm.

m. The mortality control should not exceed 10% at the end of 14-day toxicity exposure for this project. The text in Section 4.2 has been revised.

n. The computer print-outs for data associated with earthworm analysis and all raw data calculations will be provided in all data summary reports for this project.

o. The outcomes of the compost samples for earthworm analysis will be compared to the outcomes of the control pile. It is expected that all compost samples will have a higher value than the control pile to demonstrate that the treated soil is no more toxic than uncontaminated soil which has gone through the treatment process.

Comment 6: Southwest's SOP appears much more detailed than the other labs.

Response 6: Comment noted.

Comment 7: Microtox - It should be clarified what the sampling frequency really is. I am having a difficult time deciding whether the five samples will be taken during the final day interval, or whether the five samples will be taken per SWMU, at different periods in the life of the pile, including the final day interval. (Perhaps the answer to this is alluded to somewhere, but it could be clarified.)

Response 7: Comment noted. Text in Section 1.0 has been revised to clarify sampling information.

Comment 8: Table G-1: Is a 4 degree C temperature sufficient for a 3 to 14 day holding time. Also, I am having a difficult time comprehending why the samples for the pathogen test would have to be chilled. After all, won't this kill the pathogens, producing a low bias in the final results?

Response 8: The laboratory recommends samples for pathogen testing be preserved at 4 degrees C during transportation and during storage at the laboratory. Also, this requirement has been presented in Table G-1 of the approved Appendix G of the Operational Plan.

Comment 9: Section 4.1, QC Procedures: Will the field duplicate be taken once per sampling interval? While it seems sensible to collect a field duplicate, analysis of field blanks and rinse water samples may not apply. Why would it be advantageous to run toxicity tests on these QC sample types? (A field blank is just reagent grade water, and by definition would not be expected to kill microorganisms. Ditto for the rinse blank, but the analysis of these blanks for targeted speciated compounds would indicate whether any contaminant transfer has occurred. Wouldn't that be sufficient?) So isn't this overkill? What is the rationale for these QA sample types?

Response 9: Field QC will be performed as specified in Table 4-3 in Section 4, Page 10 of 18 of the approved QAPP for analytical testing only. No field QC for toxicity analysis including field blanks and rinse blanks is proposed.

Comment 10: Section 4.2: Target parameters for earthworm study analysis were not indicated.

Response 10: The mortality of the earthworms will be evaluated in this test. The parameter to be reported from the analysis is the concentration of compost which results in 50% mortality of the earthworms.

Comment 11: Section 4.2, p. G-6, last three lines: What is meant by the range of test concentrations? What is the relationship between "EC-50" and "LC-50", and in fact what do these terms stand for? (Does "EC" stand for "exposure concentration"?) Define all terms.

Response 11: This paragraph has been re-written to provide more detail to the microtox test procedure. EC-50 is the effective concentration. EC-50 is the concentration of the contaminated medium that produces a designated effect on 50% of the test organisms. LC-50 is the lethal concentration and is defined as the concentration of contaminated medium that produces mortality in 50% of the test organisms.

Comment 12: Page G-7: What is an "R value"? If this is a statistical term, then it should be defined mathematically.

Response 12: Comment noted. Text has been revised.

Comment 13: SOP/PRP/004: Is this SOP intended to be used for the microtox or the earthworm test?

Response 13: This SOP is utilized in the microtox test. The microtox test is performed in a liquid matrix. If solid samples are to be analyzed with this method, an extraction of the sample must be performed. This SOP along with SOP/PRP/005 outlines the procedure the laboratory will follow to prepare the compost samples for analysis. SOP/PRP/005 has now been included for your review.

Comment 14: Page A-2, Section 2.1: Should the term "compost" be substituted for the word, "sediment"? Won't the toxicity effects of compost be anticipated to be higher than in the case of sediment? Are these tests designed with that possibility in mind? Won't the measurement systems be 'torqued" by the really high concentrations of microorganisms?

Response 14: Comment noted. Text will be revised. The laboratory will be notified of the potential toxicity effects and as a result may use additional dilutions to bring samples within range of their measurement systems.

Comment 15: SOP/SED/201, page A-5, Section III.B: For this study, how will concentrations of specific contaminants be determined as part of exposure concentrations? The specific constituents that will be quantified for use in the earthworm test should be stated. Which compost sampling intervals will be represented in the form of earthworm data?

Response 15: The concentration of compost in artificial soil will be evaluated for this test. These concentrations will be created in the laboratory from the sample provided. As stated earlier, five dilution concentrations of 6.25%, 12.5%, 25%, 50%, and 100% will be used to evaluate the compost. These dilutions will be prepared from the day final compost of a windrow selected to have the highest day 0 explosive contaminants during the first round of windrow activity from a given SWMU.

Comment 16: Page A-6, Sections C: Will data be averaged? is there an %RPD acceptance criterion for the set of replicates representing each dilution series?

Response 16: The three replicates each consisting of ten worms will be added to one another. Therefore rather than seeing 3 replicates each with 10 earthworms, one data point will be generated based on 30 worms. Raw data for each one of the replicates will be available for review. Acceptable RPD criteria for each set of replicates is not specified in the method and will not be utilized in this study, however notes of any problems with specific replicates will be noted on the data.

Comment 17: Page A-6, Section D "Collection of Organisms for testing": Will worms which have been subjected to previous exposures in other testing be used for the Crane study?

Response 17: Worms utilized for this test will not have been subjected to other test conditions prior to use in this study. The environmental conditions of the worm batches will be documented on livestock control records from receipt in the laboratory until analysis.

Comment 18: Page A-7, Section K; How will the number of live worms added to buckets be recorded?

Response 18: The worms will be added to the testing containers not the original shipping buckets. The addition of live worms to each testing container has now been specified in 4.2 of this Appendix.

Comment 19: Page A-7, Section N: What is the rationale for performing this step? Also, does this mean that this step will be performed for each test dilution/compost concentration?

Response 19: The reference toxicant is used to verify the hardiness of the earthworms. Data collected is compared to other batches of earthworms to verify the batch of worms used in this test reacts similarly to other worms. This step will be performed at five concentration levels of KCl in artificial soil.

Comment 20: Page A-7, Section P: Referring to the last sentence, does this permit recycling of old test worms?

Response 20: Old test worms are not recycled. Live test worms will be returned to the compost/soil mixture every day after inspection for mortality and behavior.

Comment 21: Page A-8, section Q: The pH of each test mix of compost should be measured at the beginning and end of the test, (including all replicates).

Response 21: pH will be monitored for each replicate at the beginning and end of test. Data will be recorded on laboratory bench sheets.

Comment 22: Page A-8, Section R: There are two typographical errors here. Also, how will "analysis" be performed and for which constituents? Shouldn't an assay of earthworms also be analyzed before the test begins as a control blank?

Response 22: Bioaccumulation studies are not being performed for this study. This section will be removed from the laboratory standard operating procedure.

Comment 23: Page A-9: This page is intended for the Picatiny Arsenal only. Therefore, it should be deleted.

Response 23: Comment noted. The page has been deleted.

Comment 24: SOP No. TOX-1001, Page A-13, middle of page: Where is Step 1.7, referred to in the middle paragraph on page?

Response 24: The reference has been corrected.

Comment 25: Page A-15: The first sentence in the third paragraph is poorly phrased. Can it be clarified?

Response 25: Comment noted. Text has been revised.

Comment 26: Page A-15: Referring to Roman numeral V, ("Chemical analysis"), what will be measured and how? Details are needed.

Response 26: This section of Aqua Survey, Inc. Standard Operating Procedure discusses the analysis of the sample to determine the contaminant of concern. For consistency, an aliquot of this material is being sent to Southwest Laboratories for analysis of explosives. Southwest is performing the analysis of the other explosive samples.

Comment 27: Page A-15, Roman Numeral VI: In the last paragraph, does the term "B0" refer to "zero time', or the 5 minute measurement described in a previous section.

Response 27: B0 refers to the initial zero time reading. BT refers to the Blank at final time. This data reduction section will be done once for the 5-minute reading and again for the 15 minute readings. The BT will be the blank reading at the appropriate analysis time.

Comment 28: Page A-16, top of page; What is meant by "IT"? Is this the 15 minute measurement? If

so, then it should be so stated.

Response 28: The entire data reduction step is performed once for the five minute measurements and again for the fifteen minute measurements. "IT" is the light readings at the final time of the analysis.

Comment 29: Page A-16: The equation for "blank ratio" should be indicated. Is it B0/BT?

Response 29: The blank ratio is B0/BT. Text has been revised.

Comment 30: Page A-16: What does "EC-50' stand for? Show the calculation.

Response 30: EC-50 is the concentration of compost elutriate which causes the effect of a 50% decrease in the light output of the microtox reagent organisms created from a strain of the marine bacterium, *Photobacterium phosphoreum* . This calculation is performed with the assistance of the a computer program supplied by Azur Environmental, the proprietor of this method.

Comment 31: There are terms in the pathogen testing SOP which could stand definition, such as "PBS", "MPN", and "MF".

Response 31: Comment noted. Text has been revised to define these terms.

Comment 32: For all variations on the pathogen test, how many milliliters of evolved gas would define the "production of gas" on which a positive result for pathogen would be founded? The "detectable" amount? What is detectable?

Response 32: The procedure has been revised to quantify the amount of gas which will result in a detectable level of gas produced.

Comment 33: Note that the TCLP SOP is for non-volatile constituents. Will there also be a need to analyze the treated compost for TCLP parameters that are VOCs?

Response 33: Volatile contaminants could potentially be a contaminant of concern. The extraction procedure for TCLP VOC analysis has been added to this appendix.

Comment 34: It is unclear which analytical methods will be utilized in the analysis of TC parameters. Since in certain cases there are alternatives, it should be more fully defined which methods will be utilized.

Response 34: TCLP parameters to be analyzed are any SWMU-specific chemicals of concern that have been detected greater than the IM Industrial Cleanup Goals in initial pre-excavation screening at the SWMU location.

Comment 35: Does SOP SWL-IN-700 dated 5/6/98 entirely supersede SWL-IN-700, dated 12/2/97? I think this may be the case because the TCLP procedure is entirely incorporated in both SOPs. But if this is the case, then why has the earlier version been submitted? Can we delete the earlier version?

Response 35: The earlier version has been superseded and the document has been revised to replace it with a recent version.

Comment 36: It is unclear what the rationale for use of the SPLP happens to be. It just seems to be a proposed procedure, but what will the results be used for? Are SPLP results factored into the "Decision Tree" for this project?

Response 36: TCLP and SPLP procedures are being utilized to simulate leachability. The TCLP procedure will simulate leachability in a sanitary landfill while the SPLP will simulate leaching due to precipitation in a natural environment. The objectives for SPLP are the same as the ones for TCLP. Text in Section 3.0, Table G-2 has been revised.

APPENDIX G

TOXICITY TESTING AND LEACHING PROCEDURES

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1.0
INTRODUCTION

This appendix will define the testing of compost material from the Naval Surface Warfare Center (NSWC), Crane Division, Crane, Indiana to determine the toxic affects of the treated compost material when in the environment of terrestrial invertebrates. This appendix will supplement the approved *Quality Assurance Project Plan for Full-Scale Operations.* [MK, 1998]

In this study, five compost samples will be collected from one windrow on day final of composting activities. The windrow selected for sampling will contain the highest contaminant levels of explosives based on baseline (day 0 of the treatment cycle) sample results from the first set of full scale windrows for that SWMU. This pile will be representative for the SWMU and represent the worst-case scenario for treated compost material based on the selection criteria utilized.

The treated soil/compost blend from each of the four Solid Waste Management Units (SWMUs 3/10, 10/15, 12/14, and 13/14) will be sampled and analyzed to document that the treated soil meets toxicity remedial goals. This appendix provides the following information:

- Remedial Goals for Toxicity Tests
- Laboratory Standard Operating Procedures
- Laboratory Contacts and Addresses
- Quality Control Procedures
- Test report preparation

Refer to Appendix G of the approved *Full-Scale Operational Plan* [MK, 1998] for sample location and frequency information, collection and splitting procedures, sample handling, and test method descriptions.

In addition to sampling the soil/compost blend from each of the SWMUs, a control pile will be constructed for each SWMU to provide material control samples. The results from control samples will be utilized to aid in the development of background levels for the compost material. In addition, the control pile will provide a basis for determining if the amendment ratios are toxic. Appendix G of the *Full-Scale Operational Plan* [MK, 1998] Section 2.0 describes how the control windrow pile will be created.

2.0
TEST METHODS

Table G-1 summarizes the testing methods.

TABLE G-1 TOXICITY AND LEACHABILITY TESTING SUMMARY				
Test and Test Method	**Frequency**	**Holding Time**	**Preservative**	**Sample Size/Container**
Microtox (Proprietary method of AZUR Environmental)	5 per SWMU + 1 control	14 days	4°C	8 ounce glass
Earthworm Toxicity (EPA Method 600/3-88/029)	5 per SWMU + 1 control	3 days	4°C	1 gallon steel can
Pathogen Testing (40 CFR 503.32)	5 per SWMU + 1 control	3 days	4°C	8 ounce glass
Toxicity Characteristic Leaching Procedure (TCLP) (EPA Method 1311) and Explosives Analysis (EPA Method 8330)	5 per SWMU	14 days for extraction; 40 days from extraction to analysis	4°C	8 ounce glass
Synthetic Precipitation Leaching Procedure (SPLP) (EPA Method 1312) and Explosives Analysis (EPA Method 8330)	5 per SWMU	14 days for extraction; 40 days from extraction to analysis	4°C	8 ounce glass

Samples will be maintained from collection through receipt until testing at 4°C and then brought to 20°C immediately prior to testing.

3.0
REMEDIAL GOALS AND OBJECTIVES

This appendix provides the design and specifications of the toxicity testing to be performed at NSWC Crane. The testing is intended to show the toxic effects that the treated compost material may have on the environment. These effects will be studied using two toxicity methods: Microtox and Earthworm Toxicity, two pathogen tests: fecal coliform, and salmonella, and two leaching procedures: TCLP and SPLP.

For each SWMU a single sample from the control windrow will be collected to provide background data for comparison and interpretation. Toxicity data of the samples collected from the treated contaminated soil windrow will be directly compared to the background sample to determine if there is an elevation in toxicity levels due to the treated explosive contaminants.

The objective of the toxicity testing is to assess the acute toxicity of the compost to worms and the bacteria. For the Microtox assays, the EC-50 will be estimated to determine the elutriate compost concentration which causes an 50% decrease in the illuminance of the test organisms. The EC-50 for the five samples will be averaged and compared to the EC-50 for the control pile to show a comparable or lower toxicity in the compost samples than in the control pile. For the earthworm toxicity the LC-50 will be estimated to determine the compost concentration which causes 50% mortality of earthworms. The LC-50 for the five samples will be averaged and compared to the LC-50 for the control pile to show comparable or lower toxicity in the compost samples than in the control pile.

Pathogen objectives were based on specifications found in 40 CFR 503.32 which pertains to the disposal of sewage sludge. These specifications show that sewage sludge may be prepared for sale or given away as an application to the land if the sludge meets the requirements of fecal coliform and salmonella. The treated compost is intended to be returned to the land for similar purpose, and therefore these same standards will be applied to the compost material. Objectives for the density of fecal coliform shall be less than 1000 Most Probable Number per gram of dry solids, and Salmonella shall be less than 3 Most Probable Number per 4 grams of dry solids.

Two tests will be performed to demonstrate the leachability of any SWMU specific contaminant that was identified during initial excavation of the soil used to create the windrow. Toxicity Characteristic Leaching Procedure (TCLP) will simulate the leachability of contamination expected in a sanitary landfill while Synthetic Precipitation Leaching Procedure (SPLP) will simulate the leachabililty of contamination expected in a natural environment. The limits of leachability for all SWMU contaminants are defined in Section 1.0 page 10 of 44 Table 1-4C of this QAPP.

TABLE G-2 TOXICITY AND LEACHABILITY OBJECTIVE SUMMARY			
Test and Test Method	**Result to be Obtained**	**Objective**	**Result will demonstrate**
Microtox (Proprietary method of AZUR Environmental)	EC-50 for each sample and average EC-50	Average EC-50 less than EC-50 of control pile	Compost piles are no more toxic than uncontaminated soil from a borrow
Earthworm Toxicity (EPA Method 600/3-88/029)	LC-50 for each sample and average LC-50	Average LC-50 less than LC-50 of control pile	Compost piles are no more toxic than uncontaminated soil from a borrow
Pathogen Testing (40 CFR 503.32)	Fecal Coliform Salmonella	Less than 1000 Most Probable Number/g dry solids Less than 3 MPN/4g dry solids	Compost piles are no more toxic than sewage sludge
Toxicity Characteristic Leaching Procedure (TCLP) (EPA Method 1311) and Explosives Analysis (EPA Method 8330)	TCLP results for all SWMU specific contaminants	Less than leachate limits presented in Table 1-4C in Section 1.0 of the QAPP	Compost piles do not show leachability greater than Regulatory Limits
Synthetic Precipitation Leaching Procedure (SPLP) (EPA Method 1312) and Explosives Analysis (EPA Method 8330)	SPLP results for all SWMU specific contaminants	SPLP results less than leachate limits presented in Table 1-4C.	Compost piles do not show leachability greater than Regulatory Limits

4.0
QUALITY CONTROL PROCEDURES

4.1 FIELD QUALITY CONTROL

A small control compost windrow will be constructed for each SWMU using the same amendment recipe as the full scale windrows. Clean, analyzed soil from a documented borrow source selected to be of similar soil characteristics to the contaminated soil will be used to construct the control windrow which will be approximately 10 x 10 x 5 feet high located in the same building as other windrow piles. This windrow will be constructed during the first set of full-scale windrows and maintained to simulate the full-scale windrows. This windrow will be turned at the same frequency, temperature, oxygen, and moisture content of the control windrow will be measured and recorded at the same frequency as the full-scale windrows. This control will be utilized to aid in the determination of background toxicity levels as well to serve as a guide to determine if the amendment ratios are toxic. In addition, the control pile will be analyzed for explosives at the end of cycle to verify that there is no cross contamination from other windrow piles. End of cycle for control pile will be the same number of days that the pile chosen for toxicity sample analysis was processed. Other QC field procedures for decontamination of equipment is detailed in Section 5.0 of Appendix G of the *Full-Scale Operational Plan* [MK, 1998]

The collection of field blanks, rinse water samples, and field duplicates will be performed at the same frequency as other compost samples. The specifications for compost quality control is specified in Section 4.6 on page 8 through 12 of 18 of the QAPP.

4.2 ANALYTICAL TESTING QUALITY CONTROL

Detailed standard operating procedures for each analytical test are included in Attachment A of this Appendix. These procedures outline the specific quality control measures required by the analytical method.

4.2.1 Earthworm Toxicity

Earthworm analysis will be performed using the test organism, *Eisenia Foetida* supplied from Carolina Biological Supply Company. The condition and environment of the worms will be documented on the livestock record from receipt in the laboratory until use in this project. Worms used in this project will not have been previously submitted to any other testing. The worms will be uniform in size, at least 60 days of age, and weigh approximately 300 - 500 mg. Worms will be maintained at a temperature of 20 $\pm$ 2°C and acclimated for a period of five days prior to testing. During this time worms will be fed with Magic Worm Food. Feedings will be documented on the livestock record. Initiation of the test will begin after the five day stabilization has occurred. Ten worms will be placed in the testing vessels by sequential randomization, where no more than 2 worms will be added into each vessel at a time. During the duration of the analysis, the worms will not be fed.

Each vessel will contain 200 grams of testing material (compost/artificial soil). Artificial soil prepared from a homogenous mixture of 10% sphagnum peat, 20% colloidal kaolinite clay and 70% grade 70 silica sand will be used as a blank and also as a dilutant to the compost mixture. Five varying concentrations of artificial soil and compost will be homogenized using a grinder. The concentration ranges of 6.25%, 12,5%, 25%, 50%, and 100% will be created for this analysis. In addition, three replicates for each concentration of compost will analyzed be performed to determine the LC-50. (the compost concentration that kills 50% of the worms)

Additional QC includes the use KCl as a reference toxicant. The KCl will be utilized as a positive control to evaluate the sensitivity of each batch of organisms. Varying concentrations of KCl contaminated soil will be prepared to determine the LC-50 of the KCl contaminated soil. This value will represent the concentration of KCl which results in 50% mortality. The LC-50 will be compared to published acceptance limits as well as previous laboratory data to verify that the worms reacted at similar sensitivities as other batches of *Eisenia Foetida*. The artificial soil prepared in the lab will also be analyzed to verify that less than 10% of the worms died in the blank.

Initially, and again at the completion of this analysis, the pH of the testing material will be recorded for each testing vessel. The pH will be documented and measured with a calibrated meter. The temperature of the test will be monitored continually during the analysis. The temperature should remain in the window of 22$\pm$ 2 degrees Centigrade and will not fluctuate more than 3 degrees in any twenty four hour period. Testing temperatures will be documented and measured with a thermometer that has been calibrated to an ASTM traceable thermometer. Earthworm analysis will be performed by Aqua Survey, Inc.

4.2.2 Pathogen Analysis

Pathogen analysis will be performed according to the laboratory standard operating procedure. Each sample is prepared with three dilution series, each with five replicate tubes for Salmonella and four dilution series each with five replicate tubes for Fecal Coliform as stated in the laboratory SOP. All data from the various replicates will be evaluated from the raw data. All data from the various replicates will be evaluated from the raw data. The pathogen analysis will include an empty bottle shipped to the site and returned as a blank control to verify that contamination has not occurred during transport. Pathogen analysis will be performed by Pure Earth Environmental Laboratory.

4.2.3 Microtox Analysis

The EC-50 value will be determined as the concentration of elutriate compost material which causes the effect of a 50% decrease in the light output of the microtox reagent organisms created from a strain of the marine bacterium, *Photobacterium phosphoreum.* The microtox test will be performed according to the specifications of the proprietary method from Azur Environmental with the assistance of a computer program from Azur. The microtox test will utilize a blank to be analyzed with each assay. The blank will have a ratio of 0.75 or greater. A computer program will be utilized to determine the EC-50. This value must fall within the range of the test

concentrations, and the confidence limits must closely bracket the EC-50 value. In addition, the correlation coefficient of the five data points must be greater than 0.9. If any of these values fail, the assay will be re-analyzed. Microtox tests will be performed by Aqua Survey, Inc.

4.2.4 TCLP and SPLP Analysis

The TCLP and SPLP test will utilize a blank for every twenty extractions that are conducted in each extraction vessel. In addition, a matrix spike will be performed with each analytical batch. Matrix spike recoveries will be compared to the analytical procedure for limits for acceptability. Both TCLP and SPLP procedures will be performed by Southwest Laboratories.

5.0
LABORATORY INFORMATION

5.1 STANDARD OPERATING PROCEDURES

Refer to Attachment A of this Appendix for the standard operating procedures for each test performed for the toxicity analysis.

5.2 LABORATORY SHIPMENTS AND CONTACTS

Samples for the analysis of TCLP and SPLP will be shipped to Southwest Laboratories at the following location:

Mr. Keith Sims
Sample Receiving
1700 West Albany
Broken Arrow, OK 74012-1421

Samples for the analysis of Microtox and Earthworm Toxicity will be shipped to Aqua Survey, Inc., at the following location:

Mr. James Todd
Sample Receiving
499 Point Breeze Road
Flemington, NJ 08822

Samples for the analysis of Pathogens will be shipped to Pure Earth Environmental Laboratory at the following address:

Dr. Ted Passon
Sample Receiving
7184 North Park Drive
Pennsauken, NJ 08110

6.0
REFERENCES

MK, 1998. *Full-Scale Operational Plan* - Soils Bioremediation Facility - NSWC Crane, Crane, Indiana. Delivery Order Number 0009, Contract Number N62467-93-D-1106. Prepared by Morrison Knudsen Corporation, March 12, 1998.

ATTACHMENT A
LABORATORY STANDARD OPERATING PROCEDURES

STANDARD OPERATING PROCEDURE

SOP NO. SOP/PRP/004
REVISION NO. 002*
COPY NO. Original
DATE 6/29/98
* Modified for project #4324-0009-07-35

TITLE: **PREPARATION OF SEDIMENT POREWATER SAMPLES FOR USE IN TOXICITY TESTS OR MONITORING POREWATER AMMONIA IN CONCENTRATIONS IN TEST SEDIMENTS**

CONTENTS **PAGE**

I. **Objective** 1
II. **Materials and Equipment** 2
III. **Procedure** 2
Preparation 2
Procedure 2
Appendix I Benchsheet 4

I. OBJECTIVE

The objective of this procedure is to provide a standard method for the preparation of porewater samples for use in toxicity tests, also for monitoring porewater ammonia concentrations during purging for solid phase toxicity tests. (See SOP/SED/purging tests SOPs.)

II. MATERIALS and EQUIPMENT

1. Centrifuge jugs, quantity according to need.
2. Sorvall RC-5B Centrifuge with rotor models GSA and HS-4
3. Top load balance (readability ± 0.1 g)
4. 250 mL polycarbonate centrifuge bottles (Thomas #2625 - G40)

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III. PROCEDURE

1. Preparation

1.1 If the porewater extraction is for a toxicity test, the sample can be taken directly from the receiving container. If necessary, the sample can be the supernatant prepared for an elutriate test according to SOP/PRP/005

1.2 If the porewater extraction is for the purpose of monitoring concentrations of ammonia in the porewater, the sample(s) must be treated as regular test samples and allowed to settle overnight before monitoring is initiated. A set of "dummy" chambers are set-up for monitoring purposes.

1.3 If standing water is present in the sample, pour this water off before mixing. Completely homogenize the sample using a spoon, plastic rod, stainless steel trowel or equivalent.

1.4 Determine the amount of porewater sample required. Assuming approximately 25% water, calculate the amount of sediment needed to spin down. If moisture values are available these may be helpful.

2. Procedure (Consult SOP/INS110 for detailed centrifuge operation)

2.1 Fill the centrifuge bottles with compost and weigh to the nearest 0.1 gram with a top loading balance (or equivalent). Ensure that the same mass is placed in the opposing jugs so that the rotor remains balanced.

2.2 Place cups in the rotor.

2.3 Tighten down rotor cover, close door and set temperature to the test temperature.

2.4 Spin at 2480 rpm for 30 minutes with rotor GSA or 3225 rpm for 30 minutes with rotor HS-4.

2.5 Remove the samples from the centrifuge.

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2.5.1 If the sample is for toxicity testing, remove sample from the centrifuge and pour off the supernatant into a suitable container. The sample must be used the same day that it is prepared, but if it is not to be used immediately store at the test temperature.

2.5.2 If the sample is for monitoring sediment porewater ammonia, place 10 mL into a small sample cup and measure the ammonia concentration immediately. The remaining supernatant sample is used to determine porewater salinity and pH.

VI. APPROVAL SIGNATURES

Prepared By G. Stephen Hornberger — 12/3/97
G. Stephen Hornberger — Date

Approved By: _____ — 12/17/97
James Todd — Date
Vice President

Approved By: York Terrell — 12/17/97
York Terrell — Date
Laboratory Manager

Approved By: Jane Norvell — 12/17/97
Jane Norvell — Date
Quality Assurance Office

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DETERMINATION OF WATER HOLDING CAPACITY OF SOILS AND SEDIMENTS

OBJECTIVE

The objective of this procedure is to provide a standard methodology for the determination of water holding capacity of soil and sediment samples.

MATERIALS

1. Weigh boats
2. Drying oven
3. Crucible tongs or flat-tipped forceps
4. Desiccator
5. Drierite® or similar drying agent
6. 250-mL glass beakers
7. Triple Beam balance
8. Glass stir rod
9. 100-mm diameter x 95-mm overall length glass funnels
10. 185-mm diameter, coarse porosity, qualitative crepe filter paper
11. 500-mL Erlenmeyer flasks
12. Aluminum foil

PROCEDURES

1. Preparation

1.1 Prepare and label a set of weigh boats equal in number to the number of samples to be analyzed.

1.2 Place the weigh boats in a 100 $\pm$ 5° C drying oven for a minimum of 2 hours. From this point on, DO NOT manipulate the weigh boats by hand as natural skin oils will adhere to the boats and bias the weights.

1.3 Using a crucible tongs or flat-tipped forceps, transfer the boats from the oven to a desiccator containing fresh drying agent. Allow to cool to room temperature.

1.4 Calibrate the Triple Beam balance as per SOP/INS/01 .

1.5 Weigh enough sample into the weigh boats to allow a final sample dry weight of at least 100 g. The amount of sample necessary will depend on the particle size and degree of hydration of the samples.

1.6 Using a crucible tongs or flat-tipped forceps, transfer the weigh boats with sample to the drying oven. Dry the samples at 100 ± 5° C for 24 hours.

1.7 Using a crucible tongs or flat-tipped forceps, remove the samples from the oven to the desiccator and cool to room temperature.

1.8 Calibrate the Triple Beam balance as per SOP/INS/01 .

1.9 With a forceps, remove the weigh boats with sample from the desiccator. Determine and record, as the final dry weight, the weight of each sample to the nearest gram.

2. Rehydration and Analysis

2.1 Weigh 100 g dried sample into a 250-mL glass beaker.

2.2 Measure 100 mL deionized water into the beaker. Thoroughly mix the sample and water with a glass stir rod, ensuring that all sample particles are wetted, to create a homogeneous slurry.

2.3 Fold a circle of filter paper in half and then in half again. Pull open one side of the folded paper to form a cone.

2.4 Set the cone of filter paper into a glass funnel. The edges of the paper shpuld be even with the top of the funnel walls.

2.5 Using a pipet, slowly add 9 mL deionized water to the filter paper so as to wet its entire surface.

2.6 Determine and record, to the nearest 0.1 g, the combined weight of the funnel with the wetted paper. Add the weight of the dried sample (100 g) to the weight of the funnel and paper and record as the initial weight.

2.7 Set the funnel into the mouth of an Erlenmeyer flask. Slowly add the slurry of sample to the filter paper and rinse into the funnel any sample remaining in the beaker or on the stir rod with deionized water. Use as little rinse water as necessary.

2.8 Cover the funnel tightly with aluminum foil and allow the sample to drain for 3 hours at room temperature.

2.9 Determine and record, to the nearest 0.1 g, the weight of the funnel and sample as the final weight.

3. Calculation of Water Holding Capacity

3.1 To determine the sample water holding capacity as mL water per 100 g sample, subtract the initial weight from the final weight, as per the following example:

- Water Holding Capacity (mL/100 g) = [Final Weight] - [Initial Weight]
- Water Holding Capacity (mL/100 g) = [297] - [115]
- Water Holding Capacity (mL/100 g) = 182

III. PROCEDURE

1. Obtain homogenized and composited test sediment.

2. Obtain site water, near subsurface.

3. Bring both to room temperature.

4. Mix together, 4 parts water to 1 part sediment, by volume. Break up clods and clay balls by hand, remove large debris and discard appropriately.

5. Mix with stir bar and plate, or stainless steel mixer, as appropriate for 30 minutes. Allow to settle for at least one hour.

6. Decant carefully to another clean HDPE container, avoid pouring settled sediment.

7. This mixture must be used the same day it is prepared.

IV. MISCELLANEOUS

Not applicable at this time.

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STANDARD OPERATING PROCEDURE

SOP NO. SOP/PRP/005
REVISION NO. 000
COPY NO. ~~Original~~ 401-7
DATE 07/25/94

TITLE: PREPARATION OF SUSPENDED PARTICULATE PHASE ELUTRIATES FOR USE IN TOXICITY TESTS

CONTENTS PAGE

I. Objective 1
II. Materials and Equipment 1
III. Procedure 2
IV. Miscellaneous 2
V. Approval Signatures 3
VI. Appendix

I. OBJECTIVE

The objective of this procedure is to provide direction for the preparation of elutriates for use in toxicity tests. This procedure is primarily designed for use in the dredge sediment program under the NYACE but can be modified for use in other areas.

II. MATERIALS AND EQUIPMENT

1. New HDPE mixing chambers, sized according to need
2. Stainless steel mixers (for larger volumes)
3. Stir Bars (for small volumes)
4. Stir Plate
5. Site water, near subsurface, quantity according to need
6. Lab coat, gloves, glasses. Nitrile gloves and tyvek for dioxin.

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A-3d

V. APPROVAL SIGNATURES

Prepared By: _____ Date 7/25/94
Kenneth R. Hayes
Laboratory Director

Approved By: _____ Date 7/25/94
W. Scott Douglas
Project Manager

Approved By: _____ Date 7/25/94
James M. Todd
Director of Operations

Approved By: _____ Date 7/25/94
Jane Norvell
Quality Assurance Officer

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STANDARD OPERATING PROCEDURE

SOP NO. SOP/SED/201
REVISION NO. 001
COPY NO. Original
DATE 04/16/98

TITLE: SOIL TOXICITY/BIOACCUMULATION TEST USING THE EARTHWORM, *EISENIA FOETIDA*

CONTENTS PAGE

I. Objective 1
II. Test System 2
III. Procedure 2
IV. Data Handling 5
V. References 5
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Appendix

I. OBJECTIVE

This procedure is designed to determine the acute toxicity and/or bioaccumulation of toxicants/contaminants in soil and solid waste to the earthworm, *Eisenia foetida*. The procedure described herein is similar to standard and/or generally acceptable procedures such as those described by the USEPA[1] and ASTM[2] and may be used to test the toxicity/bioaccumulation of other kinds of solid and aqueous materials. Other test species may also be used.

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II. TEST SYSTEM

A. Earthworms, *Eisenia foetida*

Batches of test organisms are intermittently obtained from external sources. An organism chain of custody statement accompanies each batch and is kept in the Archive for Non-Test Data.

B. Acclimation

All organisms are acclimated to test conditions (i.e., lighting, temperature, etc.) for a reasonable period of time.

C. Artificial Soil

Soil used for diluent and/or control is artificial soil prepared from a mixture of 10% (approximately 2 mm-screened) sphagnum peat, 20% colloidal kaolinite clay and 70% grade 70 silica sand This mixture is blended by hand until it is visually determined to be homogenous.

III. PROCEDURE

A. Preparation of test Substance for Exposure

Test substance will be prepared for exposure by the method deemed most appropriate. All solid soil-like samples are homogenized by mechanical manipulation.

Studies may be conducted as "pass or fail" determinations for one exposure concentration, range-finding (order of magnitude exposure) tests, definitive exposure studies, or any combination thereof. The procedure employed in a study should be specified in the test protocol.

Soil samples are prepared for exposure by determining the water holding capacity (SOP/GEN/303) and hydrating to approximately 75 percent of its water holding capacity, using deionized water.

B. Test Concentrations

The exposure concentrations will depend on the test design and may be protocol specific. Soil samples conducted as "pass or fail' will be conducted using a single concentration (i.e., 100%). Definitive testing to determine the concentration-response curves (i.e., LC_{50}) will be

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accomplished by adding appropriate amounts of test soil to artificial soil in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 6.25, 12.5, 25, 50 and 100%).

C. Preparation of Test Vessels

One test vessel will be prepared for each replicate test concentration and control. Toxicity tests are performed in one (1) to four (4) liter borosilicate glass containers with appropriate covers (i.e., nylon screening, petri dishes, screw-tops. etc.). Small holes may be punched in the covers to allow air to enter the exposure vessels. A minimum of three (3) replicate vessels should be prepared. Bioaccumulation potential studies require more tissue mass.. The size of the test vessel must be increased to accomodate a greater number of test organisms (i.e., 39-liter tank/390 worms).

D. Collection of Organisms for Testing

Worms as uniform in size as possible are collected from holding containers. Placement of organisms in exposure vessels for testing is accomplished by sequential randomization, allocating no more than 20% of any one set of test organisms into each vessel at a time.

E. Number of Organisms

A minimum of 10 worms should be used for each replicate concentration and control.

F. Characterization of Age and Weight

No attempt is made to characterize the test organisms as to exact age or sex , however organisms should be greater than 60 days of age with a weight of approximately 300-500 mg. When appropriate the weight of the organisms should be determined at the start of testing by appropriate means.

G. Test Endpoint

The effect measured during this test is mortality which may be used to determine the LC_{50} or significant toxic effect. When appropriate, surviving organisms may be analyzed to determine the bioaccumulation of contaminants.

H. Test Duration

The duration of this test is 14 days but may be extended to 28 days when determining both the acute effects and bioaccumulation potential levels of toxicants and/or contaminants.

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I. Control Vessels

Replicate negative control vessels (artificial soil only) and when appropriate carrier solvent vessels will be prepared.

J. Feeding

Worms will not be fed during testing.

K. Loading

The biological loading will be a minimum of one (1) organism per 20g of soil based on the dry weight of the soil.

L. Temperature

The test temperature is 20±2°C.

M. Photoperiod

Lighting should be continuous with an intensity maintained at approximately 50 to 100 foot candles.

N. Reference Toxicant

A reference toxicant test using KCl will be conducted to evaluate the sensitivity of each bath of organisms

O. Control Mortality

The mortality in the controls should not exceed 10% at the end of 14-day toxicity exposure or as specified in the workplan.

P. Observations

Each vessel is inspected daily for mortality and behavioral abnormalities (i.e., avoidance of the soil). Findings are recorded on observation sheets. When appropriate, mortality may be assessed by emptying the test soil onto a tray and sorting the worms from the soil. Dead worms are discarded. Live worms may be returned to their vessel when appropriate.

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Q. Test Parameter Measurements

The pH of the soil should be measured in a replicate vessel from each treatment using a calibrated meter at the beginning and end of the test.

The temperature of the environmental control chamber should be monitored continuously (i.e., hourly).

IV DATA HANDLING

Appropriate statistical analysis (i.e., ANOVA) should be used to determine significant effects. A computer program (i.e., Stepan[3] and ASTM[4] should be utilized to calculate the LC_{50}. Other methods may be used when appropriate.

V. REFERENCES

(1.) U.S. Environmental Protection Agency (EPA), Protocols for short term toxicity screening of hazardous waste site, EPA/600/3-88/029.

(2) American Society for Testing and Materials (ASTM). Standard guide for conducting a laboratory soil toxicity test with Lumbricoid earthworm, *Eisenia foetida*, E 1676-95.

(3) Stephan, C.E. 1977. Methods for Calculating an LC_{50}. In: F.L. Mayer and J.L. Hamelink, Eds; Aquatic Toxicology and Hazard Evaluation, Spec. Techn. Publ. No. 634, American Society for Testing and Materials, Philadelphia, PA, pp. 65-84.

(4) American Society for Testing Materials. 1988. Proposed New Standard Practice for Using Probit Analysis. ASTM Subcommittee E-47.07. Draft No. 4, June, 1988.

Original Issue:	10/25/91
Revision No.01:	04/16/98
Revision No. 02	06/29/98

VI. MISCELLANEOUS

Not applicable at this time.

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VII. APPROVAL SIGNATURES

Prepared By: _____ Date 4/16/98
York Terrell
GLP Manager

Approved By: _____ Date 4/16/98
James Todd
Vice President

Approved By: _____ Date 4/16/98
Jane Norvell
Quality Assurance Officer

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TITLE: MICROTOX® STANDARD ASSAY

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I. Introduction

The objective of this procedure is to determine the effective toxicity of a sample to the bioluminescent marine bacterium, *Photobacterium phosphoreum*, using the Microtox® test system. The concentration of the test substance which results in fifty percent (50%) inhibition of bioluminescence in a concentration series during a 15-minute exposure will be calculated from observations of light emission. When no observed effects are noted below a test concentration, the results are expressed as the highest acute No Observed Effect Concentration (NOEC). The information from this primary sensitivity test can be used to evaluate the likelihood of a secondary community effect if the test substance enters an aquatic environment.

The procedure described herein is similar to standard and/or generally accepted procedures such as those described by the Microbics Corporation[1,2,3].

II. Materials

1. Microtox® Model 500
2. Microtox® glass cuvettes

3. 10-μL automatic pipettor
4. 250-μL automatic pipettor
5. 500-μL automatic pipettor
6. 10-μL pipettor tips
7. 200/500-μL pipettor tips
8. Microtox® Reagent
9. Microtox® Reconstitution Solution (Recon Solution)
10. Microtox® Diluent
11. Microtox® Osmotic Adjusting Solution (MOAS)
12. Microtox® data reduction software

III. Test System

A. Test Organism

The Microtox® test system utilizes a specially developed strain of the bioluminescent marine bacterium, *Photobacterium phosphoreum*. The organisms are obtained as lyophilized cultures, each containing approximately one hundred million cells. Lyophilized cultures have a shelf life of one year when stored at minus 20° C.

B. Diluent

A standardized test system diluent, obtained from Microbics Corporation, Carlsbad, CA, is used for the Microtox® test system. Diluent is a two percent (2%) solution of sodium chloride in distilled water.

IV. Test Procedures

A. Preparation

Place a cuvette in the REAGENT well. Place a cuvette in each well of row A and of row B of the incubator block.

Using the 500-μL pipettor fitted with a clean pipettor tip, dispense 1 mL (1000 μL) Recon Solution into the cuvette in the REAGENT well.

Using the 500-μL pipettor, dispense 500-μL Diluent into each cuvette in wells B1 through B5.

Dispense 1 mL Diluent into each cuvette in wells A1 through A4. Do not add diluent to the cuvette in well A5.

Prepare the sample dilutions as follows:

1. Using the 250-µL pipettor fitted with a clean pipettor tip, dispense 250 µL MOAS into the cuvette in well A5. Discard the pipettor tip.

2. Using the 500-µL pipettor, add 2.5 mL (2500 µL) sample to well A5 and mix thoroughly by alternately aspirating and ejecting the sample with the pipettor.

3. Transfer 1 mL from A5 to A4 and thoroughly mix A4.

4. Transfer 1 mL from A4 to A3 and thoroughly mix A3.

5. Transfer 1 mL from A3 to A2 and thoroughly mix A2. Do not transfer from A2 to A1, as A1 will be the Blank, or control, for the assay. Discard the pipettor tip.

If the sample toxicity is unknown or is suspected of being high, perform a rangefinding assay. Where necessary, prepare a primary dilution of the sample in Microtox® Diluent and continue as in steps 1-5 above.

Allow five minutes for temperature equilibration.

Remove a vial of Reagent from frozen storage and quickly remove the seal and stopper from the vial, avoiding contact with the glass surface. If necessary, seat the reagent pellet on the bottom of the vial by gently tapping the vial.

The following functions MUST be performed as quickly and as carefully as possible to ensure the integrity of the reagent:

1. Remove the cuvette from the REAGENT well and, with the lip of the cuvette seated firmly on the rim of the vial, pour the contents of the cuvette into the Reagent vial.

2. Immediately swirl the contents of the vial, pour the reagent suspension into the cuvette and return the cuvette to the REAGENT well.

3. Using the 250-µL pipettor fitted with a clean pipettor tip, thoroughly mix the reconstituted Reagent suspension by alternately aspirating and ejecting. Discard the pipettor tip.

Using the 10-µL pipettor fitted with a clean pipettor tip, dispense 10 µL Reagent suspension into each cuvette in row B of the incubator block as follows:

1. Do not remove the cuvettes from the wells while dispensing Reagent.

2. Place the pipettor tip under the surface of the liquid but DO NOT set the tip on the bottom of the cuvette.

3. Rest the pipettor tip against the inside rim of the cuvette and slide the tip down until the ridge along its surface rests on the rim of the cuvette. Carry out pipettor functions with the pipette tip in this position.

Thoroughly mix the contents of the cuvettes in row B using one of the following methods:

1. Using the 250-μL pipettor fitted with a clean pipettor tip, aspirate and eject the contents of each cuvette 2 or 3 times; or

2. Working quickly, remove a cuvette from its well and, holding the cuvette firmly between thumb and index finger, tap the cuvette near its base with a second finger to create a vortex. Replace the cuvette in the well and continue until all cuvettes are mixed.

Wait 15 minutes for reagent stabilization.

B. Assay

Set a timer for 15 minutes but do not yet start the timer.

Take the cuvette from well B1 and set it in the READ turret well. Press the SET pad. Wait for the Ready green symbol to appear in the display. DO NOT press the SET pad again during the assay procedure.

Press the READ pad and wait for the light level, or "gamma", value to appear in the display. Read and record the value as the initial light level (I_0) for cuvette B1. Repeat for cuvettes B2, B3, B4 and B5, in that order.

Immediately start the timer.

Using the 500-μL pipettor fitted with a clean pipettor tip, make the following 500-μL transfers, mixing thoroughly after each transfer: From A1 to B1; from A2 to B2; from A3 to B3; from A4 to B4; from A5 to B5.

The resulting assay concentrations are, in respective order, 0.0 (Blank), 5.6, 11.3, 22.5 and 45.0 percent of the undiluted sample or primary dilution, as applicable. If the addition of MOAS was omitted, the resulting concentrations are 0.0, 6.3, 12.5, 25.0 and 50.0 percent of the primary dilution.

At 5 minutes and 15 minutes post timer initiation, read and record the light level readings for the 5-minute and the 15-minute exposures, respectively, from cuvettes B1 through B5.

Light readings may be determined for intervals other than, or in addition to, 5 and 15 minutes, as dictated by purpose. With samples of unknown toxicity, it is recommended that the 5-minute and 15-minute light values be determined.

Exposure to some toxicants, such as organic compounds, will result in immediate light loss followed by a period of recovery or stabilization. In such cases the 5-minute values prove most useful.

With other classes of toxicants, such as metals, the light loss from the bacterium may still be occurring, or has not yet begun at the 5 minute interval. In such cases, the 15-minute data may be more useful.

Reduce the data (see section VI., below).

C. Observations

All observations of organism light emission and any other observations will be recorded on a Microtox® Data Sheet or an equivalent data form.

V. Chemical Analysis

The test substance concentration will be determined by a suitable analytical technique unless specified otherwise in the study protocol. The analytical procedure will be described in the protocol specific for the test substance.

No measurements of test substance exposure solution parameters (e.g., temperature, pH, dissolved oxygen) will be made.

VI. Data Reduction

Working at a computer station, initiate the Microtox® data reduction program from the general menu by entering the program code. Refer to Appendix I for a complete set of sample program displays and output.

Upon initiating the program, the Master Menu Screen will be displayed. Choose the option, 4) INPUT DATA FROM KEYBOARD.

The program will prompt for a sample description. Enter a description consisting of at least the study number, sample identification and test date.

The program will then prompt for Blank values. Enter the initial (B0) and the final (BT) light readings for the Blank, (cuvette B1).

The program will then prompt for sample treatment data. Enter the initial (I0) and the final (IT) light readings for each sample concentration, in ascending concentration order, in accordance with the format in the display.

The normalized data will be displayed along with a prompt to continue. The ensuing display will reveal the results of the data reduction and a second prompt to continue.

The MICROTOX CALCULATIONS MENU will be displayed. To initiate a printout of the data analysis, choose option 2.....HARD COPY. The information will be stored in the printer.

Instructions for printing a graph of the reduced data will appear in the display along with a prompt to produce and display the graph. Initiate the procedure and the graph will be created in the display. Use the print screen key to print the graph and then press ENTER. A hard copy of the reduced data and the graph will be produced.

The calculations menu will appear in the display. If another data set is to be reduced, choose the option, 6) RUN NEW DATA SET, and repeat Section 3. If no more data is to be analyzed, enter E to exit the program, and return to the general menu.

Examine the data printout for the following:

1. Blank Ratio - This should be 0.75 or greater. If the ratio is less than 0.75, it is an indication of poor reagent quality or poor reagent transfer technique. Blank ratio is calculated as BO/BT.

2. EC 50 - The EC 50 value should fall within the range of the test concentration values. If the EC 50 value falls outside of the range of concentrations, the accuracy of the test results becomes questionable.

3. EC 50 Confidence Limits - Found in parentheses to the right of the EC 50 value, the confidence limits express the range of precision of the reported EC 50. As such, they should closely bracket the EC 50.

4. R Value - The R Value indicates the degree to which the distribution of the data points correlates to a straight line, and should be as close to a value of 1.0 as possible. If the value is less than 0.9, it is an indication of a poor correlation.

If any one of the above parameters appears questionable, the assay should be repeated.

VII. Responsibility

The name of the person primarily responsible (and that of an alternate) for ensuring adherence to all procedures described in this SOP are documented in records maintained by the Unit Leaders. These records are currently located in the central file in the main office of ASI.

VIII. References

1. Microbics Corporation. 1985. How to Run Toxicity Tests Using the Microtox® Model 500. Microbics Corporation, Carlsbad, California.

2. Microbics Corporation. 1989. Microtox® Data Capture and Reduction Program, An Interim Manual. Microbics Corporation, Carlsbad, California.

3. Microbics Corporation. 1991. Microtox® Manual, Model 500 Toxicity Test System. Microbics Corporation, Carlsbad, California.

Original Issue: 09/17/92

APPENDIX I

Data Reduction Software Displays and Printout

Display One

```
                    MICROBICS CORPORATION

     MASTER MENU SCREEN


1)   SELECT PROCEDURE.
2)   REVIEW/CHANGE PARAMETERS.
3)   START TESTING.
4)   INPUT DATA FROM KEYBOARD.
5)   DISPLAY PARAMETER FILES.
6)   TIMER.
7)   SOFTWARE TEST.
8)   EXIT PROGRAM.

     PLEASE ENTER CHOICE:
```

Display Two

MICROTOX CALCULATIONS

ENTER SAMPLE DESCRIPTION, DATE, ETC. (ONE LINE ONLY):

Display Three

```
                    MICROTOX CALCULATIONS

ENTER SAMPLE DESCRIPTION, DATE, ETC. (ONE LINE ONLY):
SAMPLE RUN, DATA REDUCTION PROGRAM

ENTER BLANKS -- BO,BT

? 100,88
```

Display Four

```
                    MICROTOX CALCULATIONS

ENTER SAMPLE DESCRIPTION, DATE, ETC. (ONE LINE ONLY):
SAMPLE RUN, DATA REDUCTION PROGRAM

ENTER BLANKS -- B0,BT

? 100,88

BLANK RATIO=    .88

ENTER DATA -- CONCENTRATION, I0, IT
(enter 0,0,0 as prompt when finished)

?
```

Display Five

```
                    MICROTOX CALCULATIONS

ENTER SAMPLE DESCRIPTION, DATE, ETC. (ONE LINE ONLY):
SAMPLE RUN, DATA REDUCTION PROGRAM

ENTER BLANKS -- B0,BT

? 100,88

BLANK RATIO=    .88

ENTER DATA -- CONCENTRATION, I0, IT
(enter 0,0,0 as prompt when finished)

? 5.6,98,74
? 11.3,99,66
? 22.5,98,53
? 45,97,41
? 0,0,0
```

Display Six

SAMPLE #	CONCENTRATION	GAMMA
1	5.6	0.165
2	11.3	0.320
3	22.5	0.627
4	45	1.082

TO CONTINUE PRESS ANY KEY

Display Seven

SAMPLE RUN, DATA REDUCTION PROGRAM

PAIR #	CONC.	G-OBS	G-EST
1	5.600	0.165	0.169
2	11.300	0.320	0.319
3	22.500	0.627	0.597
4	45.000	1.082	1.120

EC 50 = 39.652 (33.629 TO 46.755)

R =0.99898 SLOPE = 1.0981 INTERCEPT = +3.6802

TO CONTINUE PRESS ANY KEY

Display Eight

```
MICROTOX CALCULATIONS MENU

1.....REPEAT RESULTS

2.....HARD COPY

3.....DELETE DATA POINTS

4.....ADD DATA POINTS

5.....ABSORBANCE CORRECTIONS

6.....RUN NEW DATA SET

7.....PLOT DATA

8.....CALCULATE ECXX

9.....RETURN TO MAIN MENU

PLEASE ENTER CHOICE  (E TO EXIT)
```

Data as Sent to Printer

SAMPLE RUN, DATA REDUCTION PROGRAM

PAIR #	CONC.	G-OBS	G-EST
1	5.600	0.165	0.169
2	11.300	0.320	0.319
3	22.500	0.627	0.597
4	45.000	1.082	1.120

BLANK Bo/Bt= 100 / 88
BLANK RATIO= 0.8800

EC 50 = 39.652 (33.629 TO 46.755)

R =0.99898 SLOPE = 1.0981 INTERCEPT = +3.6802

Display Nine

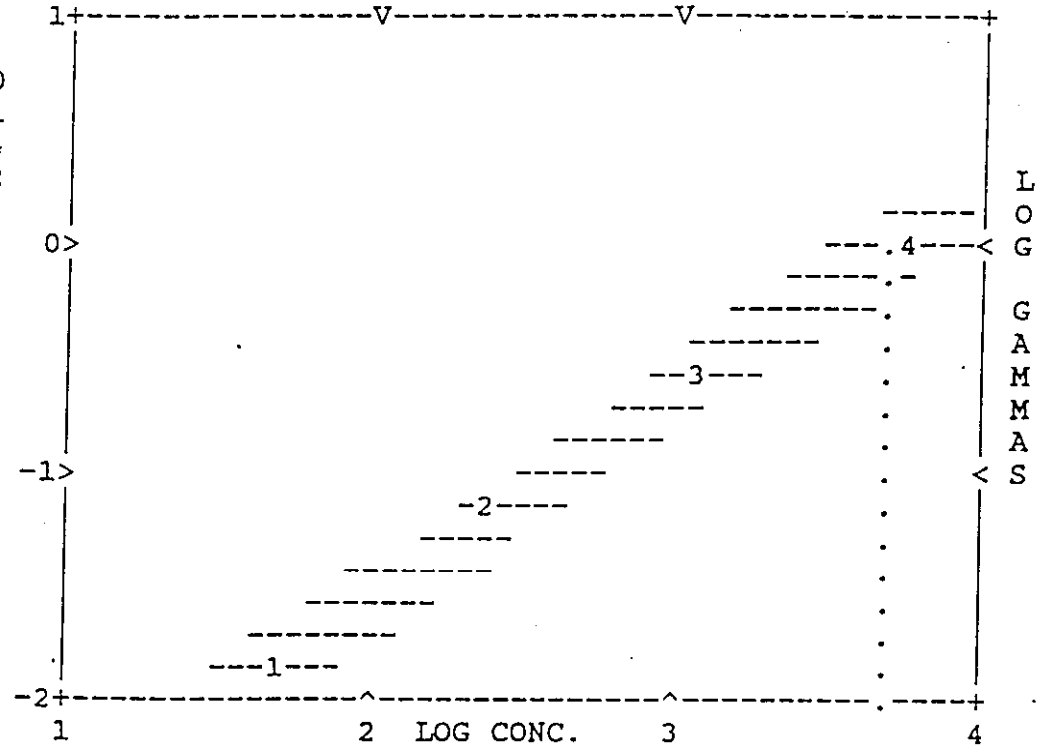
HOW TO PRINT A GRAPH

1. Wait for the graph to be displayed on the screen.
2. Use the Print Screen Key to print the graph.
3. Then, touch any key
   to return to the MICROTOX CALCULATIONS MENU.

TOUCH ANY KEY
And the graph will be displayed on the screen.

Display Ten

##	RES.	PROB.
1	0.181	100
2	0.116	41
3	0.120	44
4	0.174	92



Data Printout

SAMPLE RUN, DATA REDUCTION PROGRAM

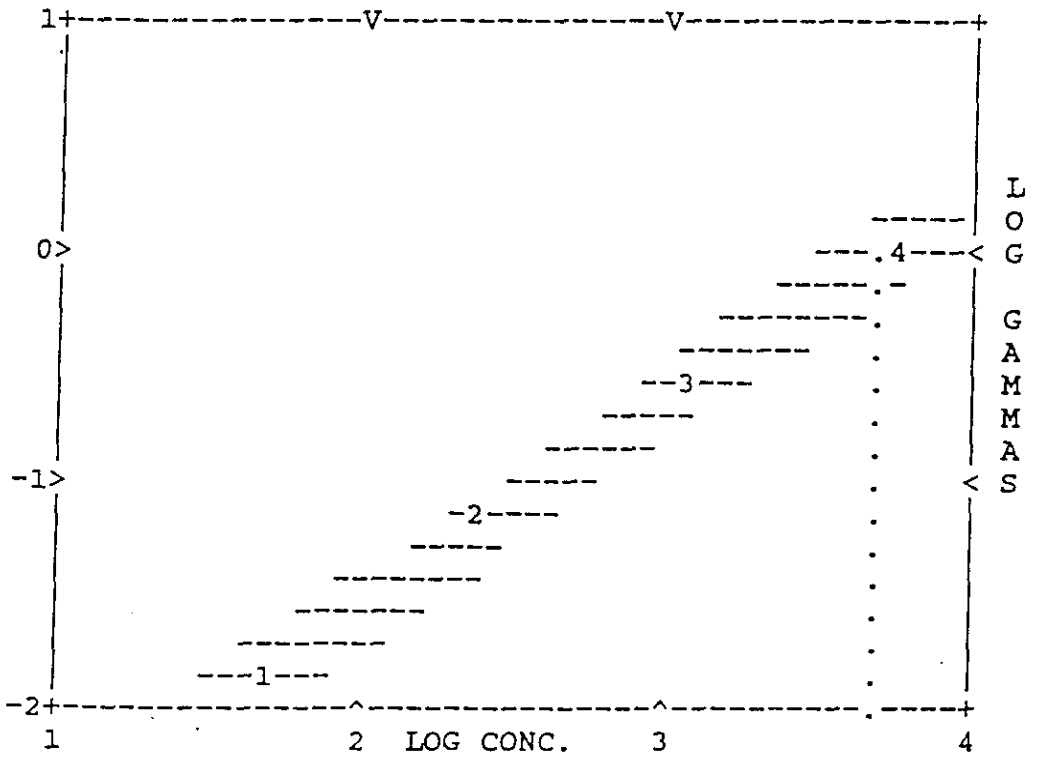
PAIR #	CONC.	G-OBS	G-EST
1	5.600	0.165	0.169
2	11.300	0.320	0.319
3	22.500	0.627	0.597
4	45.000	1.082	1.120

BLANK Bo/Bt= 100 / 88
BLANK RATIO= 0.8800

EC 50 = 39.652 (33.629 TO 46.755)

R =0.99898 SLOPE = 1.0981 INTERCEPT = +3.6802

##	RES.	PROB.
1	0.181	100
2	0.116	41
3	0.120	44
4	0.174	92



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Attachment 1

Microtox® Data Sheet

AQUA SURVEY, INC.
Microtox Data Sheet

ASI Number	Test Material	Test Start Date	Test Start Time

Nominal Concentration		Light Reading Interval - Minutes					
(%)	(mg/L)	Initial					
	A						
	B						
	C						
	Mean						
	A						
	B						
	C						
	Mean						
	A						
	B						
	C						
	Mean						
	A						
	B						
	C						
	Mean						
	A						
	B						
	C						
	Mean						
Date/Time and Initial							

Comments:

Signature	Date	Reviewed by	Date	Page ____
				of

SOP NO.	TOX-1001
REVISION NO.	00
DATE	09/17/92

Prepared By: _____ 09/16/92
Date

Approved: _____ 9/16/92
York Terrell
GLP Testing Manager
Date

Approved: _____ 9/16/92
James Todd
Director of Operations
Date

Approved: _____ 09/16/92
Carl Bretton
Quality Assurance Unit
Date

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40 CFR 503 SLUDGE PATHOGEN TESTING SAMPLING SUGGESTIONS

SAMPLE VOLUME:

It is important that volume is based on the Total Solids percentage of the biosolid to be tested or analyzed.

Sample to achieve at least 4 grams dry weight equivalent for Salmonella and Helminth Ova testing.

At least 8 grams dry weight equivalent for Enteric Viruses and 1 gram dry weight equivalent for Fecal Coliform analysis.

TO NOTE - EXAMPLE:

> If Salmonella, Helminth Ova and Enteric Viruses are required and your Total Solids percentage is 50%, you should provide 8 grams wet weight for Salmonella, 8 grams wet weight for Helminth Ova and 16 grams wet weight for Enteric Viruses.

It is important the Total Minimum wet weight sample to be provided = 32 grams.

Optimal wet weight would be approximately 100 grams. This would allow for a suitable margin of error.

SAMPLE CONTAINER:

The Sample Container must be sterile and properly marked with sample data, location, time, Total Solids percentage (or performed on Pure Earth Environmental Laboratory) sample (with signature change of custody) and your ID# and PO#.

SAMPLE SHIPMENT:

1. Ship same day as sampling, on wet/blue ice, via overnight delivery.

2. Ship sample to arrive PREFERABLY ON TUESDAY OR WEDNESDAY.

Please make every effort to give 48 hours notice of sample arrival to Pure Earth Environmental Laboratory in order for us to put you on our busy schedule.

SHIP TO: Pure Earth Environmental Laboratory, Inc.
7184 North Park Drive
Pennsauken, NJ 08110

SAMPLING:

Use aseptic technique and sample according to general accepted methods from the 40 CFR 503 protocol.

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DETERMINATION OF TOTAL DRY SOLIDS
Method D4994-89,ASTM (1992)

1. Weigh a dry weigh pan at a constant weight. Place 50 ml or 50 grs. sludge portion for solids determination into the pan and weigh.

2. Place the pan and its contents into an oven maintained at 103-105 C for at least 1 hour.

3. Cool the sample to room temperature in a desiccator and weigh again.

4. Repeat the drying (1 hr each), cooling , and weighing steps until the loss in weight is no more than 4% of the previous weight.

5. Calulate the fraction of total dry solids (T) using the formula: T=A/B

   A=weight of the sample after drying (Pan dish is T off on the balance)
   B= weight of the sample before drying

   example: A=3.5
   B=5.0 0.7 x 100=70%

FECAL COLIFORMS, BIOSOLID, 503 METHOD

I. PURPOSE

1.0 This procedure outlines the steps involved in utilizing the Most Probable Number (MPN) method to analyze biosolid samples for the presence of fecal coliforms as per EPA regulations. The 503 method is described for liquid and solid samples of Class A, Alternative 1 or Class B, Alternative 1.

2.0 The 503 method describes guidelines for reducing pathogen levels in biosolids (sludge). Class A designation is given to biosolids in which the pathogens are below detectable levels. Biosolids are designated Class B if pathogens are detectable but have been reduced to levels which allow restricted use.

3.0 Class A, Alternative 1 is defined to signify that those biosolids have been thermally treated by being subjected to one of four time-temperature regimes. There are a total of seven alternatives for achieving Class A pathogen levels.

4.0 Class B, Alternative 1 is defined to signify that those biosolids may contain some pathogens, but the indicator organisms are being monitored. There are a total of three alternatives for meeting Class B pathogen requirements.

5.0 CLASS A, ALTNERATIVE 1, SOLID SAMPLES

5.1 This procedure is a modification of the 503 method. The procedure is modified because it has been noted by this laboratory that when testing solid samples the following is found.

5.1.1 If 10.0 g or 5.0 g of solid sample is added to 10.0 ml of broth, ALL the liquid culture medium is absorbed and the results cannot be determined.

5.1.2 It has been found by this laboratory that 1.0 g should replace all steps requesting 10.0 g and that 0.5 g should replace all steps requesting 1.0 g. The results obtained with this adjustment yield results consistent with the standards set forth in the method 503.

5.2 Four series (rows) of 5 tubes of EC broth are set up in a rack and allowed to warm to room temperature.

5.2.1 1st series: Double strength EC broth
5.2.2 2nd series: Double strength EC broth
5.2.3 3rd series: Double strength EC broth
5.2.4 4th series: Single strength EC broth

5.3 Blend 50 g of sample in 450 ml of PBS on high speed for 2 minutes. This blended sample is a 0.1 dilution of original sample.

5.4 Place 11 ml of sample into 99 ml of PBS. This is Dilution A, which is a 1×10^{-2} dilution of the original sample.

5.5 Inoculate the tubes with the following aliquots:

5.5.1 1st series: 10 ml blended sample (dilution factor = 1)
5.5.2 2nd series: 5 ml blended sample (dilution factor = 0.5)
5.5.3 3rd series: 10 ml Dilution A (dilution factor = 0.1)
5.5.4 4th series: 1 ml Dilution A (dilution factor = 0.01)

5.6 Incubate tubes in 44.5° C water bath for 24 hours.

5.7 After incubation view tubes for turbidity and gas production in fermentation vials. Positive reactions are indicative of fecal coliforms.

5.8 Only three of the four series will be used for estimating MPN. Choose the greatest dilution that gives a positive result in all five tubes and then the next two higher dilutions.

5.8.1 The greatest dilution equals the most diluted sample, which is that of Series 4.

5.8.2 If Series 2, 3 or 4 have all 5 tubes with positive growth, then the combination of positives will be Series 2,3,4.

5.8.3 If there are no positives, or if only Series 1 has all five tubes with positive growth, then the combination of positives will be 1,2,3.

5.9 Select MPN index/100 ml based on combination of positives. MPN index/100 ml is contained in the table on Form SAP 330B.

5.10 <u>Calculation:</u> Compute MPN/g using the following equation.

$$\text{MPN/g} = \frac{10 \times \text{MPN index/100 ml}}{\text{largest volume used} \times \%\ \text{total solids}}$$

10 = factor of equation
MPN index/100 ml = value obtained from Form SAP 330B
Largest volume used = least diluted sample from combination of positives
If used 2,3,4 combo, then largest volume is 0.5 g

If used 1,2,3 combo, then largest volume is 1 g
% Total solids = value obtained from determining % total solids (separate assay)

5.11 <u>Specifications:</u> Class A Alternatives are allowed 1,000 MPN/g of total solids (dry weight).

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FECAL COLIFORM TEST FOR SLUDGE

(All steps are performed aseptically)

Class B Alternative 1

The geometric mean of the Fecal Coliform density must not exceed 2 million CFU (colony-forming uni) or MPN (most probabl number) per gram of sewage sludge solids (dry weight basis).

LIQUID SAMPLES

1.) Transfer 1.0 ml of well mixed sample to 99.0 ml of PBS (.....) in a screw cap bottle. Shake a minimum of 25 times. (this is dilution "A", and is 0.010 ml of the original sample).

2.) Transfer 1.0 ml of dilution "A" to a second 99.0 ml blank of PBS, and mix as before. (this is dilution "B", and is 0.00010 ml of the original sample).

3.) Transfer 1.0 ml of dilution "B" to a third 99.0 ml blank of PBS, and mix as before. (this is dilution "C", and is 0.0000010 ml of the original sample). GO TO MPN OR MF ANALYSIS.

SOLID SAMPLES

1.) Weigh out 50.0 grams of well-mixed sample and place in a blender.

2.) Add 450.0 ml of PBS to the blender and blend on high for 2 minutes. (1.0 ml of this sample contains 0.10 grams of the original sample).

3.) Transfer 11.0 ml of the blender contents to a screw cap bottle containing 99.0 ml of PBS. Shake a minimum of 25 times. (this is dilution "A", and 1.0 ml of this sample contains 0.010 grams of the original sample).

4.) Follow the procedure for liquid samples starting at step 2.

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MPN FOR FECAL COLIFORM CLASS B, ALTERNATIVE 1.

FOUR SERIES OF FIVE TUBES ARE TO BE USED.

1.) Inoculate the first series of 5 tubes containing EC broth with 10.0 ml of dilution "B" (this is 0.0010 of the original sample).
2.) Inoculate the second series of 5 tubes containing EC broth with 1.0 ml of dilution "B" (0.00010).
3.) Inoculate the third series of 5 tubes containing EC broth with 10.0 ml of dilution "C" (0.000010).
4.) Inoculate the fourth series of 5 tubes containing EC broth with 1.0 ml of dilution "C" (0.0000010).
5.) Incubate the inoculated tubes in a water bath at 44.5° +/- 0.2° for 24 +/- hours. (place tubes in the water bath within 30 minutes of inoculation. The depth should be sufficent to cover all liquid in tubes.).
6.) A positive result for fecal coliforms is the production of gas within 24 +/- 2 hours.

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FECAL COLIFORM TEST FOR SLUDGE

(All steps are performed aseptically)

Class A Alternative 1

Fecal coliform density must be less then 1,000 MPN fecal coliform per gram of total solids (dry weight basis).

LIQUID SAMPLES

(four series of five tubes containing EC broth and inverted vial will be used for analysis).

1.) Transfer 10.0 ml of well mixed sample directly into the first series of five tubes.

2.) Transfer 1.0 ml of well mixed sample directly into the second series of five tubes.

3.) Transfer 1.0 ml of well mixed sample to a 99.0 ml PBS blank. Mix by vigorously shaking a minimum of 25 times. This is dilution "A".

4.) Transfer 10.0 ml of dilution "A" to the third series of five tubes.

5.) Transfer 1.0 ml of dilution "A" to a fourth series of five tubes. GO TO STEP 5 OF MPN FOR FECAL COLIFORM CLASS B, ALTERNATIVE 1.

SOLID SAMPLES

(four series of five tubes containing Lauryl Tryptose broth of various concentrations with inverted vials)

1.) Transfer 10.0 grams of well mixed sample directly into the first series of five tubes containing 10.0 ml of double strength Lauryl Tryptose broth.

2.) Transfer 1.0 gram of well mixed sample directly into the second series of five tubes containing 10.0 ml of single strength Lauryl Tryptose broth.

3.) Prepare dilution "A" as described for CLASS B, ALTERNATIVE 1: "SOLID SAMPLE".

4.) Transfer 10.0 ml of dilution "A" to the third series of five tubes containing single strength Lauryl Tryptose broth.

5.) Transfer 1.0 ml of dilution "A" to the fourth series of five tubes containing single strength Lauryl Tryptose broth.

6.) Incubate the inoculated tubes at 35.0° +/- 0.5 for 24 +/- 2 h.

7.) A presumptive identification of coliform organisms is the production of gas within 24 +/- 2 h. Any negative tubes are to be reincubated and reexamined at the end of 48 +/- 3 h.

8.) All positive tubes are to be inoculated into tubes of EC broth containing an inverted

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(step 8 continued from sludge, FC Class A, Alternative 1)
vial. Incubate at 44.5°+/- 0.2 for 24 +/- 2 h in a water bath which covers the liquid in the tubes completely.

how much gas ?

9.) A positive result for fecal coliforms is the production of gas within 24 +/- 2 h.

10.) Proceed with identification of MPN (MPN chart included).

9.) A positive result . . . . the production of at least [volume] of gas within 24 ± 2h.

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SALMONELLA SP. TEST FOR SLUDGE
(all steps are performed aseptically)

CLASS A CRITERIA

Salmonella sp. density must be below 3 MPN per 4 grams (dry weight basis) in order to qualify for Class A.

LIQUID SAMPLES

1.) Follow the same procedure as for liquid samples of fecal coliform analysis CLASS A, ALTERNATIVE 1 (therefore obtain the "A" dilution). Only three series of five tubes will be used for MPN. The tubed medium should be as follows:

A.) Series 1: 10.0 ml of double strength DSE broth.
B.) Series 2: 10.0 ml of single strength DSE broth.
C.) Series 3: 10.0 ml of double strength DSE broth.

2.) Inoculate SERIES 1 with 10.0 ml of well mixed liquid sample.
3.) Inoculate SERIES 2 with 1.0 ml of well mixed liquid sample.
4.) Inoculate SERIES 3 with 10.0 ml of DILUTION "A".
5.) Incubate all tubes at 35.0° +/- 0.5 for 24 +/- 2 h.
6.) A positive result will show the presence of growth (turbidity).
7.) All positive results are to be inoculated on Hektoen enteric and XLD media. Incubate at plates at 35.0° +/- 0.5 for 24 +/- 2 h.
8.) A positive Hektoen enteric media result is a colony which is blue-green with a black center. A positive XLD media result is a colony which is red with a black center.
9.) All positives are then to be used for the standard determination of MPN.

SOLID SAMPLES

1.) Follow the same procedure as for solid samples of fecal coliform analysis CLASS "A", ALTERNATIVE 1 (therefore obtain dilution "A"). Only three series of five tubes will be used for MPN. The tubed media should be as follows:

A.) Series 1: 10.0 ml of double strength DSE broth.
B.) Series 2: 10.0 ml of single strength DSE broth.
C.) Series 3: 10.0 ml of double strength DSE broth.

2.) Inoculate SERIES 1 with 10.0 grams of well mixed solid sample.
3.) Inoculate SERIES 2 with 1.0 gram of well mixed sample.

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4.) Inoculate SERIES 3 with 10.0 ml of dilution "A".

5.) Go to step 5 for liquid samples, Class "A", ALTERNATIVE 1 for Salmonella sp. in sludge.

* It has been noted by this laboratory that when testing solid samples the following is found:

If 10.0 grams or 5.0 grams of solid sample is added to 10.0 ml of broth, ALL liquid is absorbed and the results can not be determined. It has been found by this laboratory that 1.0 gram should replace all steps requesting 10.0 grams, and that 0.5 grams should replace all steps requesting 1.0 grams. The results obtained with this adjustment yield results consistent with the standards set forth in the method 503.

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PREPARATION OF DULCITOL SELENITE CYSTINE BROTH (DSE).

(all steps should be performed aseptically)

SINGLE STRENGTH FORMULA

1.) Place 11.5 grams of the Selinite Cystine broth (SE) medium into a sterile 500 ml screw top bottle.
2.) Add 2.5 grams of Dulcitol to the SE broth.
3.) Add 500 ml of reagent grade water.
4.) Dissolve fully (with frequent agitation) but AVOID HEATING.
5.) Dispense in 10 ml amounts into 50 ml sterile test tubes.

DOUBLE STRENGTH FORMULA

1.) Simply double the amount of SE broth medium (23.0 grams) and Dulcitol (5.0 grams) placed in 500 ml of water.

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Standard Operating Procedure

Zero Headspace Extraction for TCLP / SPLP

Document No.: SWL-IN-701

Rev No. / Date: Rev. 4.0 — 05/06/98

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APPROVALS	DATES
Chuck Hoover Procedure Prepared By	5/6/98 Date
Program Manager or Section Supervisor	5/7/98 Date
Laboratory Safety Officer	5/6/98 Date
Chuck Hoover Laboratory QA/QC Officer	5/6/98 Date

(Effective Date is 5 calendar days after the last signature above - QA/QC Officer)

Document Status

[ ] Controlled	DC No. _____	Issued to: _____	Date: _____	QA: _____
[✓] Official Copy	OC No.: 2084	Issued to: Alan Fosdick	Date: 5/8/98	QA: MM

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ZERO HEADSPACE EXTRACTION FOR TCLP / SPLP

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1.0 SCOPE AND APPLICATION

1.1 The Toxicity Characteristic Leaching Procedure (TCLP) and Synthetic Precipitation Leaching Procedure (SPLP) is to determine the mobility of both organic and inorganic analytes in liquid, solid, and multiphasic wastes.

1.2 If a total analysis of the waste demonstrates that individual analytes are not present in the waste, or that they are present but at such low concentrations that the appropriate regulatory levels could not be exceeded, a TCLP need not be run.

1.3 If an analysis of any one of the liquid fractions of a TCLP extract indicates that a regulated compound is present at such high concentrations that, even after accounting for dilution from the other fractions of the extract, the concentration would be above the regulatory level for that compound, then the waste is hazardous and it is not necessary to analyze the remaining fractions of the extract.

2.0 METHOD SUMMARY

2.1 For liquid waste (i.e., those containing less than 0.5% dry solid materials), after filtration through a 0.6 to 0.8um glass fiber filter, is defined as the TCLP / SPLP extract.

2.2 For wastes containing greater than or equal to 0.55% solids, the liquid, if any, is separated from the solid phase and stored for later analysis. The solid phase is extracted with an amount of extraction fluid equal to 20 times the weight of the solid phase. Following extraction, the liquid phase is separated from the solid phase by filtration through a 0.6 to 0.8 um glass fiber filter.

2.3 If compatible, the initial liquid of the waste is added to the liquid extract, and these are analyzed together. If incompatible, the liquids are analyzed separately and the results are mathematically combined to yield a volume-weighted average concentration.

3.0 HEALTH AND SAFETY.

Refer to the Lab Safety Plan (SWL-GA-111) for precautions.

4.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING & STORAGE.

N/A

5.0 INTERFERENCES AND POTENTIAL PROBLEMS.

Potential interferences that may be encountered during analysis are discussed in the individual analytical SOP's.

6.0 EQUIPMENT / APPARATUS

6.1 Agitation apparatus: Associated Design and MFG. Co., Model No. 3740-24-BRE-TM. Capable of maintaining 30 ± 2rpm.

6.2 Zero-Headspace Extraction Vessel. This device is for use only when the waste is being tested for the mobility of volatile analytes. The ZHE allows for liquid/solid separation within the device, and effectively precludes headspace. This type of vessel allows for

initial liquid/solid separation, extraction, and final extract filtration without opening the vessel. The ZHE should be checked for leaks after every extraction. Pressurize the device to 50psi, allow it to stand unattended for one hour, and recheck the pressure. If the pressure is lost, check all fittings and inspect and replace O-rings, if necessary. Retest the device.

6.3 Filter. Glass fiber filters with a pore size of 0.6 to 0.8 um.

6.4 pH meter. Accumet Model 915.

6.5 Top Loading Balance. Mettler PM2000.

6.6 Magnetic stirrer.

6.7 ZHE Extract Collection Devices. PTFE gas-tight syringe

7.0 REAGENTS

7.1 Reagent Water. Millipore Super-Q (Carbon Filtration)

7.2 Sodium hydroxide (1N). Dissolve 40g of NaOh in 500 ml distilled water, dilute to 1L.

7.3 Glacial acetic acid, concentration. ACE reagent grade.

7.4 TCLP Extraction fluid #1. Add 5.7ml glacial acetic acid (see Section 7.3) to 500ml reagent water (see Section 7.1), add 64.3ml of 1N NaOH (see Section 7.2), and dilute to 1 liter. When correctly prepared, the pH of this solution will be 4.93 ± 0.05. If pH is outside these limits the fluid will be discarded and fresh extraction fluid prepared.

7.5 SPLP Extraction Fluid: This fluid is reagent water (Step 7.1) and is used to determine cyanide and volatiles leachability.

8.0 PROCEDURE

8.1 Preliminary determination of percent solids:

8.1.1 If the waste will obviously yield no liquid when subjected to pressure filtration proceed to Section 8.2.

8.1.2 Pre-weigh the filter and the container that will receive the filter. Record weight on TCLP Percent Moisture Worksheet (Attachment 1).

8.1.3 Assemble the filter holder and filter. Place the filter on the support screen and secure.

8.1.4 Weigh out a sub-sample of the waste (100gram minimum) and record the weight.

8.1.5 Allow slurries to stand to permit the solid phase to settle. Transfer the waste sample to the filter holder and gradually apply vacuum of 1-10psi, until air moves through the filter. Slowly increase the vacuum in 10psi increments to 50psi. When liquid flow ceases stop the filtration.

8.1.6 The material in the filter holder is defined as the solid phase and the filtrate is defined as the liquid phase.

8.1.7 Some waste such as oil wastes and paints will not filter and is defined as a solid. Use only one filter for this procedure and do not replace.

8.1.8 Determine the weight of the liquid phase by subtracting the weight of the filtrate container from the total weight of the filtrate-filled container. Determine the weight of the solid phase by subtracting the weight of the liquid phase from the weight of the total waste sample.

8.1.9 Determine the percent solids as follows:

$$\text{Percent solids} = \frac{\text{Weight of solid}}{\text{Total weight of waste}} \times 100$$

8.1.10 If the percent solid is equal to or greater than 0.5%, then proceed to Section 8.2 to determine if particle size reduction is necessary or to Section 8.1.11 if it's noticed that water is entrapped in the solid material. If the percent solid is less than 0.5%, proceed to 8.3 with a fresh portion of the waste.

8.1.11 Remove the solid phase and filter from the filtration apparatus.

8.1.12 Dry the filter and solid phase at 100 ± 20°C until the two successive weighings yield the same value within 1%. Record the final weight.

8.1.13 Calculate the percent solids as follows:

$$\text{Percent solids} = \frac{\text{(Wt. Of dry waste + filter) – tared wt. of filter}}{\text{Initial weight of waste}} \times 100$$

8.1.14 If the percent solids is less than 0.5% proceed to Section 8.3. If the percent dry solid is equal to or greater than 0.5%, proceed to Section 8.2 and determine if particle size reduction is necessary.

8.2 Determination if particle size reduction is required.

8.2.1 Particle size reduction is not required if the waste is smaller than 1cm in its narrowest dimensions. Proceed to Section 8.3.1.

8.2.2 If the waste is larger than 1cm in its narrowest dimension, prepare the waste for extraction by cutting, crushing or grinding to reduce the particle size. When complete proceed to Section 8.3.1.

8.2.3 Wastes and appropriate reduction equipment should be refrigerated to 4°C prior to particle size reduction. The means used to effect particle size reduction must not generate heat. If reduction is necessary, exposure of the waste to the atmosphere should be avoided to the extent possible.

8.3 Extraction procedure

8.3.1 The ZHE device has approximately a 500ml internal capacity. The ZHE can accommodate a maximum of 25 grams of solid, due to the need to add an amount of extraction fluid equal to 20 times the weight of the solid phase.

8.3.2 Do not allow the waste, the initial liquid phase, or the extract to be exposed to the atmosphere for any more time than is absolutely necessary. Any manipulation of these materials should be done when cold (4°C) to minimize loss of volatiles.

8.3.3 If the waste contains <0.5% dry solids (Section 8.1), the liquid portion of the waste, after filtration, is defined as the extract. Filter enough of the sample so that the amount of filtered liquid will support all of the volatile analysis required. The recommended sample size is as follows:

8.3.3.1 For waste containing <0.5% solids, weigh out a 500 gram sub-sample of waste and record the weight.

8.3.3.2 For wastes containing >0.5% solids, determine the amount of waste to charge into the ZHE as follows:

$$\text{weight of waste to charge ZHE} = \frac{25}{\text{percent solids (see Section 8.1)}} \times 100$$

8.3.3.3 Measure out waste to required weight ± 0.1g.

8.3.4 Waste slurries need not be allowed to stand to permit the solid phase to settle. Do not centrifuge wastes prior to filtration.

8.3.5 Transfer the entire sample (liquid and solid phases) quickly to the ZHE. Secure the filter and support screens onto the top flange of the device and secure the top flange to the ZHE body. Tighten all ZHE fitting sand place the device in the vertical position (gas inlet/outlet flange on the bottom).

8.3.6 Attach a gas line to the gas inlet/outlet valve and, with the liquid inlet/outlet valve open, apply gentle pressure of 1-10psi to force all headspace slowly out of the ZHE into a hood. At the first sign of liquid from the liquid inlet/outlet valve, quickly close the valve and discontinue pressure. If the waste is 100% solid, slowly increase pressure to a maximum of 50psi to force most of the headspace out of the device and proceed to Section 8.3.9.

8.3.7 Attach the evacuated pre-weighed gas-tight syringe to the liquid inlet/outlet valve and open the valve. Begin applying gentle pressure of 1-10psi to force the liquid phase of the sample into the syringe. If no additional liquid has passed through the filter in any 2 minute interval, slowly increase the pressure in 10psi increments to a maximum of 50psi. When liquid flow has ceased such that continued pressure filtration at 50psi does not result in any additional filtrate within a 2 minutes period, stop the filtration. Close the liquid inlet/outlet valve, discontinue pressure, and disconnect and weight the syringe.

8.3.8 The material in the ZHE is defined as the solid phase of the waste and the filtrate is defined as the liquid phase. If the original waste contained <0.5% dry solids, this filtrate is defined as the extract and is analyzed directly. Store the liquid phase at 4°C with zero-headspace till time of analysis.

8.3.9 Determine the amount of the extraction fluid to add to the extractor vessel as follows:

$$\text{Amount Of Extraction Fluid} = \frac{20 \times \text{percent solids (see Section 8.1)} \times \text{weight of waste filtered}}{100}$$

8.3.10 Determination of Extraction Fluid

8.3.10.1 For TCLP-ZHE use TCLP extraction fluid #1. (See Section 7.5)

8.3.10.2 SPLP Extraction Fluid is used for volatile leachability. (See Section 7.5)

8.3.11 With the ZHE in the vertical position, attach a line from the extraction fluid reservoir to the liquid inlet/outlet valve. The line must contain fresh extraction fluid and be pre-flushed with fluid to eliminate any air pockets in the line. Release gas pressure on the ZHE piston (from the gas inlet/outlet valve), open the liquid inlet outlet valve, and begin transferring extraction fluid into the ZHE.

8.3.12 After the extraction fluid has been added, close the liquid inlet/outlet valve and disconnect the extraction fluid line. Check the ZHE to ensure that all valves are closed. Manually rotate the device end-over-end 2 or 3 times. Pressurize the ZHE to 5-10psi and slowly open the liquid inlet/outlet valve to bleed out any headspace. Re-pressurize the ZHE with 5-10psi and check all fittings to ensure that they are closed.

8.3.13 Place the ZHE in the rotary extractor and rotate at 30 ± 2 rpm for 18 ± 2 hours. Ambient temperature must be maintained at 23 ± 2°C during agitation.

8.3.14 Following the 18hour extraction, check the pressure of the ZHE piston by quickly opening and closing the gas inlet/outlet valve and noting the escape of gas. If the pressure has not been maintained, the device is leaking. Check the ZHE for leaking as specified in Section 6.2, and perform the extraction again with a new sample of waste. If the pressure within the device has been maintained, the material in the extractor vessel is ready to be separated into its liquid and solid phases. Filter through the glass fiber filter, using the ZHE device as discussed in Section 8.1.

8.3.15 If the waste contained an initial liquid phase, the liquid should be combined. A separate container must be used if combining would create multiple phases.

8.3.16 If the original waste contained no liquid the filtered extract obtained in Section 8.3 is defined as the TCLP / SPLP extract. If the waste contained an initial liquid phase, the filtered material obtained from Section 6.2 and the initial liquid phase (Section 8.3.6) are collectively defined as the TCLP / SPLP extract.

8.3.17 Following collection of the TCLP / SPLP extract, immediately prepare the extract for analysis and store with minimal headspace at 4°C until analysis.

9.0 CALCULATIONS. N/A

10.0 QUALITY ASSURANCE / QUALITY CONTROL

10.1 A minimum of one blank (using the same extraction fluid as used for samples) must be analyzed for every 20 extractions that have been conducted in an extraction vessel.

10.2 A matrix spike shall be performed for each waste type. A minimum of one matrix spike must be analyzed for each analytical batch. Matrix spikes are to be added after filtration of the TCLP extract and before preservation. Matrix spikes should be added at a concentration equivalent to the corresponding regulatory limit.

10.3 All quality control measures described in the appropriate analytical methods shall be followed.

11.0 WASTE DISPOSAL. N/A

12.0 METHOD PERFORMANCE. N/A

13.0 REFERENCES. USEPA, SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. Method 1311 & 1312.

14.0 DEFINITIONS. N/A

15.0 ATTACHMENTS. Percent Moisture Worksheet

ATTACHMENT 1
TCLP / SPLP Percent Moisture Worksheet

TCLP/SPLP Extraction Log, Ref: SWL-IN-700 / IN-701

TCLP Extraction Log (700-ATT1.DOC) SWLO SOP rev. #____ Book # IN700-LOG-1 Page _____

Type of Extraction (circle): TCLP / Zero Head Space

Analyst Initials:_____ **Date:**_____ **Time:**_____ **Batch ID:**_____

#	SWL ID#	Particle Reduction Necessary?	INITIAL pH	ACID pH	FINAL pH	AMOUNT EXTRACTED	START TEMP & TIME	END TEMP & TIME
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
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COMMENTS:_____

Southwest Laboratory of Oklahoma, Inc.

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SOUTHWEST LABORATORY OF OKLAHOMA, INC.

Standard Operating Procedure

TCLP / SPLP Non-Volatile Extractions

Document No.: SWL-IN-700

Rev No. / Date: Rev. 4.0 — 05/06/98

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APPROVAL	DATES
Procedure Prepared By	5/6/98
Laboratory Manager or Director	5/8/98
Laboratory Safety Officer	5/6/98
Laboratory QA/QC Officer	5/6/98

(Effective Date for is 5 calendar days after the last signature above - QA/QC Officer)

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TCLP / SPLP NON-VOLATILE EXTRACTIONS

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1.0 SCOPE AND APPLICATION

1.1 The purpose of this SOP, Toxicity Characteristic Leaching Procedure / Synthetic Precipitation Leaching Procedures (TCLP / SPLP) is to determine the mobility of both organic and inorganic analytes in liquid, solid, and multiphasic wastes.

1.2 If a total analysis of the waste demonstrates that individual analytes are not present in the waste, or that they are present but at such low concentrations that the appropriate regulatory levels could not be exceeded, the TCLP / SPLP need not be run.

1.3 If an analysis of any one of the liquid fractions of the TCLP / SPLP extract indicates that a regulated compound is present at such high concentrations that, even after accounting for dilution from the other fractions of the extract, the concentration would be above the regulatory level for that compound, then the waste is hazardous and it is not necessary to analyze the remaining fractions of the extract.

2.0 METHOD SUMMARY

2.1 For liquid wastes (i.e., those containing less than 0.5% dry solid material), the waste, after filtration through a 0.6 to 0.8 um glass fiber filter, is defined as the TCLP / SPLP extract.

2.2 For wastes containing greater than or equal to 0.5% solids, the liquid, if any, is separated from the solid phase and stored for later analysis. The solid phase is extracted with an amount of extraction fluid equal to 20 times the weight of the solid phase. Following extraction, the liquid phase is separated from the solid phase by filtration through a 0.6 to 0.8 um glass fiber filter.

2.3 If compatible, the initial liquid of the waste is added to the liquid extract, and these are analyzed together. If incompatible, the liquids are analyzed separately and the results are mathematically combined to yield a volume-weighted average concentration.

3.0 HEALTH AND SAFETY.

Refer to the Lab Safety Plan (SWL-GA-111) for precautions.

4.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING & STORAGE.

N/A

5.0 INTERFERENCES.

Potential interferences that may be encountered during analysis are discussed in the individual analytical SOP's.

6.0 APPARATUS

6.1 Agitation apparatus: Associated Design and MFG. Co., Model No. 3740-24-BRE-TM. Capable of maintaining 30 +/-2 rpm.

6.2 Bottle Extraction Vessel. Two liter borosilicate glass jar.

6.3 Filtration Device.

6.4 Filters. Glass fiber filters with a pore size of 0.6 to 0.8 um. When analyzing for metals, the filters are rinsed with 1N nitric acid, followed by three consecutive rinses with deionized water.

6.5 pH meter. Accumet Model 915.

6.6 Top Loading Balance. Mettler PM2000, or equivalent — All measurements shall be ± 0.1g of the desired weight. All balances used shall be able to read to 0.01g.

6.7 Beakers. 250 ml glass.

6.8 Watchglass.

6.9 Magnetic stirrer.

7.0 REAGENTS

7.1 Reagent Water. Millipore – ASTM Type II (by conductivity).

7.2 Hydrochloric acid (1N). Add 83 ml conc. HCl to 500 ml distilled water, dilute to 1 L.

7.3 Nitric acid (1N). Add 64 ml conc. HNO_3 to 500 ml distilled water, dilute to 1 L.

7.4 Sodium hydroxide (1N). Dissolve 40 g of NaOH in 500 ml distilled water, dilute to 1 L.

7.5 Glacial acetic acid, conc. , ACS reagent grade.

7.6 TCLP Extraction fluid #1. Add 5.7 ml glacial acetic acid(7.5) to 500 ml reagent water(7.1), add 64.3 ml of 1N NaOH(7.4), and dilute to 1 liter. When correctly prepared, the pH of this solution will be 4.93+/- 0.05. If pH is outside these limits the fluid will be discarded and fresh extraction fluid prepared.

7.7 TCLP Extraction fluid #2. Dilute 5.7 ml glacial acetic acid(7.5) with reagent water(7.1) to a volume of 1 L. When correctly prepared, the pH of this fluid will be 2.88 +/- 0.05. If pH is outside limits the fluid will be discarded and fresh fluid extraction fluid prepared.

7.8 SPLP Extraction Fluid #1: This fluid is made by adding the 60/40 weight percent mixture of sulfuric and nitric acids (or a suitable dilution) to reagent water (Step 7.1) until the pH is 4.20 ± 0.05. This fluid is used to determine the leachability of soil from a site that is east of the Mississippi River, and the leachability of wastes and wastewaters.

NOTE: Solutions are unbuffered and exact pH may not be attained.

7.9 SPLP Extraction Fluid #2: This fluid is made by adding the 60/40 weight percent mixture of sulfuric and nitric acids to reagent water (Step 7.1) until the pH is 5.00 ± 0.05. The fluid is used to determine the leachability of soil from a site that is west of the Mississippi River.

7.10 SPLP Extraction Fluid #3: This fluid is reagent water (Step 7.1) and is used to determine cyanide and volatiles leachability.

NOTE: These extraction fluids should be monitored frequently for impurities. The pH should be checked prior to use to ensure that these fluids are made up accurately. If impurities are found or the pH is not within the above specifications, the fluid shall be discarded and fresh extraction fluid prepared.

8.0 PROCEDURE

8.1 Preliminary determination of percent solids:

8.1.1 If the waste will obviously yield no liquid when subjected to pressure filtration proceed to Section 8.2.

8.1.2 Pre-weigh the filter and the container that will receive the filter. Record weight on TCLP / SPLP Percent Moisture Log (Attachment 1).

8.1.3 Assemble the filter holder and filter. Place the filter on the support screen and secure.

8.1.4 Weigh out a subsample of the waste (100 gram minimum) and record the weight.

8.1.5 Allow slurries to stand to permit the solid phase to settle. Transfer the waste sample to the filter holder and gradually apply vacuum of 1-10 psi, until air moves through the filter. Slowly increase the vacuum in 10 psi increments to 50 psi. When liquid flow ceases stop the filtration.

8.1.6 The material in the filter holder is defined as the solid phase and the filtrate is defined as the liquid phase.

8.1.7 Some wastes such as oily wastes and paints will not filter and is defined as a solid. Use only one filter for this procedure and do not replace.

8.1.8 Determine the weight of the liquid phase by subtracting the weight of the filtrate container from the total weight of the filtrate-filled container. Determine the weight of the solid phase by subtracting the weight of the liquid phase from the weight of the total waste sample.

8.1.9 Determine the percent solids as follows:

$$\text{Percent solids} = \frac{\text{weight of solid}}{\text{total weight of waste}} \times 100$$

8.1.10 If the percent solid is equal to or greater than 0.5%, then proceed to Section 8.2 to determine if particle size reduction is necessary or to Section 8.1.11 if it's noticed that water is entrapped in the solid material. If the percent solid is less than 0.5%, then the filtrate will be the TCLP / SPLP extract.

8.1.11 Remove the solid phase and filter from the filtration apparatus.

8.1.12 Dry the filter and solid phase at 100 +/- 20° C until the two successive weighings yield the same value within 1%. Record the final weight.

8.1.13 Calculate the percent solids as follows:

$$\text{Percent solids} = \frac{\text{(Wt. of dry waste + filter) - tared wt. of filter}}{\text{Initial weight of waste}} \times 100$$

8.1.14 If the percent solid is less than 0.5 % then the filtrate from Section 8.1.5 will be the TCLP / SPLP extract. If the percent dry solid is equal to or greater than 0.5%,return to Section 8.1.1 with a fresh portion of waste and determine if particle size reduction is necessary.

8.2 Determination if particle size reduction is required.

8.2.1 Particle size reduction is not required if the waste will pass through a 9.5 mm (0.375 in.) sieve. Proceed to Section 8.3.1.

8.2.2 If the waste will not pass through a 9.5 mm sieve then prepare the waste for extraction by cutting, crushing or grinding to reduce the particle size. When complete proceed to Section 8.3.1.

8.3 Determination of appropriate extraction fluid. If the solid content of the waste is greater than or equal to 0.5% determine the appropriate fluid for TCLP extraction as follows:

8.3.1 Weigh out a small subsample of the solid phase, reduce the solid, if necessary, to a particle size of 1mm in diameter or less. Transfer 5.0 g of the waste to a 250 ml beaker.

8.3.2 Add 96.5 ml of reagent water to the beaker, cover with a watchglass, and stir vigorously for 5 minutes on a magnetic stirrer. Measure and record the pH on the TCLP pH Run Log (Attachment 1). If the pH is <5.0 use extraction fluid #1 and proceed to section 8.4.1.

8.3.3 If the pH was greater than 5.0, add 3.5 ml 1N HCl, cover with a watch glass, heat to 50° C, and hold at 50° C for 10 minutes.

8.3.4 Let the solution cool to room temperature and record the pH. If the pH is <5.0, use extraction fluid #1. If the pH is >5.0, use extraction fluid #2.

8.3.5 For SPLP the following extraction fluid determination is made:

8.3.5.1 For soils, if the sample is from a site that is east of the Mississippi River, extraction fluid #1 should be used. If the sample is from a site that is west of the Mississippi River, extraction fluid #2 should be used.

8.3.5.2 For wastes and wastewater, extraction fluid #1 should be used.

8.3.5.3 For cyanide-containing wastes and/or soils, extraction fluid #3 (reagent water) must be used because leaching of cyanide-containing samples under acidic conditions may result in the formation of hydrogen cyanide gas.

8.4 Extraction procedure

8.4.1 Enough solids should be generated for extraction such that the volume of extract will be sufficient to support all of the analyses required. A good rule of thumb is 1-L per analyses.

8.4.2 Determine the amount of the raw sample needed to provide combined filtrate and extract totaling 2000mL. The following formula should be used to determine this amount:

$$(1-S/100)\,(RS) + 20(S/100)\,(RS) = 2000$$

where: S = % solids
RS = raw sample wieght

e.g. if the raw sample were 20% solids:

$$(1-20/100)\,(RS) + 20(20/100)\,(RS) = 2000$$
$$0.8RS + 4RS = 2000$$
$$RS = 417 \text{ grams } \pm .1 \text{ g}$$

8.4.3 Determine the amount of the extraction fluid to add to the extractor vessel a follows:

$$\text{Amount of extraction fluid} = \frac{20 \times \text{percent solids (8.1)} \times \text{weight of waste filtered}}{100}$$

8.4.4 Add the solids and appropriate amount of extraction fluid to the extractor vessel. Record information on TCLP / SPLP non-volatile extraction log (Attachment 1).

8.4.5 Close the extractor bottle tightly, secure in the rotary agitation device, and rotate at 30 +/- 2 rpm for 18 +/- 2 hours. Ambient temperature shall be maintained at 23 +/- 2^{0}C during the extraction. Record both the time start and temperature at start on extraction log.

8.4.6 Following the 18 hour extraction, separate the material in the extractor vessel into its component liquid and solid phase by filtering through a new glass fiber filter as outlined in Section 8.1. For final filtration of the extract, the glass fiber filter may be changed, if necessary, to facilitate filtration. Filters shall be acid-washed (6.4) if evaluating the mobility of metals.

8.5 Prepare the extract as follows:

8.5.1 If the waste contained no initial liquid phase, the filtered liquid material is the extract.

8.5.2 If compatible, combine the filtered liquid obtained in Step 8.4 with the initial liquid phase of the waste obtained in Section 8.1. This combined liquid is defined as the extract.

8.5.3 If the initial liquid phase of the waste, obtained in Section 8.1, is not compatible with the filtered liquid resulting from Section 8.4, do not combine these liquids. Analyze these liquids, collectively defined as the extract, and combine the results mathematically. Determine the volume of the individual phases and combine the results by using the volume-weighted average:

$$\text{Final Analyte Concentration} = \frac{(V_1)(C_1) + (V_2)(C_2)}{V_1 + V_2}$$

where: V_1 = The volume of the first phase (liters).
C_1 = The concentration of the analyte of concern in the first phase (mg/l).
V_2 = The volume of the second phase (liters).
C_2 = The concentration of the analyte of concern in the second phase (mg/l).

8.5.4 Following collection of the extract, the pH of the extract should be recorded.

8.5.5 Immediately aliquot and preserve the extract for analysis. Metals aliquots must be acidified with nitric acid to pH <2. Metal matrix spike samples are to be spiked prior to the addition of HNO_3. All other aliquots must be stored at 4°C until analyzed.

9.0 CALCULATIONS. Not Applicable

10.0 QUALITY ASSURANCE

10.1 A minimum of one blank (using the same extraction fluid as used for samples) must be analyzed for every 20 extractions that have been conducted in an extraction vessel.

10.2 A matrix spike shall be performed for each waste type. A minimum of one matrix spike must be analyzed for each analytical batch. Matrix spikes are to be added after filtration of the extract and before preservation. Matrix spikes should be added at a concentration equivalent to the corresponding regulatory limit.

10.3 The method of standard additions (MSA) shall be used for metals if:

10.3.1 Recovery of the matrix spike for an analyte is < 50% and the concentration does not exceed the regulatory limit.

10.3.2 The concentration of the contaminant measured in the extract is within 20% of the regulatory level.

10.4 All quality control measures described in the appropriate analytical methods shall be followed.

11.0 **WASTE DISPOSAL.** Not Applicable

12.0 **METHOD PERFORMANCE.** Not Applicable

13.0 **REFERENCES**

- USEPA, SW-846, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. Method 1311 & 1312.

14.0 **DEFINITIONS** — Not Applicable

15.0 **ATTACHMENTS** — TCLP / SPLP WORKSHEET

ATTACHMENT 1
TCLP / SPLP Worksheet

TCLP/SPLP Extraction Log, Ref: SWL-IN-700 / IN-701

TCLP Extraction Log (700-ATT1.DOC) SWLO SOP rev. #____ Book # IN700-LOG-1 Page _____

Type of Extraction (circle): TCLP / Zero Head Space

Analyst Initials:_____ **Date:**_____ **Time:**_____ **Batch ID:**_____

#	SWL ID#	Particle Reduction Necessary?	INITIAL pH	ACID pH	FINAL pH	AMOUNT EXTRACTED	START TEMP & TIME	END TEMP & TIME
1								
2								
3								
4								
5								
6								
7								
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COMMENTS:_____

Southwest Laboratory of Oklahoma, Inc.

Verified:_____