Tennessee Valley Authority, Post Office Box 2000, Soddy-Daisy, Tennessee 37384-2000

February 11, 2003

State of Tennessee
Department of Environment and Conservation
Division of Water Pollution Control
Enforcement & Compliance Section
6th Floor, L & C Annex
401 Church Street
Nashville, Tennessee 37243-1534

Attention: Mr. Chip Hannah

Dear Mr. Hannah:

TENNESSEE VALLEY AUTHORITY - DISCHARGE MONITORING REPORT FOR SEQUOYAH NUCLEAR PLANT

Please find enclosed the Discharge Monitoring Report (DMR) of January 2003 for Sequoyah. Please contact me at (423) 843-7324 if you have any questions or comments.

Sincerely,

M. B

Michael G. Beavers
Acting Environmental Supervisor
Signatory Authority for
Richard T. Purcell
Site Vice President
Sequoyah Nuclear Plant

Enclosure
cc (Enclosure):

Chattanooga Environmental Assistance Center
Division of Water Pollution Control
State Office Building, Suite 550
540 McCallie Avenue
Chattanooga, Tennessee 37402-2013

U.S. Nuclear Regulatory Commission
ATTN: Document Control Desk
Washington, D.C. 20555

IE25

Printed on recycled paper

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P O BOX 2000
(INTEROFFICE SB-2A)
SODDY - DAISY TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN. Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 101 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
DIFFUSER DISCHARGE
EFFLUENT

*** NO DISCHARGE ☐ ***

Form Approved
OMB No 2040-0004

NOTE Read instructions before completing this form

| PARAMETER | | QUANTITY OR LOADING AVERAGE | QUANTITY OR LOADING MAXIMUM | QUANTITY OR LOADING UNITS | QUALITY OR CONCENTRATION MINIMUM | QUALITY OR CONCENTRATION AVERAGE | QUALITY OR CONCENTRATION MAXIMUM | QUALITY OR CONCENTRATION UNITS | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TEMPERATURE, WATER DEG. CENTIGRADE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 9.2 | 04 | 0 | 31 / 31 | MODELD |
| 00010 Z 0 0 INSTREAM MONITORING | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 30.5 DAILY MX | DEG. C. | | SEE PERMIT | CK REQ |
| TEMPERATURE, WATER DEG. CENTIGRADE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 23.2 | 04 | 0 | 31 / 31 | RCORDR |
| 00010 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | REPORT DAILY MX | DEG. C. | | SEE PERMIT | CK REQ |
| PH | SAMPLE MEASUREMENT | ******** | ******** | ** | 7.6 | ******** | 8.0 | 12 | 0 | 10 / 31 | GRAB |
| 00400 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 6.0 MINIMUM | ******** | 9.0 MAXIMUM | SU | | WEEKLY | GRAB |
| SOLIDS, TOTAL SUSPENDED | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | 4 | 5 | 19 | 0 | 5 / 31 | GRAB |
| 00530 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | 30 MO AVG | 100 DAILY MX | MG/L | | WEEKLY | GRAB |
| OIL AND GREASE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | <5 | <5 | 19 | 0 | 5 / 31 | GRAB |
| 00556 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | 15 MO AVG | 20 DAILY MX | MG/L | | WEEKLY | GRAB |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | ******** | 1614 | 03 | ******** | ******** | ******** | ** | 0 | 31 / 31 | RCORDR |
| 50050 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | REPORT DAILY MX | MGD | ******** | ******** | ******** | **** | | CONTIN UOUS | RCORDR |
| CHLORINE, TOTAL RESIDUAL | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | <0.005 | 0.016 | 19 | 0 | 38 / 31 | GRAB |
| 50060 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | 0.036 | 0.058 INST MAX | MG/L | | WEEK-DAYS | CALCTD |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | M. Bu<br>Environmental Supervisor<br>SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

No closed mode operation. The following injections occured 1. PCL-222 (max. calc. conc. was 0 026mg/L--limit 0 100mg/L) 2. CL-363 (max. calc. conc. was 0 009mg/L--limit 0 100mg/L) 3. PCL-222/PCL-401 (max. calc conc. was 0 020mg/L--limit 0.100mg/L)

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P O BOX 2000
(INTEROFFICE SB-2A)
SODDY - DAISY TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN. Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 101 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
DIFFUSER DISCHARGE
EFFLUENT

*** NO DISCHARGE [ ] ***

Form Approved
OMB No 2040-0004

NOTE: Read instructions before completing this form.

| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| TEMPERATURE - C, RATE OF CHANGE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 0.2 | 62 | 0 | 31 / 31 | CALCTD |
| 82234 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 2.0 | DEG C /HR | | CONTIN UOUS | CALCTD |
| TEMP. DIFF. BETWEEN SAMP. & UPSTRM DEG.C | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 2.4 | 04 | 0 | 31 / 31 | CALCTD |
| 00016 1 W 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 5.0 | DEG. C. | | CONTIN UOUS | CALCTD |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| BORON, TOTAL | SAMPLE MEASUREMENT | ******** | ******** | ** | <0.2 | <0.2 | <0.2 | 19 | 0 | 1 / 31 | GRAB |
| 01022 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | REPORT | REPORT | REPORT | MG/L | | ONCE/ MONTH | GRAB |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | M. Brown<br>Environmental Supervisor | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

CCW data for Jan 2003 is attached

CCW Data forJanuary 2003

| CCW CHANNEL | | |
|---|---|---|
| DATE | EXTRACTABLE PETROLEUM HYDROCARBONS (mg/L) | COMMENTS |
| January 23, 2003 | < 0.5 | |

| CCW TRENCH DISCHARGE | | |
|---|---|---|
| DATE | EXTRACTABLE PETROLEUM HYDROCARBONS (mg/L) | COMMENTS |
| January 23, 2003 | 2.3 | |

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Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN. Michael G Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 101 T |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
BIOMONITORING FOR OUTFALL 101
EFFLUENT

*** NO DISCHARGE [ ] ***

Form Approved
OMB No 2040-0004

NOTE. Read instructions before completing this form

| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| IC25 STATRE 7DAY CHR CERIODAPHNIA | SAMPLE MEASUREMENT | ******** | ******** | ** | >100 | ******** | ******** | 23 | 0 | 1 / 90 | COMPOS |
| TRP3B 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 43.9 MINIMUM | ******** | ******** | PERCENT | | QUART-ERLY | COMPOS |
| IC25 STATRE 7DAY CHR PIMEPHALES | SAMPLE MEASUREMENT | ******** | ******** | ** | >100 | ******** | ******** | 23 | 0 | 1 / 90 | COMPOS |
| TRP6C 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 43.9 MIMINUM | ******** | ******** | PERCENT | | QUART-ERLY | COMPOS |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is , to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | M. Beaver<br>Environmental Supervisor<br>SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

Toxicity was sampled from Jan 12, 2003 to Jan 17, 2003. The report is attached.

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P O BOX 2000
(INTEROFFICE SB-2A)
SODDY - DAISY TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| PERMIT NUMBER | DISCHARGE NUMBER |
|---|---|
| TN0026450 | 103 G |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
LOW VOL. WASTE TREATMENT POND
EFFLUENT

*** NO DISCHARGE ☐ ***

Form Approved
OMB No 2040-0004

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|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | SAMPLE MEASUREMENT | ******** | ******** | ** | 7.6 | ******** | 8.9 | 12 | 0 | 14 / 31 | GRAB |
| 00400 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | ** | 6.0 MINIMUM | ******** | 9.0 MAXIMUM | SU | | THREE/ WEEK | GRAB |
| SOLIDS, TOTAL SUSPENDED | SAMPLE MEASUREMENT | 127 | 193 | 26 | ******** | 13 | 20 | 19 | 0 | 5 / 31 | GRAB |
| 00530 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | 380 MO AVG | 1250 DAILY MX | LBS/DY | ******** | 30 MO AVG | 100 DAILY MX | MG/L | | WEEKLY | GRAB |
| OIL AND GREASE | SAMPLE MEASUREMENT | <48 | <51 | 26 | ******** | <5 | <5 | 19 | 0 | 5 / 31 | GRAB |
| 00556 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | 190 MO AVG | 250 DAILY MX | LBS/DY | ******** | 15 MO AVG | 20 DAILY MX | MG/L | | WEEKLY | GRAB |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | 1.140 | 1.430 | 03 | ******** | ******** | ******** | ** | 0 | 31 / 31 | TOTAL |
| 50050 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | REPORT MO AVG | REPORT DAILY MX | MGD | ******** | ******** | ******** | ** | | DAILY | TOTAL |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
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| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is , to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | Environmental Supervisor<br>SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
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(INTEROFFICE SB-2A)
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Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 107 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
METAL CLEANING WASTE POND
EFFLUENT

*** NO DISCHARGE XX ***

Form Approved
OMB No 2040-0004

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| PARAMETER | | QUANTITY OR LOADING AVERAGE | QUANTITY OR LOADING MAXIMUM | QUANTITY OR LOADING UNITS | QUALITY OR CONCENTRATION MINIMUM | QUALITY OR CONCENTRATION AVERAGE | QUALITY OR CONCENTRATION MAXIMUM | QUALITY OR CONCENTRATION UNITS | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | SAMPLE MEASUREMENT | ******** | ******** | ** | | ******** | | 12 | | | |
| 00400 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 6.0 MINIMUM | ******** | 9.0 MAXIMUM | SU | | DAILY | GRAB |
| PHOSPHORUS, TOTAL (AS P) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00665 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1.0 DAILY MX | MG/L | | DAILY | COMPOS |
| SOLIDS, TOTAL SUSPENDED | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00530 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 30 DAILY MX | MG/L | | DAILY | COMPOS |
| OIL AND GREASE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00556 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 15 DAILY MX | MG/L | | DAILY | GRAB |
| COPPER, TOTAL (AS CU) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 01042 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1.0 DAILY MX | MG/L | | DAILY | COMPOS |
| IRON, TOTAL (AS FE) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 01045 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1.0 DAILY MX | MG/L | | DAILY | COMPOS |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | | | 03 | ******** | ******** | ******** | ** | | | |
| 50050 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | REPORT MO AVG | REPORT DAILY MX | MGD | ******** | ******** | ******** | **** | | DAILY | CALCTD |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is , to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | M. B<br>Environmental Supervisor | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

No phosphate bearing chemicals were employed. No Discharge this Period

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P O BOX 2000
(INTEROFFICE SB-2A)
SODDY - DAISY TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 110 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
RECYCLED COOLING WATER
EFFLUENT

*** NO DISCHARGE XX ***

Form Approved
OMB No 2040-0004

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|---|---|---|---|---|---|---|---|---|---|---|---|
| PH | SAMPLE MEASUREMENT | ******** | ******** | ** | | ******** | | 12 | | | |
| 00400 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 6.0 MINIMUM | ******** | 9.0 MAXIMUM | SU | | DAILY | GRAB |
| SOLIDS, TOTAL SUSPENDED | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00530 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 30 DAILY MX | MG/L | | DAILY | COMPOS |
| OIL AND GREASE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00556 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 15 DAILY MX | MG/L | | DAILY | COMPOS |
| COPPER, TOTAL (AS CU) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 01042 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1.0 DAILY MX | MG/L | | DAILY | COMPOS |
| IRON, TOTAL (AS FE) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 01045 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1.0 DAILY MX | MG/L | | DAILY | COMPOS |
| PHOSPHORUS, TOTAL (AS P) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00665 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1.0 DAILY MX | MG/L | | DAILY | COMPOS |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | | | 03 | ******** | ******** | ******** | ** | | | |
| 50050 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | REPORT MO AVG | REPORT DAILY MX | MGD | ******** | ******** | ******** | **** | | DAILY | CALCTD |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is , to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | M. Purcell<br>Environmental Supervisor | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

No Discharge this Period

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P O BOX 2000
(INTEROFFICE SB-2A)
SODDY - DAISY TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 116 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
BACKWASH
EFFLUENT

*** NO DISCHARGE ☐ ***

Form Approved
OMB No 2040-0004

NOTE: Read instructions before completing this form

| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| DEBRIS, FLOATING (SEVERITY) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 0 | 9A | 0 | 1 / 31 | VISUAL |
| 01345 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | REPORT MO TOTAL | PASS=0 FAIL=1 | | SEE PERMIT | VISUAL |
| OIL AND GREASE VISUAL | SAMPLE MEASUREMENT | ******** | 0 | 94 | ******** | ******** | ******** | ** | 0 | 1 / 31 | VISUAL |
| 84066 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | REPORT MO TOTAL | YES=1 NO=0 | ******** | ******** | ******** | **** | | SEE PERMIT | VISUAL |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | Environmental Supervisor<br>SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

Operations performs visual inspections for floating debris and oil and grease during all backwashes.

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P O BOX 2000
(INTEROFFICE SB-2A)
SODDY - DAISY TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN· Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 117 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
BACKWASH
EFFLUENT

*** NO DISCHARGE [ ] ***

Form Approved
OMB No 2040-0004

NOTE· Read instructions before completing this form

| PARAMETER | | QUANTITY OR LOADING | | | QUALITY OR CONCENTRATION | | | | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AVERAGE | MAXIMUM | UNITS | MINIMUM | AVERAGE | MAXIMUM | UNITS | | | |
| DEBRIS, FLOATING (SEVERITY) | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | 0 | 9A | 0 | 1 / 31 | VISUAL |
| 01345 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | REPORT MO TOTAL | PASS=0 FAIL=1 | | SEE PERMIT | VISUAL |
| OIL AND GREASE VISUAL | SAMPLE MEASUREMENT | ******** | 0 | 94 | ******** | ******** | ******** | ** | 0 | 1 / 31 | VISUAL |
| 84066 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | REPORT MO TOTAL | YES=1 NO=0 | ******** | ******** | ******** | **** | | SEE PERMIT | VISUAL |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
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| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is , to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | Environmental Supervisor<br>SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

Operations performs visual inspections for floating debris and oil and grease during all backwashes

PERMITTEE NAME/ADDRESS *(Include Facility Name/Location if Different)*

Name TVA - SEQUOYAH NUCLEAR PLANT
Address P O BOX 2000
(INTEROFFICE SB-2A)
SODDY - DAISY TN 37384
Facility TVA - SEQUOYAH NUCLEAR PLANT
Location HAMILTON COUNTY

ATTN: Michael G. Beavers

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM *(NPDES)*
DISCHARGE MONITORING REPORT *(DMR)*

| TN0026450 | 118 G |
|---|---|
| PERMIT NUMBER | DISCHARGE NUMBER |

| MONITORING PERIOD | YEAR | MO | DAY | | YEAR | MO | DAY |
|---|---|---|---|---|---|---|---|
| From | 03 | 01 | 01 | To | 03 | 01 | 31 |

MAJOR
(SUBR 01)
F - FINAL
WASTEWATER & STORM WATER
EFFLUENT

*** NO DISCHARGE XX ***

Form Approved
OMB No 2040-0004

NOTE Read instructions before completing this form

| PARAMETER | | QUANTITY OR LOADING AVERAGE | QUANTITY OR LOADING MAXIMUM | QUANTITY OR LOADING UNITS | QUALITY OR CONCENTRATION MINIMUM | QUALITY OR CONCENTRATION AVERAGE | QUALITY OR CONCENTRATION MAXIMUM | QUALITY OR CONCENTRATION UNITS | NO. EX | FREQUENCY OF ANALYSIS | SAMPLE TYPE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| OXYGEN, DISSOLVED (DO) | SAMPLE MEASUREMENT | ******** | ******** | ** | | ******** | ******** | 19 | | | |
| 00300 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | 2.0 DAILY MN | ******** | ******** | MG/L | | TWICE/ WEEK | GRAB |
| SOLIDS, TOTAL SUSPENDED | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 19 | | | |
| 00530 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 100 DAILY MX | MG/L | | TWICE/ WEEK | GRAB |
| SOLIDS, SETTLEABLE | SAMPLE MEASUREMENT | ******** | ******** | ** | ******** | ******** | | 25 | | | |
| 00545 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | ******** | ******** | **** | ******** | ******** | 1.0 DAILY MX | ML/L | | ONCE/ MONTH | GRAB |
| FLOW, IN CONDUIT OR THRU TREATMENT PLANT | SAMPLE MEASUREMENT | | | 03 | ******** | ******** | ******** | ** | | | |
| 50050 1 0 0 EFFLUENT GROSS VALUE | PERMIT REQUIREMENT | REPORT MO AVG | REPORT DAILY MX | MGD | ******** | ******** | ******** | * | | ONCE/ BATCH | ESTIMA |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |
| | SAMPLE MEASUREMENT | | | | | | | | | | |
| | PERMIT REQUIREMENT | | | | | | | | | | |

| NAME/TITLE PRINCIPAL EXECUTIVE OFFICER | | | TELEPHONE | | DATE | | |
|---|---|---|---|---|---|---|---|
| Richard T. Purcell<br>Site Vice President | I Certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment for knowing violations | M. Bar<br>Environmental Supervisor | 423 | 843-6700 | 03 | 02 | 13 |
| TYPED OR PRINTED | | SIGNATURE OF PRINCIPAL EXECUTIVE OFFICER OR AUTHORIZED AGENT | AREA CODE | NUMBER | YEAR | MO | DAY |

COMMENTS AND EXPLANATION OF ANY VIOLATIONS *(Reference all attachments here)*

During this reporting period, there has been no flow from the Dredge Pond other than that resulting from rainfall

February 7, 2003

Richard D. Nunley, SB 2A-SQN

SEQUOYAH NUCLEAR PLANT (SQN) TOXICITY BIOMONITORING, NPDES PERMIT NO. TN0026450, COMPLIANCE TOXICITY TESTS, JANUARY, 2003

Attached are two copies of the subject report for submission to the state of Tennessee and a copy of the report for your records. The report provides results of compliance testing using fathead minnows and daphnids. Outfall 101, samples collected January 12-17, showed no toxic effects to fathead minnows or daphnids. The resulting $IC_{25}$ values for both species were > 100 percent. Exposure of fathead minnows and daphnids to intake samples resulted in no significant differences from controls during this study period.

In addition to the routine compliance test, fathead minnows were also tested in Outfall 101 and intake samples which were treated using UV exposure for pathogen removal prior to introduction of test organisms. Fish pathogens present in intake water have been the suspected cause of anomalous dose responses and high variability among replicates in previous toxicity testing at Sequoyah. At the time this study was conducted, mortality which occurred in minnows exposed to routine compliance samples and UV treated samples was not sufficient to jeopardize statistical validity, although survival was improved in the UV treated test.

Call me at (256) 386-2755 if you have any questions or comments following your review of the report.

Cynthia L. Russell
Biologist
Environmental Engineering Services- West
CEB 3A-M

Attachment
cc (Attachment):
Files, ER&TA, CEB 1B-M

SQN January 2003m

# TENNESSEE VALLEY AUTHORITY
# TOXICITY TEST REPORT

## INTRODUCTION / EXECUTIVE SUMMARY

Report Date: February 7, 2003

1. Facility / Discharger: Sequoyah Nuclear Plant / TVA
2. County / State: Hamilton / Tennessee
3. NPDES Permit #: TN 0026450
4. Type of Facility: Nuclear-Fueled Electric Generating Plant
5. Design Flow (MGD): 3,266
6. Receiving Stream: Tennessee River (TRM 483.6)
7. 1Q10: 2,992.4
8. Outfall Tested: 101
9. Dates Sampled: January 12 - 17, 2003
10. Average Flow on Days Sampled (MGD): 1605, 1578, 1601
11. Pertinent Site Conditions: No unusual conditions reported. (Production / operation data will be provided upon request.)
12. Test Dates: January 14 - 21, 2003
13. Test Type: Short-term Chronic Definitive
14. Test Species: Fathead Minnows (*Pimephales promelas*)
    Daphnids (*Ceriodaphnia dubia*)
15. Concentrations Tested (%): Outfall 101: 10.98, 22, 43.9, 72, 100
    Intake: 100
    *Pimephales promelas*: UV treated Outfall 101: 10.98, 22, 43.9, 72, 100
    UV treated Intake: 100
16. Permit Limit Endpoint(%): Outfall 101: $IC_{25} = 43.9\%$
17. Test Results: Outfall 101: *Pimephales promelas:* $IC_{25} > 100$
    *Ceriodaphnia dubia:* $IC_{25} > 100$
    UV treated Outfall 101: *Pimephales promelas:* $IC_{25} > 100$

18. Facility Contact: Richard D. Nunley
Phone #: (423) 843-7748

19. Consulting / Testing Lab: Environmental Testing Solutions, LLC

20. Lab Contact: Jim Sumner
Phone #: (828) 862-8193

21. TVA Contact: Cynthia L. Russell
Phone #: (256) 386-2755

22. Notes: Outfall 101, samples collected January 12-17, showed no toxic effects to fathead minnows or daphnids. The resulting $IC_{25}$ values for both species were > 100 percent. Exposure of fathead minnows and daphnids to intake samples resulted in no significant differences from controls during this study period.

In addition to the routine compliance test, fathead minnows were also tested in Outfall 101 and intake samples which were treated using UV exposure for pathogen removal prior to introduction of test organisms. Fish pathogens present in intake water have been the suspected cause of anomalous dose responses and high variability among replicates in previous toxicity testing at Sequoyah. At the time this study was conducted, mortality which occurred in minnows exposed to routine compliance samples and UV treated samples was not sufficient to jeopardize statistical validity, although survival was improved in the UV treated test.

## METHODS SUMMARY

Samples:

1. Sampling Point: Outfall 101, Intake

2. Sample Type: Composite

3. Sample Information:

| Sample ID | Date (MM/DD/YY)/ Time (EST) Collected | Date (MM/DD/YY)/ Time (EST) Received | Arrival Temp. (°C) | Initial TRC* (mg/L) | Date (MM/DD/YY)/ Time (EST) Used |
|---|---|---|---|---|---|
| 101 | 01/12/03 1350 to 01/13/03 1250 | 01/14/03 0941 | 0.7† | <0.10 | 01/14/03 1517<br>01/15/03 1500 |
| Intake | 01/12/03 1329 to 01/13/03 1229 | 01/14/03 0941 | 0.7 | <0.10 | 01/14/03 1517<br>01/15/03 1500 |
| 101 | 01/14/03 1254 to 01/15/03 1154 | 01/16/03 0956 | 0.6† | <0.10 | 01/16/03 1426<br>01/17/03 1431 |
| Intake | 01/14/03 1248 to 01/15/03 1148 | 01/16/03 0956 | 0.3 | <0.10 | 01/16/03 1426<br>01/17/03 1431 |
| 101 | 01/16/03 1301 to 01/17/03 1201 | 01/18/03 1000 | 0.7† | <0.10 | 01/18/03 1430<br>01/19/03 1502<br>01/20/03 1436 |
| Intake | 01/16/03 1254 to 01/17/03 1154 | 01/18/03 1000 | 0.2 | <0.10 | 01/18/03 1430<br>01/19/03 1502<br>01/20/03 1436 |

*TRC = Total Residual Chlorine
†Collected in two 2.5-gallon cubitainers. Temperature was measured in each cubitainer upon arrival

4. Sample Manipulation: Samples from Outfall 101 and the Intake were warmed to test temperature (25.0 ± 1.0°C) in a warm water bath.

Aliquots of Outfall 101 and Intake samples were UV-treated through a 40-watt Rainbow Lifeguard® UV Sterilizer for 2 minutes.

| | *Pimephales promelas* | *Ceriodaphnia dubia* |
|---|---|---|
| **Test Organisms:** | | |
| 1. Source: | Aquatic BioSystems, Inc. | In-house Cultures |
| 2. Age: | 24.0-27.0 hours old | < 24-hours old |
| **Test Method Summary:** | | |
| 1. Test Conditions: | Static, Renewal | Static, Renewal |
| 2. Test Duration: | 7 days | Until at least 60% of control females have 3 broods |
| 3. Control / Dilution Water: | Moderately Hard Synthetic | Moderately Hard Synthetic |
| 4. Number of Replicates: | 4 | 10 |
| 5. Organisms per Replicate: | 10 | 1 |
| 6) Test Initiation: (Date/Time) | | |
| Outfall 101 | 01/14/03-1450 EST | 01/14/03-1517 EST |
| UV Treated Outfall 101 | 01/14/03-1420 EST | |
| 7) Test Termination: (Date/Time) | | |
| Outfall 101 | 01/21/03-1440 EST | 01/21/03-1504 EST |
| UV Treated Outfall 101 | 01/21/03-1423 EST | |
| 8) Test Temperature: Outfall 101: | Mean =24.8 °C (24.4-25.4°C) | Mean = 24.8°C (24.3-25.6°C) |
| Test Temperature: UV-Treated Outfall 101: | Mean = 24.7°C (24.3-25.3°C) | |

9. Physical / Chemical Measurements: Alkalinity, hardness, total residual chlorine, and conductivity were measured at the laboratory in each 100% sample. Daily temperatures were measured in one replicate for each test concentration. Pre- and post-exposure test solutions were analyzed daily for pH and dissolved oxygen.

10. Statistics: Statistics were performed according to methods prescribed by EPA using ToxCalc version 5.0 statistical software (Tidepool Scientific Software, McKinneyville, CA).

**TOXICITY TEST RESULTS** (see Appendix C for Bench Sheets)

1. Results of a *Pimephales promelas* Chronic 7-day Toxicity Test.
(Genus species) (Type / Duration)

Conducted January 14 -21, 2003 using effluent from Outfall 101.

| Test Solutions (% Effluent) | Percent Surviving (time interval used – days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10.98% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 22% | 100 | 98 | 98 | 98 | 98 | 98 | 95 |
| 43.9% | 100 | 100 | 100 | 100 | 100 | 100 | 98 |
| 72% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 100% | 100 | 100 | 98 | 90 | 85 | 85 | 83 |
| Intake | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

| Test Solutions (% Effluent) | Mean Dry Weight (mg) (replicate number) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Mean |
| Control | 0.688† | 0.580 | 0.620 | 0.676 | 0.641 |
| 10.98% | 0.626 | 0.625 | 0.591 | 0.671 | 0.628 |
| 22% | 0.624 | 0.703 | 0.707 | 0.633 | 0.667 |
| 43.9% | 0.560 | 0.568 | 0.606 | 0.648 | 0.596 |
| 72% | 0.627 | 0.561 | 0.652 | 0.677 | 0.629 |
| 100% | 0.181 | 0.693 | 0.665 | 0.560 | 0.525 |
| Intake | 0.508 | 0.580 | 0.718 | 0.681 | 0.622 |

| | |
|---|---|
| $IC_{25}$ Value: ≥ 100%<br>Permit Limit: 43.9<br><br>95% Confidence Limits: NA<br>Upper Limit: NA<br>Lower Limit: NA | Calculated TU Estimates: < 1.0 TUc*<br><br>Permit Limit: 2.3 TUc |

*TUa = 100/$LC_{50}$: TUc = 100/ $IC_{25}$

†One animal missing. Replicate was initiated with 9 animals for statistical analysis.

TOXICITY TEST RESULTS (see Appendix C for Bench Sheets)

2. Results of a *Ceriodaphnia dubia* (Genus species) Chronic 7-day Toxicity Test. (Type / Duration)
Conducted January 14 - 21, 2003 using effluent from Outfall 101.

| Test Solutions (% Effluent) | Percent Surviving (time interval used – days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10.98% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 22% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 43.9% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 72% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 100% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Intake | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

| Test Solutions (% Effluent) | Reproduction (#young/female/7 days) Data (replicate number) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Mean |
| Control | 29 | 35 | 32 | 36 | 34 | 36 | 38 | 35 | 32 | 34 | 34.1 |
| 10.98% | 36 | 36 | 33 | 37 | 35 | 34 | 42 | 34 | 39 | 37 | 36.3 |
| 22% | 33 | 40 | 40 | 39 | 38 | 36 | 39 | 39 | 35 | 32 | 37.1 |
| 43.9% | 41 | 37 | 34 | 41 | 40 | 40 | 38 | 39 | 33 | 36 | 37.9 |
| 72% | 40 | 38 | 44 | 39 | 36 | 37 | 40 | 42 | 45 | 40 | 40.1 |
| 100% | 42 | 42 | 40 | 38 | 40 | 38 | 39 | 37 | 43 | 39 | 39.8 |
| Intake | 37 | 34 | 40 | 40 | 40 | 34 | 34 | 37 | 37 | 39 | 37.2 |

| | |
|---|---|
| $IC_{25}$ Value: > 100%<br>Permit Limit: 43.9<br><br>95% Confidence Limits: NA<br>Upper Limit: NA<br>Lower Limit: NA | Calculated TU Estimates: < 1.0 TUc*<br><br>Permit Limit: 2.3 TUc |

*TUa = $100/LC_{50}$: TUc = $100/IC_{25}$

TOXICITY TEST RESULTS, UV-TREATED (see Appendix C for Bench Sheets)

3. Results of a *Pimephales promelas* Chronic 7-day Toxicity Test.
(Genus species) (Type / Duration)

Conducted January 14 - 21, 2003 using effluent from UV Treated Outfall 101.

| Test Solutions (% Effluent) | Percent Surviving (time interval used – days) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 10.98% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 22% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 43.9% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 72% | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 100% | 100 | 100 | 100 | 100 | 100 | 98 | 98 |
| Intake | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

| Test Solutions (% Effluent) | Mean Dry Weight (mg) (replicate number) | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | Mean |
| Control | 0.532 | 0.686 | 0.720 | 0.603 | 0.635 |
| 10.98% | 0.759 | 0.626 | 0.689 | 0.644 | 0.680 |
| 22% | 0.664 | 0.650 | 0.653 | 0.645 | 0.653 |
| 43.9% | 0.702 | 0.576 | 0.556 | 0.634 | 0.617 |
| 72% | 0.741 | 0.608 | 0.661 | 0.660 | 0.668 |
| 100% | 0.624 | 0.706 | 0.686 | 0.619 | 0.659 |
| Intake | 0.699 | 0.632 | 0.652 | 0.669 | 0.663 |

| | |
|---|---|
| $IC_{25}$ Value: > 100%<br>Permit Limit: 43.9<br><br>95% Confidence Limits: NA<br>Upper Limit: NA<br>Lower Limit: NA | Calculated TU Estimates: < 1.0 TUc*<br><br>Permit Limit: 2.3 TUc |

*TUa = 100/$LC_{50}$: TUc = 100/ $IC_{25}$

Reference Toxicant Test Results (see Appendix A and D)

| Species | Date | Time | Duration | Toxicant | Results ($IC_{25}$) |
|---|---|---|---|---|---|
| ***Pimephales promelas*** | January 14, 2003 | 1400 | 7-days | KCl | 607.9 mg/L |
| ***Ceriodaphnia dubia*** | January 7, 2003 | 1200 | 7-days | NaCl | 962.4 mg/L |

PHYSICAL/CHEMICAL SUMMARY

Water Chemistry Mean Values and Ranges for *Pimephales promelas* and *Ceriodaphnia dubia* Tests, Sequoyah Nuclear Plant Effluent (SQN), Outfall 101, January 14-21, 2003.

| Test | Sample ID | Temperature (°C) | | Dissolved Oxygen (mg/L) | | pH (S.U.) | | Conductance | Alkalinity | Hardness | Total Residual |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | (µmhos/cm) | (mg/L $CaCO_3$) | (mg/L $CaCO_3$) | Chlorine (mg/L) |
| *Pimephales promelas* | Control | 24.5<br>24.4 - 24.7 | 24.9<br>24.6 - 25.3 | 7.8<br>7.6 - 8.0 | 7.4<br>6.4 - 8.1 | 8.13<br>8.06 - 8.19 | 7.84<br>7.62 - 8.11 | 295<br>278 - 308 | 62<br>60 - 64 | 85<br>84 - 86 | -<br>- - |
| | 10.98% | 24.6<br>24.4 - 24.8 | 24.9<br>24.6 - 25.3 | 7.9<br>7.5 - 8.5 | 7.2<br>6.2 - 8.0 | 8.15<br>8.05 - 8.22 | 7.81<br>7.58 - 8.03 | 289<br>278 - 298 | -<br>- - | -<br>- - | -<br>- - |
| | 22% | 24.6<br>24.4 - 24.8 | 24.9<br>24.6 - 25.4 | 8.0<br>7.5 - 8.5 | 7.2<br>6.3 - 8.1 | 8.15<br>8.07 - 8.20 | 7.81<br>7.61 - 8.08 | 279<br>266 - 288 | -<br>- - | -<br>- - | -<br>- - |
| | 43.9% | 24.6<br>24.4 - 24.8 | 24.9<br>24.6 - 25.4 | 8.1<br>7.5 - 8.6 | 7.4<br>6.3 - 8.2 | 8.14<br>8.07 - 8.19 | 7.84<br>7.64 - 8.09 | 259<br>246 - 266 | -<br>- - | -<br>- - | -<br>- - |
| | 72% | 24.7<br>24.4 - 25.0 | 24.9<br>24.6 - 25.4 | 8.2<br>7.9 - 8.6 | 7.4<br>6.5 - 8.1 | 8.14<br>8.08 - 8.19 | 7.86<br>7.67 - 8.16 | 231<br>220 - 240 | -<br>- - | -<br>- - | -<br>- - |
| | 100% | 24.7<br>24.4 - 25.2 | 24.9<br>24.6 - 25.4 | 8.3<br>7.8 - 8.6 | 7.5<br>6.8 - 8.0 | 8.14<br>8.07 - 8.18 | 7.93<br>7.75 - 8.22 | 203<br>196 - 213 | 71<br>68 - 73 | 88<br>86 - 89 | <0.10<br><0.10 - <0.10 |
| | Intake | 24.6<br>24.4 - 24.9 | 24.9<br>24.6 - 25.4 | 8.5<br>8.2 - 8.8 | 7.6<br>6.9 - 8.3 | 8.12<br>8.01 - 8.19 | 7.91<br>7.72 - 8.18 | 203<br>193 - 214 | 72<br>70 - 74 | 81<br>80 - 82 | <0.10<br><0.10 - <0.10 |
| *Ceriodaphnia dubia* | Control | 24.5<br>24.3 - 24.7 | 25.0<br>24.8 - 25.3 | 7.8<br>7.6 - 8.0 | 8.1<br>7.8 - 8.3 | 8.13<br>8.06 - 8.19 | 8.18<br>8.14 - 8.23 | 295<br>278 - 308 | 62<br>60 - 64 | 85<br>84 - 86 | -<br>- - |
| | 10.98% | 24.6<br>24.5 - 24.6 | 25.0<br>24.8 - 25.3 | 7.9<br>7.5 - 8.5 | 8.1<br>7.9 - 8.3 | 8.15<br>8.05 - 8.22 | 8.19<br>8.16 - 8.23 | 289<br>278 - 298 | -<br>- - | -<br>- - | -<br>- - |
| | 22% | 24.6<br>24.5 - 24.7 | 25.0<br>24.8 - 25.3 | 8.0<br>7.5 - 8.5 | 8.2<br>8.0 - 8.3 | 8.15<br>8.07 - 8.20 | 8.21<br>8.16 - 8.26 | 279<br>266 - 288 | -<br>- - | -<br>- - | -<br>- - |
| | 43.9% | 24.7<br>24.5 - 24.9 | 25.0<br>24.8 - 25.4 | 8.1<br>7.5 - 8.6 | 8.2<br>8.0 - 8.3 | 8.14<br>8.07 - 8.19 | 8.21<br>8.16 - 8.24 | 258<br>246 - 266 | -<br>- - | -<br>- - | -<br>- - |
| | 72% | 24.8<br>24.5 - 25.1 | 25.0<br>24.8 - 25.3 | 8.2<br>7.9 - 8.6 | 8.2<br>8.0 - 8.3 | 8.14<br>8.08 - 8.19 | 8.26<br>8.21 - 8.31 | 231<br>220 - 240 | -<br>- - | -<br>- - | -<br>- - |
| | 100% | 24.9<br>24.4 - 25.6 | 25.0<br>24.8 - 25.3 | 8.3<br>7.8 - 8.6 | 8.2<br>7.8 - 8.4 | 8.14<br>8.07 - 8.18 | 8.28<br>8.22 - 8.30 | 203<br>196 - 213 | 71<br>68 - 73 | 88<br>86 - 89 | <0.10<br><0.10 - <0.10 |
| | Intake | 24.7<br>24.4 - 24.9 | 25.0<br>24.8 - 25.3 | 8.5<br>8.2 - 8.8 | 8.1<br>8.0 - 8.3 | 8.12<br>8.01 - 8.19 | 8.26<br>8.22 - 8.30 | 203<br>193 - 214 | 72<br>70 - 74 | 81<br>80 - 82 | <0.10<br><0.10 - <0.10 |

PHYSICAL/CHEMICAL SUMMARY

Water Chemistry Mean Values and Ranges for the *Pimephales promelas* Test, Sequoyah Nuclear Plant Effluent (SQN), UV Treated Outfall 101, January 14-21, 2003.

| Test | Sample ID | Temperature (°C) | | Dissolved Oxygen (mg/L) | | pH (S.U.) | | Conductance | Alkalinity | Hardness | Total Residual |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | (µmhos/cm) | (mg/L $CaCO_3$) | (mg/L $CaCO_3$) | Chlorine (mg/L) |
| *Pimephales promelas* | Control | 24 5<br>24 3 - 24 8 | 24.8<br>24 5 - 25 1 | 8.3<br>8 1 - 8 6 | 7.7<br>6 9 - 8 4 | 8.11<br>8.03 - 8 17 | 7 87<br>7 66 - 8 08 | 299<br>286 - 309 | 62<br>60 - 64 | 85<br>84 - 86 | |
| | 10.98% | 24 6<br>24 4 - 24 8 | 24 8<br>24 5 - 25 1 | 8.3<br>8 1 - 8 6 | 7 4<br>6 5 - 8 2 | 8.11<br>8 02 - 8 18 | 7 85<br>7 63 - 8 07 | 293<br>283 - 300 | | | |
| | 22% | 24 6<br>24 3 - 24 8 | 24 8<br>24 4 - 25.1 | 8 2<br>7.9 - 8 6 | 7 5<br>6 8 - 8 2 | 8.10<br>8 04 - 8 18 | 7.86<br>7 66 - 8 07 | 281<br>273 - 286 | | | |
| | 43.9% | 24 6<br>24 4 - 24 9 | 24.8<br>24 4 - 25 1 | 8 2<br>7 8 - 8 5 | 7 6<br>6 9 - 8.3 | 8.10<br>8 04 - 8 18 | 7.87<br>7 66 - 8 07 | 260<br>254 - 264 | | | |
| | 72% | 24 7<br>24.5 - 25.1 | 24 8<br>24 4 - 25 1 | 8.2<br>7 8 - 8 5 | 7 6<br>6 9 - 8 3 | 8.11<br>8 05 - 8.18 | 7 87<br>7.67 - 8 09 | 231<br>226 - 235 | | | |
| | 100% | 24.7<br>24 5 - 25 3 | 24 8<br>24 4 - 25 1 | 8 1<br>7 8 - 8 5 | 7 6<br>6 9 - 8.4 | 8 11<br>8 04 - 8 18 | 7.90<br>7 70 - 8 13 | 203<br>198 - 209 | 71<br>68 - 73 | 88<br>86 - 89 | < 0.10<br>< 0 10 - < 0 10 |
| | Intake | 24 6<br>24 4 - 24 9 | 24 8<br>24 4 - 25.1 | 8 2<br>7 8 - 8 6 | 7.7<br>6 9 - 8 6 | 8.10<br>8 03 - 8 18 | 7.91<br>7 71 - 8 10 | 202<br>197 - 207 | 72<br>70 - 74 | 81<br>80 - 82 | < 0.10<br>< 0 10 - < 0.10 |

## SUMMARY / CONCLUSIONS

Outfall 101, samples collected January 12-17, showed no toxic effects to fathead minnows or daphnids. The resulting $IC_{25}$ values for both species were > 100 percent. Exposure of fathead minnows and daphnids to intake samples resulted in no significant differences from controls during this study period.

In addition to the routine compliance test, fathead minnows were also tested in Outfall 101 and intake samples which were treated using UV exposure for pathogen removal prior to introduction of test organisms. Fish pathogens present in intake water have been the suspected cause of anomalous dose responses and high variability among replicates in previous toxicity testing at Sequoyah. At the time this study was conducted, mortality which occurred in minnows exposed to routine compliance samples and UV treated samples was not sufficient to jeopardize statistical validity, although survival was improved in the UV treated test.

## Appendix A

## ADDITIONAL TOXICITY TEST INFORMATION

### SUMMARY OF METHODS

1. *Pimephales promelas*

   Tests were conducted according to EPA-821-R-02-013 (October 2002) using four replicates, each containing ten test organisms, per treatment. Test vessels consisted of 400-mL polypropylene beakers, each containing 250-mL of test solution.

2. *Ceriodaphnia dubia*

   Tests were conducted according to EPA-821-R-02-013 (October 2002) using ten replicates, each containing one test organism, per treatment. Test vessels consisted of 30-mL polypropylene cups, each containing 15-mL of test solution.

### DEVIATIONS / MODIFICATIONS TO TEST PROTOCOL

1. *Pimephales promelas*

   None

2. *Ceriodaphnia dubia*

   None

### DEVIATIONS / MODIFICATIONS TO PRETEST CULTURE OR HOLDING OF TEST ORGANISMS

1. *Pimephales promelas*

   None

2. *Ceriodaphnia dubia*

   None

## PHYSICAL AND CHEMICAL METHODS

1. Regents, Titrants, Buffers, etc.: All chemicals were certified products used before expiration dates (where applicable).

2. Instruments: All identification, service, and calibration information pertaining to laboratory instruments is recorded in calibration and maintenance logbooks.

3. Temperature was measured using EPA Method 170.1.

4. Dissolved oxygen was measured using EPA Method 360.1.

5. The pH was measured EPA Method 150.1.

6. Conductance was measured EPA Method 120.1.

7. Alkalinity was measured using EPA Method 310.1.

8. Total Hardness was measured EPA Method 130.2.

9. Total residual chlorine was measured using EPA Method 330.5.

## QUALITY ASSURANCE

Toxicity Test Methods: All phases of the study including, but not limited to, sample collection, handling and storage, glassware preparation, test organism culturing/acquisition and acclimation, test organism handling during test, and maintaining appropriate test conditions were conducted according to the protocol as described in this report and EPA-821-R-02-013. Any known deviations were noted during the study and are reported herein.

## REFERENCE TOXICANT TESTS (See Appendix D for control chart information)

1. Test Type: 7-day chronic tests with results expressed as $IC_{25}$ values in g KCl or NaCl.

2. Standard Toxicant: Potassium Chloride (KCl crystalline) for *Pimephales promelas*.
   Sodium Chloride (NaCl crystalline) for *Ceriodaphnia dubia*.

3. Dilution Water Used: Moderately hard synthetic water.

4. Statistics: ToxCalc software Version 5.0 was used for statistical analyses.

## REFERENCES


1. NPDES Permit No. TN 0026450

2. USEPA. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013 (October 2002).

3. Methods for Chemical Analysis of Water and Wastes, EPA/600/4-79/020 (March 1983).

Sequoyah Nuclear Plant Biomonitoring
January 14 - 21, 2003

Appendix B

Diffuser Discharge Concentrations of Total Residual Chlorine,

Diffuser Discharge Concentrations of Chemicals Used to Control Growth of Microbiologically Induced Bacteria and Asiatic Clams, During Toxicity Test Sampling,

Table B-1. Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of Chemicals Used to Control Growth of Microbiologically Induced Bacteria and Asiatic Clams, During Toxicity Test Sampling, March 12, 1998-January 17, 2003

| Date | Sodium hypochlorite mg/L TRC | Towerbrom mg/L TRC | PCL-222 mg/L Phosphate | PCL-401 mg/L Copolymer | CL-363 mg/L DMAD | Cuprostat-PF mg/L Azole | H-130M mg/L Quat |
|---|---|---|---|---|---|---|---|
| 03/12/1998 | 0.016 | - | - | - | - | - | - |
| 03/13/1998 | 0.015 | - | - | - | - | - | - |
| 03/14/1998 | 0.013 | - | - | - | - | - | - |
| 03/15/1998 | 0.030 | - | - | - | - | - | - |
| 03/16/1998 | 0.013 | - | - | - | - | - | - |
| 03/17/1998 | 0.020 | - | - | - | - | - | - |
| 03/18/1998 | 0.018 | - | - | - | - | - | - |
| 09/08/1998 | 0.015 | - | 0.014 | 0.005 | - | - | 0.021 |
| 09/09/1998 | 0.003 | - | 0.031 | 0.011 | - | - | - |
| 09/10/1998 | 0.014 | - | 0.060 | 0.021 | - | - | - |
| 09/11/1998 | 0.013 | - | 0.055 | 0.019 | - | - | - |
| 09/12/1998 | < 0.001 | - | 0.044 | 0.015 | - | - | - |
| 09/13/1998 | < 0.001 | - | 0.044 | 0.015 | - | - | - |
| 09/14/1998 | 0.008 | - | 0.044 | 0.015 | - | - | - |
| 02/22/1999 | < 0.001 | - | - | - | - | - | - |
| 02/23/1999 | 0.005 | - | - | - | - | - | - |
| 02/24/1999 | 0.009 | - | - | - | - | - | - |
| 02/25/1999 | 0.012 | - | - | - | - | - | - |
| 02/26/1999 | 0.008 | - | - | - | - | - | - |
| 02/27/1999 | < 0.001 | - | - | - | - | - | - |
| 02/28/1999 | < 0.001 | - | - | - | - | - | - |
| 08/18/1999 | - | 0.015 | 0.069 | 0.024 | 0.006 | - | - |
| 08/19/1999 | - | 0.012 | 0.068 | 0.024 | - | - | - |
| 08/20/1999 | - | 0 023 | 0.070 | 0.024 | - | 0.120 | - |
| 08/21/1999 | - | 0.022 | 0.068 | 0.024 | - | - | - |
| 08/22/1999 | - | 0.022 | 0.068 | 0.024 | - | - | - |
| 08/23/1999 | - | 0.025 | 0.068 | 0.024 | 0.006 | - | - |
| 08/24/1999 | - | 0.016 | 0.067 | 0.023 | 0.020 | - | - |

Table B-1 (continued). Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of Chemicals Used to Control Growth of Microbiologically Induced Bacteria and Asiatic Clams, During Toxicity Test Sampling, March 12, 1998-January 17, 2003

| Date | Sodium hypochlorite mg/L TRC | Towerbrom mg/L TRC | PCL-222 mg/L Phosphate | PCL-401 mg/L Copolymer | CL-363 mg/L DMAD | Cuprostat-PF mg/L Azole | H-130M mg/L Quat |
|---|---|---|---|---|---|---|---|
| 01/31/2000 | - | < 0.002 | 0.026 | 0.009 | - | - | - |
| 02/01/2000 | - | 0.011 | 0.026 | 0.028 | - | - | - |
| 02/02/2000 | - | 0.028 | 0.026 | 0.009 | 0.006 | - | - |
| 02/03/2000 | - | 0.008 | 0.027 | 0.009 | - | - | - |
| 02/04/2000 | - | 0.006 | 0.027 | 0.009 | 0.005 | 0.109 | - |
| 02/05/2000 | - | < 0.002 | 0.027 | 0.009 | - | - | - |
| 02/06/2000 | - | < 0.002 | 0.027 | 0.009 | - | - | - |
| 07/26/2000 | - | < 0.0057 | 0.055 | 0.019 | - | - | 0.021 |
| 07/27/2000 | - | 0.019 | 0.055 | 0.019 | - | - | - |
| 07/28/2000 | - | 0.0088 | 0.053 | 0.018 | 0.004 | 0.108 | - |
| 07/29/2000 | - | < 0.0088 | 0.055 | 0.019 | - | - | - |
| 07/30/2000 | - | < 0.0076 | 0.055 | 0.019 | - | - | - |
| 07/31/2000 | - | < 0.0152 | 0.055 | 0.019 | 0.006 | - | - |
| 08/01/2000 | - | < 0.0141 | 0.055 | 0.019 | 0.005 | - | - |
| 12/11/2000 | - | 0.0143 | 0.025 | 0.020 | 0.005 | - | - |
| 12/12/2000 | - | 0.0092 | 0.025 | 0.020 | 0.005 | - | - |
| 12/13/2000 | - | < 0.0120 | 0.025 | 0.020 | - | - | - |
| 12/14/2000 | - | < 0.0087 | 0.025 | 0.020 | - | - | - |
| 12/15/2000 | - | 0.0120 | 0.025 | 0.020 | 0.005 | - | - |
| 12/16/2000 | - | < 0.0036 | 0.025 | 0.020 | - | - | - |
| 12/17/2000 | - | < 0.0036 | 0.025 | 0.020 | - | - | - |
| 08/26/2001 | - | 0.017 | 0.06 | 0.021 | 0.006 | - | - |
| 08/27/2001 | - | <0.0096 | 0.06 | 0.021 | 0.005 | - | 0.021 |
| 08/28/2001 | - | <0.0085 | 0.06 | 0.021 | - | - | - |
| 08/29/2001 | - | <0.0094 | 0.059 | 0.020 | 0.005 | - | 0.021 |
| 08/30/2001 | - | <0.0123 | 0.06 | 0.021 | 0.005 | - | - |
| 08/31/2001 | - | <0.005 | 0.059 | 0.020 | - | - | - |
| 11/25/2001 | - | <0.0044 | - | - | - | - | - |
| 11/26/2001 | - | <0.0119 | 0.024 | 0.02 | 0.005 | - | - |
| 11/27/2001 | - | 0.0137 | 0.023 | 0.019 | 0.007 | - | - |
| 11/28/2001 | - | <0.0089 | 0.022 | 0.019 | 0.006 | - | - |
| 11/29/2001 | - | 0.0132 | 0.024 | 0.02 | 0.007 | - | - |
| 11/30/2001 | - | < 0.0043 | 0.024 | 0.02 | - | - | - |
| 12/09/2001 | - | <0.0042 | - | - | - | - | - |
| 12/10/2001 | - | <0.0042 | - | - | - | - | - |
| 12/11/2001 | - | <0.0104 | - | - | - | - | - |
| 12/12/2001 | - | 0.0128 | 0.024 | 0.02 | 0.008 | - | - |
| 12/13/2001 | - | <0.0088 | 0.024 | 0.02 | - | - | - |
| 12/14/2001 | - | 0.0134 | 0.024 | 0.02 | 0.007 | - | - |

Table B-1 (continued). Sequoyah Nuclear Plant Diffuser (Outfall 101) Discharge Concentrations of Chemicals Used to Control Growth of Microbiologically Induced Bacteria and Asiatic Clams, During Toxicity Test Sampling, March 12, 1998-January 17, 2003

| Date | Sodium hypochlorite mg/L TRC | Towerbrom mg/L TRC | PCL-222 mg/L Phosphate | PCL-401 mg/L Copolymer | CL-363 mg/L DMAD | Cuprostat-PF mg/L Azole | H-130M mg/L Quat |
|---|---|---|---|---|---|---|---|
| 01/02/2002 | - | < 0.0079 | 0.023 | 0.02 | 0.006 | - | - |
| 01/03/2002 | - | < 0.0042 | 0.023 | 0.014 | - | - | - |
| 01/04/2002 | - | 0.0124 | 0.024 | 0.014 | 0.009 | - | - |
| 01/05/2002 | - | < 0.0042 | - | - | - | - | - |
| 01/06/2002 | - | < 0.0042 | - | - | - | - | - |
| 01/07/2002 | - | < 0.0089 | 0.024 | 0.014 | 0.006 | - | - |
| 02/24/2002 | - | < 0.004 | - | - | - | - | - |
| 02/25/2002 | - | < 0.004 | 0.023 | 0.023 | - | - | - |
| 02/26/2002 | - | 0.0143 | 0.023 | 0.023 | 0.007 | - | - |
| 02/27/2002 | - | < 0.0041 | 0.023 | 0.023 | - | - | - |
| 02/28/2002 | - | < 0.0041 | 0.024 | 0.008 | - | - | - |
| 03/01/2002 | - | <0.0041 | 0.024 | 0.008 | - | - | - |
| 05/05/2002 | - | | - | - | - | - | - |
| 05/06/2002 | - | | 0.058 | 0.02 | 0.014 | - | - |
| 05/07/2002 | - | | 0.058 | 0.02 | 0.015 | - | - |
| 05/08/2002 | - | | 0.056 | 0.019 | - | - | - |
| 05/09/2002 | - | | 0.057 | 0.02 | 0.014 | - | - |
| 05/10/2002 | - | | 0.056 | 0.019 | - | - | - |
| 08/04/2002 | | <0.0058 | | | | | |
| 08/05/2002 | | <0.0058 | 0.053 | 0.018 | | | 0.025 |
| 08/06/2002 | | 0.0092 | 0.053 | 0.018 | | | |
| 08/07/2002 | | <0.0107 | 0.055 | 0.019 | 0.007 | | |
| 08/08/2002 | | <0.0061 | 0.055 | 0.019 | | | |
| 08/09/2002 | | 0.0152 | 0.054 | 0.018 | 0.008 | | |
| 10/06/2002 | - | <0.00497 | - | - | - | - | - |
| 10/07/2002 | - | 0.0153 | 0.054 | 0.018 | 0.009 | - | |
| 10/08/2002 | - | <0.0092 | 0.054 | 0.018 | 0.007 | - | - |
| 10/09/2002 | - | 0.0124 | 0.053 | 0.018 | 0.009 | - | - |
| 10/10/2002 | - | 0.0134 | 0.054 | 0 018 | 0.009 | - | - |
| 10/11/2002 | - | <0.0042 | 0.054 | 0 018 | - | - | - |
| 01/12/2003 | - | <0.0035 | - | - | - | - | - |
| 01/13/2003 | - | <0.006 | 0.025 | 0.019 | 0.009 | - | |
| 01/14/2003 | - | <0.0118 | 0.026 | 0.020 | | - | - |
| 01/15/2003 | - | <0.0063 | 0.026 | 0.020 | 0.009 | - | - |
| 01/16/2003 | - | <0.0034 | 0.026 | 0 020 | | - | - |
| 01/17/2003 | - | <0.0034 | 0.026 | 0.009 | - | - | - |

Sequoyah Nuclear Plant Biomonitoring
January 14 - 21, 2003

Appendix C

Chain of Custody Records and
Toxicity Test Bench Sheets

# BIOMONITORING CHAIN OF CUSTODY RECORD



Client: TVA

Project Name: TVA SQN

P O. Number. PO BOX 2000

Facility Sampled: SEQUOYAH NUCLEAR PLANT

NPDES Number: 0026450

Collected By: WANDA ALLEN

**Environmental Testing Solutions, LLC**

**Research Building**

351 DEPOT STREET
ASHEVILLE. NC 28801

**Phone:** 828-350-9364

**Fax:** 256-386-2963

Delivered By (Circle One):

FedEx UPS Bus Client

Other (specify):_ _____

General Comments:

* Custody seals intact. Samples received in good condition
JL

| Field Identification / Sample Description | Grab/ Comp. | Ship. Temp ( °C) | Collection Date/Time | | Container Number & Volume Collected | Flow (MGD) | Rain Event? (Mark as Appropriate) | | | | Laboratory Use Project # 571 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Start | End | | | Y- | If Yes, Inches | No | Trace | EST Log Number | Arrival Temp. ( °C) | By | Time | Appear-ance |
| SQN-101-TOX | comp | 4 | 1350 1/12/03 | 1/13/03 1250 | 2(2.5g) | | | | ✓ | | 030114.01 | 0.7°C | KEK | 0941 * | see comments |
| SQN-INT-TOX | comp | 4 | 1329 1/12/03 | 1/13/03 1229 | 1 (2 5g) | | | | ✓ | | 030114.02 | 0.7°C | KEK | 0941 | ↓ |
| | | | | | | | | | | | | | | | JL |

Sample Custody – Fill In From Top Down

| Relinquished By (Signature): | Date/Time | Received By (Signature): | Date/Time |
|---|---|---|---|
| Wanda Allen | 1/13/03 1500 | Fedex | 01-13-03 1500 |
| Fedex | 01-14-03 0941 | KEKeenan | 01-14-03 0941 |
| | | JL | |

Instructions· Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 4°C and shipping them in ice. The hold time for each sample is 36 hours from the time of collection. Therefore, please collect and ship in such a way that the laboratory will receive the samples with ample time to initiate testing within that time frame. Samples shipped overnight on Friday via FedEx or UPS must be marked for Saturday delivery or they will not arrive until the following Monday.

# BIOMONITORING CHAIN OF CUSTODY RECORD



Client: TVA

Project Name: TVA SQN

P O. Number: PO BOX 2000

Facility Sampled: SEQUOYAH NUCLEAR PLANT

NPDES Number: 0026450

Collected By: WANDA ALLEN

**Environmental Testing Solutions, LLC**

**Research Building**

351 DEPOT STREET
ASHEVILLE. NC 28801

**Phone:** 828-350-9364

**Fax:** 256-386-2963

Delivered By (Circle One):

FedEx UPS Bus Client

Other (specify):_____

General Comments:

*Samples received in good condition
Custody seals intact.
JHumber

| Field Identification / Sample Description | Grab/ Comp. | Ship Temp. ( °C) | Collection Date/Time | | Container Number & Volume Collected | Flow (MGD) | Rain Event? (Mark as Appropriate) | | | | Laboratory Use PROJECT 571 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Start | End | | | Y- | If Yes, Inches | No | Trace | EST Log Number | Arrival Temp. ( °C) | By | Time | Appear-ance |
| SQN-101-TOX | comp | 4 | 1254 1/14/03 | 1/15/03 1154 | 2(2.5g) | | | | ✓ | | 030116.01 | 0.6°C | JH | 0956 | *See COMMENTS |
| SQN-INT-TOX | comp | 4 | 1248 1/14/03 | 1/15/03 1148 | 1 (2.5g) | | | | ✓ | | 030116.02 | 0.3°C | JH | 0956 | ↓ |
| | | | | | | | | | | | | | | | JH |

Sample Custody – Fill In From Top Down

| Relinquished By (Signature): | Date/Time | Received By (Signature): | Date/Time |
|---|---|---|---|
| Wanda Allen | 1/15/03 1600 | Fedex | 01-15-03 1600 ~~01-16-03 0956~~ JH |
| JS Fedex | 01-16-03 0956 | JHumber | 01-16-03 0956 |
| | | JH | |
| | | | |

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 4°C and shipping them in ice. The hold time for each sample is 36 hours from the time of collection. Therefore, please collect and ship in such a way that the laboratory will receive the samples with ample time to initiate testing within that time frame. Samples shipped overnight on Friday via FedEx or UPS must be marked for Saturday delivery or they will not arrive until the following Monday.

# BIOMONITORING CHAIN OF CUSTODY RECORD



Client: TVA

Project Name: TVA SQN

P.O. Number: PO BOX 2000

Facility Sampled: SEQUOYAH NUCLEAR PLANT

NPDES Number: 0026450

Collected By: WANDA ALLEN

**Environmental Testing Solutions, LLC**

**Research Building**

351 DEPOT STREET
ASHEVILLE, NC 28801

**Phone:** 828-350-9364

**Fax:** 256-386-2963

Delivered By (Circle One):

FedEx UPS Bus Client

Other (specify):_____

General Comments:

* Custody seals intact. Samples received in good condition. JL

| Field Identification / Sample Description | Grab/ Comp. | Ship Temp (°C) | Collection Date/Time | | Container Number & Volume Collected | Flow (MGD) | Rain Event? (Mark as Appropriate) | | | | Laboratory Use | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Start | End | ---- | ---- | Y- | If Yes, Inches | No | Trace | EST Log Number | Arrival Temp (°C) | By | Time | Appearance |
| SQN-101-TOX | comp | 4 | ~~1257~~ 1134 1/16/03 | 1/17/03 ~~1135~~ 1201 | 2(2 5g) | | | | ✓ | | 030118.01 | 0.7°C | JL | 1000 | *See COMMENTS |
| SQN-INT-TOX | comp | 4 | 1254 1/16/03 | 1/17/03 1154 | 1 (2 5g) | | | | ✓ | | 030118.02 | 0.2°C | JL | 1000 | ↓ |
| | | | | | | | | | | | | | | | JL |

Project #571

Sample Custody – Fill In From Top Down

| Relinquished By (Signature). | Date/Time | Received By (Signature): | Date/Time |
|---|---|---|---|
| Wanda Allen | 1/17/03 1600 | Fedex | 01-17-03 1600 |
| Fedex | 01-18-03 1000 | J/Lumner ETS | 01-18-03 1000 |
| | | JL | |

Instructions: Clients should fill in all areas except those in the "Laboratory Use" block. Biomonitoring samples are preserved by storing them at 4°C and shipping them in ice The hold time for each sample is 36 hours from the time of collection. Therefore, please collect and ship in such a way that the laboratory will receive the samples with ample time to initiate testing within that time frame Samples shipped overnight on Friday via FedEx or UPS must be marked for Saturday delivery or they will not arrive until the following Monday

# Sample Receipt Log

| Date Received | Time Received | Received By | Received From | Sample Temperature (°C) | Project Number | Sample Number | Sample Name and Description | State | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 01-09-03 | 1045 | Jumper | Fedex | 0.1°C | 570 | 030109.12 | TVA WCF Acutes Phase III Test #9 FAP | | |
| 01-09-03 | 1045 | Jumper | Fedex | 0.1°C | 570 | 030109.13 | Test # 10 FGD | | |
| 01-09-03 | 1045 | Jumper | Fedex | 0.1°C | 570 | 030109.14 | Test # 11 FAP | | |
| 01-09-03 | 1048 | Jumper | Fedex | 0.5°C | 560 | 030109.15 | Anderson County Sewer Authority | SC | |
| 01-10-03 | 1056 | Jumper | K. Deaver | 1.4°C | 563 | 030110.01 | RFS Ecusta, Inc | NC | |
| 01-10-03 | 1130 | Jumper | L. Carreiro | 3.3°C | 562 | 030110.02 | City of Asheville North Fork WTP | NC | |
| 01-10-03 | 1613 | Jumper | M. Ladd | 3.0°C | 564 | 030110.03 | Test America - Earth Env. Haggie Valley WWTP | NC | |
| 01-10-03 | 1539 | Jumper | D. Ellerbe | 1.9°C | 565 | 030110.04 | CP&L Asheville Ash Pond | NC | |
| 01-11-03 | 1002 | Jumper | Fedex | 0.4°C | 560 | 030111.01 | Anderson County Sewer Auth. | SC | |
| 01-11-03 | 1002 | Jumper | Fedex | 0.6°C | 567 | 030111.02 | Duke Energy Corp. Riverbend SS. | NC | |
| 01-11-03 | 1002 | Jumper | Fedex | 0.2°C | 568 | 030111.03 | Aquasource, Inc. Briarwood Subdivision | NC | |
| 01-11-03 | 1936 | Jumper | Fedex | 0.3°C | 558 | 030111.04 | Tritest Inc. Franklin Co. W&S | NC | |
| 01-14-03 | 0941 | KEKeenan | Fedex | 0.7°C | 571 | 030114.01 | TVA - Sequoyah Nuclear Plant Outfall 101 | TN | |
| 01-14-03 | 0941 | KEKeenan | Fedex | 0.7°C | 571 | 030114.02 | TVA - Sequoyah Nuclear Plant Intake | TN | |
| 01-14-03 | 0943 | KEKeenan | Fedex | 1.2°C | 572 | 030114.03 | Georgetown Steel Co. LLC Scale Pit Grab | SC | |
| 01-14-03 | 0943 | KEKeenan | Fedex | 1.2°C | ~~572~~ 573 | 030114.04 | Scale Pit Composite | SC | |
| 01-15-03 | 1003 | KEKeenan | Fedex | 1.0°C | 574 | 030115.01 | Duke Energy Corp. McGuire Nuclear Station | NC | |
| 01-15-03 | 1003 | KEKeenan | Greyhound | 0.4°C | 575 | 030115.02 | Three R's MHP | NC | |
| 01-15-03 | 1004 | KEKeenan | Fedex | 0.5°C | 576 | 030115.03 | Duke Energy Corp. Allen Steam Station | NC | |
| 01-15-03 | 1026 | KEKeenan | D. Wike | 0.4°C | 577 | 030115.04 | Env. Inc. Bryson City WWTP | NC | |
| 01-16-03 | 0956 | Jumper | Fedex | 0.6°C | 571 | 030116.01 | TVA - Sequoyah Nuclear Plant Outfall 101 | TN | |

# Sample Receipt Log

| Date Received | Time Received | Received By | Received From | Sample Temperature (°C) | Project Number | Sample Number | Sample Name and Description | State | Comments |
|---|---|---|---|---|---|---|---|---|---|
| 01-16-03 | 0956 | Jlumner | Fedex | 0.3°C | 571 | 030116.02 | TVA- SEQUOYAH NUCLEAR PLANT INTAKE | TN | |
| 01-18-03 | 1000 | Jlumner | Fedex | 0.7°C | 571 | 030118.01 | TVA- SEQUOYAH NUCLEAR PLANT OUTFALL 101 | TN | |
| 01-18-03 | 1000 | Jlumner | Fedex | 0.2°C | 571 | 030118.02 | TVA- SEQUOYAH NUCLEAR PLANT INTAKE | TN | |
| 01-18-03 | 1000 | KEKeenan | Fedex | 3.2°C | 574 | 030118.03 | DUKE ENERGY CORP. McGUIRE NUCLEAR STATION | NC | |
| 01-18-03 | 1000 | KEKeenan | Fedex | 0.1°C | 576 | 030118.04 | DUKE ENERGY CORP. ALLEN STEAM STATION | NC | |
| 01-18-03 | 1001 | KEKeenan | Greyhound | 2.4°C | 575 | 030118.05 | THREE R'S HHP | NC | |
| 01-18-03 | 1023 | KEKeenan | A. Wike | 2.4°C | 577 | 030118.06 | ENV. INC. BAYSON CITY WWTP | NC | |
| 01-20-03 | 1000 | Jlumner | UPS | NA | 578 | 030120.01 | COGNIS TEXTILE Co. ADASIL J&S | NC | PRODUCT TEST |
| 01-20-03 | 1000 | Jlumner | UPS | NA | 578 | 030120.02 | ADASIL ESF | NC | |
| 01-20-03 | 1000 | Jlumner | UPS | NA | 578 | 030120.03 | SYNTERGENT APW | NC | |
| 01-22-03 | 0953 | KEKeenan | Fedex | 0.8°C | 579 | 030122.01 | PRISM LABS. SOUTH CENTRAL OIL Co. | NC | |
| 01-~~23~~ 24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.01 | TVA- WCF ACUTES PHASE III TEST 1 - FGD | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.02 | TEST 1 ~~2~~ - FAP | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.03 | TEST 4 | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.04 | TEST 5 #1 | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.05 | TEST 5 #2 | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.06 | TEST 5 #3 | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.07 | TEST 6 | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.08 | TEST 7 | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.09 | TEST 3 - #1 w/o N | | |
| 01-24-03 | 1136 | KEKeenan | Fedex | 0.9°C | 580 | 030124.10 | TEST 3 - #2 w/N | | |

# Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013 Method 1000.0)
## Species: *Pimephales promelas*

Client: TVA
Facility: SEQUOYAH NUCLEAR PLANT
NPDES #: TN 0026450
Project #: 571

NONTREATED

| *Dilution preparation information:* | | | | | | *Comments:* |
|---|---|---|---|---|---|---|
| Dilution prep (%) | 10.98 | 22 | 43.9 | 72 | 100 | |
| Effluent volume (mL) | 164.7 | 330 | 658.2 | 1080 | 1500 | |
| Diluent volume (mL) | 1335.3 | 1170 | 841.5 | 420 | 0 | |
| Total volume (mL) | 1500 | 1500 | 1500 | 1500 | 1500 | |

| *Test organism information:* | | *Test information:* | |
|---|---|---|---|
| Organism age: | 24 HOURS OLD | Randomizing template: | GREEN |
| Date and times organisms were born between: | 01-13-03 1230 TO 1530 MDT | Incubator number: | 2 |
| Organism source: | ABS BATCH 01-13-03 | *Artemia* lot number: | BG 18096 |
| Transfer bowl information: | pH = 7.96 Temperature = 24.2 °C | Total drying time: | 17.25 HOURS |
| | | Date / Time in: | 01-21-03 1450 |
| Average transfer volume. | 10.4 mL | Date / Time out: | 01-22-03 0800 |
| | | Oven temperature: | 101°C |

*Daily feeding and renewal information:*

| Day | Date | Morning feeding time | Afternoon feeding time | Test initiation, renewal, or termination time | Control water batch used | Sample numbers used 101 | INTAKE | Analyst |
|---|---|---|---|---|---|---|---|---|
| 0 | 01-14-03 | — | 1530 | 1450 | MHS 01-12-03 | 030114.01 | 030114.02 | JJ |
| 1 | 01-15-03 | 0914 | 1520 | 1430 | MHS 01-12-03 | 030114.01 | 030114.02 | JJ |
| 2 | 01-16-03 | 0841 | 1450 | 1400 | MHS 01-12-03 | 030116.01 | 030116.02 | JJ |
| 3 | 01-17-03 | 0908 | 1510 | 1356 | MHS 01-15-03 | 030116.01 | 030116.02 | JJ |
| 4 | 01-18-03 | 0910 | 1517 | 1400 | MHS 01-15-03 | 030118.01 | 030118.02 | JJ |
| 5 | 01-19-03 | 0918 | 1520 | 1402 | MHS 01-18-03 | 030118.01 | 030118.02 | JJ |
| 6 | 01-20-03 | 0837 | 1500 | 1400 | MHS 01-18-03 | 030118.01 | 030118.02 | JJ |
| 7 | 01-21-03 | | | 1440 | — | — | | JJ |

| *Control information:* | | Acceptance criteria |
|---|---|---|
| % Mortality: | 0% | ≤ 20% |
| Average weight per initial larvae: | 0.6411 | |
| Average weight per surviving larvae: | 0.6411 | ≥ 0.25 mg/larvae |

| *Summary of test endpoints:* | |
|---|---|
| 7-day $LC_{50}$ | >100% |
| NOEC | 100% |
| LOEC | >100% |
| ChV | >100% |
| $IC_{25}$ | >100% |

**Species:** *Pimephales promelas*

**Client:** SEQUOYAH- NONTREATED **Date:** 01-14-03

*Survival and Growth Data*

| Day | CONTROL | | | | 10.98% | | | | 22% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 id |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 |
| 7 | 9 MISSING | 10 | 10 | 10 | 10 | 10 | 10 | 1 SMALL 10 | 9 id | 10 | 10 | 9 |
| A = Pan weight (mg) Black | 14.777 | 14.647 | 15.049 | 14.832 | 14.908 | 15.076 | 15.097 | 14.990 | 14.854 | 14.881 | 14.855 | 15.089 |
| B = Pan + Larvae weight (mg) | 20.97 | 20.45 | 21.25 | 21.59 | 20.97 | 21.33 | 21.01 | 21.70 | 21.07 | 21.72 | 21.92 | 21.37 |
| Larvae weight (mg) = A – B | 6.193 | 5.803 | 6.201 | 6.758 | 6.262 | 6.254 | 5.913 | 6.710 | 6.236 | 7.029 | 7.065 | 6.331 |

Calculations and data reviewed dl

*Comments:*

**Species:** *Pimephales promelas*

**Client:** SEQUOYAH - NONTREATED

**Date:** 01-14-03

*Survival and Growth Data*

| Day | 43.9% | | | | 72% | | | | 100% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T | *U | V | W | X |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 1d | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 6 3d | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 4 2d | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 4 | 10 | 10 | 10 |
| 7 | 9 1d | 10 | 1 SMALL 10 | 10 | 10 | 10 | 10 | 10 | 3 1d | 10 | 10 | 1 SMALL 10 |
| A = Pan weight (mg) | 15.150 | 14.690 | 14.970 | 14.849 | 14.819 | 15.148 | 14.992 | 14.714 | 15.013 | 14.909 | 15.340 | 14.660 |
| B = Pan + Larvae weight (mg) | 20.75 | 20.37 | 21.04 | 21.35 | 21.09 | 20.76 | 21.51 | 21.48 | 16.82 | *21.92 | 21.99 | 20.27 |
| Larvae weight (mg) = A – B | 5.600 | 5.680 | 6.064 | 6.481 | 6.271 | 5.612 | 6.518 | 6.766 | 1.807 | 6.931 | 6.650 | 5.604 |

Calculations and data reviewed JH

*Comments:* * Pathogenic growth observed. Dead minnows were heavily fungused.

**Species:** *Pimephales promelas*

**Client:** SEQUOYAH - NONTREATED

**Date:** 01-14-03

*Survival and Growth Data*

| Day | INTAKE | | | |
|---|---|---|---|---|
| | Y | Z | AA | BB |
| 0 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 |
| 7 | 1 SMALL 10 | 10 | 10 | 10 |
| A = Pan weight (mg) | 15.017 | 14.685 | 15.129 | 14.716 |
| B = Pan + Larvae weight (mg) | 20.10 | 20.48 | 22.31 | 21.53 |
| Larvae weight (mg) = A – B | 5.083 | 5.795 | 7.181 | 6.814 |

Calculations and data reviewed: JH

| *Comments:* |
|---|
| |
| |
| |
| |
| |

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1000.0)
## Species: *Pimephales promelas*

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

**Client:** TVA Sequoyah Nuclear Plant, Nontreated

**Test dates:** January 14 - 21, 2003

**Project number:** 571

**Reveiwed by:** J Turner

| Concentration (%) | Replicate | Initial number of larvae | Final number of larvae | A = Pan weight (mg) | B = Pan + Larvae weight (mg) | Larvae weight (mg) = A - B | Weight / Initial number of larvae (mg) | Mean survival (%) | Mean weight (mg) | Coefficient of variation (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | A | 9 | 9 | 14 777 | 20 970 | 6 193 | 0,6881 | 100 0 | 0 6411 | 7 8 | Not applicable |
| | B | 10 | 10 | 14 647 | 20 450 | 5 803 | 0 5803 | | | | |
| | C | 10 | 10 | 15 049 | 21 250 | 6 201 | 0 6201 | | | | |
| | D | 10 | 10 | 14 832 | 21 590 | 6 758 | 0 6758 | | | | |
| 10 98% | E | 10 | 10 | 14 708 | 20 970 | 6 262 | 0 6262 | 100 0 | 0 6285 | 5 2 | 2 0 |
| | F | 10 | 10 | 15 076 | 21 330 | 6 254 | 0 6254 | | | | |
| | G | 10 | 10 | 15 097 | 21 010 | 5 913 | 0 5913 | | | | |
| | H | 10 | 10 | 14 990 | 21.700 | 6 710 | 0 6710 | | | | |
| 22% | I | 10 | 9 | 14 834 | 21 070 | 6 236 | 0 6236 | 95 0 | 0 6665 | 6 6 | -4 0 |
| | J | 10 | 10 | 14 691 | 21 720 | 7 029 | 0 7029 | | | | |
| | K | 10 | 10 | 14 855 | 21 920 | 7 065 | 0 7065 | | | | |
| | L | 10 | 9 | 15 039 | 21 370 | 6 331 | 0 6331 | | | | |
| 43 9% | M | 10 | 9 | 15 150 | 20 750 | 5 600 | 0 5600 | 97 5 | 0 5956 | 6 8 | 7.1 |
| | N | 10 | 10 | 14 690 | 20 370 | 5 680 | 0 5680 | | | | |
| | O | 10 | 10 | 14 976 | 21 040 | 6 064 | 0 6064 | | | | |
| | P | 10 | 10 | 14 869 | 21 350 | 6 481 | 0 6481 | | | | |
| 72% | Q | 10 | 10 | 14 819 | 21 090 | 6 271 | 0 6271 | 100 0 | 0 6292 | 7 9 | 1 9 |
| | R | 10 | 10 | 15 148 | 20 760 | 5 612 | 0 5612 | | | | |
| | S | 10 | 10 | 14 992 | 21 510 | 6 518 | 0 6518 | | | | |
| | T | 10 | 10 | 14 714 | 21 480 | 6 766 | 0 6766 | | | | |
| 100% | U | 10 | 3 | 15 013 | 16 820 | 1 807 | 0 1807 | 82 5 | 0.5248 | 45 0 | 18 1 |
| | V | 10 | 10 | 14 989 | 21 920 | 6 931 | 0 6931 | | | | |
| | W | 10 | 10 | 15 340 | 21 990 | 6 650 | 0 6650 | | | | |
| | X | 10 | 10 | 14 666 | 20 270 | 5 604 | 0 5604 | | | | |
| 100% Intake | Y | 10 | 10 | 15 017 | 20 100 | 5 083 | 0 5083 | 100 0 | 0 6218 | 15 4 | 3 0 |
| | Z | 10 | 10 | 14 685 | 20 480 | 5 795 | 0 5795 | | | | |
| | AA | 10 | 10 | 15 129 | 22 310 | 7 181 | 0 7181 | | | | |
| | BB | 10 | 10 | 14 716 | 21 530 | 6 814 | 0 6814 | | | | |

**Outfall 101:**
**Dunnett's MSD value:** 0.1781
**PMSD** 27.8

**Intake:**
**Dunnett's MSD value:** 0 105
**PMSD** 16 4

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test On average, a significant difference occurs for Environmental Testing Solutions, LLC chronic toxicity tests when a toxicant reduces Pimephales growth by 17 9% from the control (determined through reference toxicant testing)
Lower PMSD bound determined by USEPA (10th percentile) = 9 4%.
Upper PMSD bound determined by USEPA (90th percentile) = 35%
The lower and upper bounds were calculated by the USEPA using 205 tests conducted from 19 laboratories for Pimephales growth in chronic reference toxicant tests

USEPA. 2000 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003 US Environmental Protection Agency, Cincinnati, OH.

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Larval Fish Growth and Survival Test-7 Day Growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID: | PpFRCR | Sample ID: | TVA, Sequoyah Nuclear Plant |
| End Date | 1/21/03 | Lab ID: | ETS-Env. Testing Solutions | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date | | Protocol. | EPAF 91-EPA Freshwater | Test Species | PP-Pimephales promelas |
| Comments: | | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 0 6881 | 0 5803 | 0 6201 | 0 6758 |
| 10 98 | 0 6262 | 0 6254 | 0.5913 | 0 6710 |
| 22 | 0 6236 | 0 7029 | 0 7065 | 0 6331 |
| 43.9 | 0 5600 | 0.5680 | 0 6064 | 0.6481 |
| 72 | 0 6271 | 0.5612 | 0 6518 | 0 6766 |
| 100 | 0 1807 | 0.6931 | 0 6650 | 0.5604 |

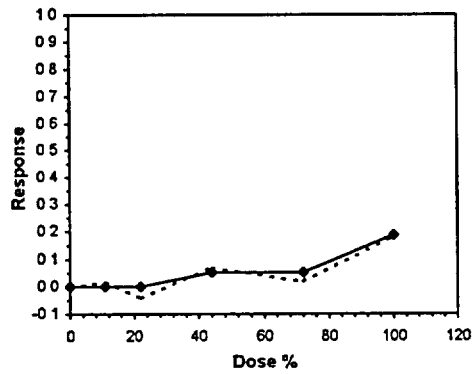
| | | | Transform Untransformed | | | | | Rank | 1-Tailed | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Mean | N-Mean |
| D-Control | 0 6411 | 1 0000 | 0 6411 | 0 5803 | 0 6881 | 7.826 | 4 | | | 0 6454 | 1 0000 |
| 10 98 | 0 6285 | 0 9803 | 0 6285 | 0.5913 | 0 6710 | 5 201 | 4 | 17 00 | 10 00 | 0 6454 | 1 0000 |
| 22 | 0 6665 | 1 0397 | 0.6665 | 0 6236 | 0 7065 | 6 643 | 4 | 22.00 | 10 00 | 0 6454 | 1 0000 |
| 43.9 | 0 5956 | 0 9291 | 0.5956 | 0.5600 | 0 6481 | 6 787 | 4 | 13 00 | 10 00 | 0 6124 | 0 9489 |
| 72 | 0 6292 | 0 9814 | 0 6292 | 0.5612 | 0 6766 | 7 886 | 4 | 17 00 | 10 00 | 0 6124 | 0 9489 |
| 100 | 0 5248 | 0 8186 | 0.5248 | 0 1807 | 0 6931 | 45 045 | 4 | 16 00 | 10 00 | 0.5248 | 0 8132 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0 01) | 0 785154283 | 0 884 | -2 002403144 | 8.376526546 |
| Bartlett's Test indicates unequal variances (p = 1 69E-03) | 19 2979126 | 15 08631706 | | |

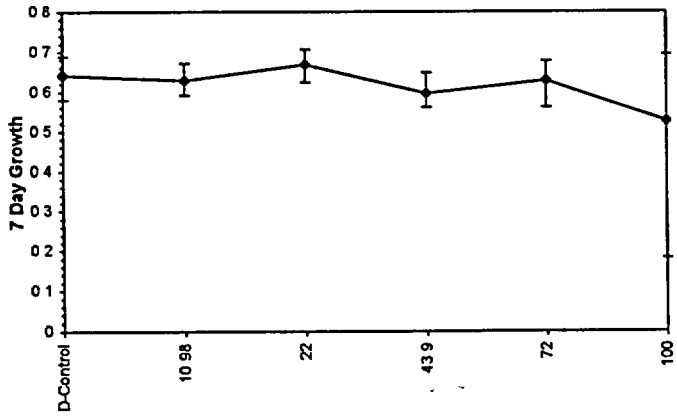
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 100 | >100 | | 1 |

**Linear Interpolation (80 Resamples)**

| Point | % | SD | 95% CL(Exp) | Skew |
|---|---|---|---|---|
| IC05 | 43 441 | | | |
| IC10 | 82 093 | | | |
| IC15 | 92 407 | | | |
| IC20 | >100 | | | |
| IC25 | >100 | | | |
| IC40 | >100 | | | |
| IC50 | >100 | | | |




**Dose-Response Plot**




*sqn_01-14-03data.xls*

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Larval Fish Growth and Survival Test-7 Day Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID: | PpFRCR | Sample ID: | TVA, Sequoyah Nuclear Plant |
| End Date: | 1/21/03 | Lab ID: | ETS-Env Testing Solutions | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date | | Protocol. | EPAF 91-EPA Freshwater | Test Species | PP-Pimephales promelas |
| Comments | | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1 0000 | 1 0000 | 1 0000 | 1 0000 |
| 10.98 | 1 0000 | 1 0000 | 1 0000 | 1 0000 |
| 22 | 0 9000 | 1 0000 | 1 0000 | 0 9000 |
| 43 9 | 0.9000 | 1 0000 | 1 0000 | 1 0000 |
| 72 | 1 0000 | 1 0000 | 1 0000 | 1 0000 |
| 100 | 0.3000 | 1 0000 | 1 0000 | 1 0000 |

| | | | Transform: Arcsin Square Root | | | | | Rank | 1-Tailed |
|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical |
| D-Control | 1 0000 | 1 0000 | 1 4098 | 1 4033 | 1 4120 | 0.307 | 4 | | |
| 10 98 | 1 0000 | 1 0000 | 1 4120 | 1 4120 | 1 4120 | 0 000 | 4 | 20 00 | 10 00 |
| 22 | 0 9500 | 0 9500 | 1.3305 | 1 2490 | 1 4120 | 7 072 | 4 | 15 00 | 10 00 |
| 43 9 | 0 9750 | 0 9750 | 1.3713 | 1 2490 | 1 4120 | 5 942 | 4 | 17.50 | 10 00 |
| 72 | 1 0000 | 1 0000 | 1 4120 | 1 4120 | 1 4120 | 0 000 | 4 | 20 00 | 10 00 |
| 100 | 0 8250 | 0 8250 | 1 2039 | 0 5796 | 1 4120 | 34 569 | 4 | 17.50 | 10 00 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0 01) | 0 671914518 | 0 884 | -2 675882645 | 11.2806056 |
| Equality of variance cannot be confirmed | | | | |

| Hypothesis Test (1-tail, 0 05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 100 | >100 | | 1 |

# Environmental Testing Solutions, LLC

## Statistical Analyses

| Used for PMSD calculation only | | | | Larval Fish Growth and Survival Test-7 Day Growth | | |
|---|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID | PpFRCR | | Sample ID | TVA, Sequoyah Nuclear Plant |
| End Date | 1/21/03 | Lab ID | ETS-Env Testing Solutions | | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date | | Protocol. | EPAF 91-EPA Freshwater | | Test Species | PP-Pimephales promelas |
| Comments | | | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 0 6881 | 0.5803 | 0 6201 | 0 6758 |
| 10 98 | 0 6262 | 0 6254 | 0.5913 | 0 6710 |
| 22 | 0 6236 | 0 7029 | 0 7065 | 0 6331 |
| 43 9 | 0 5600 | 0 5680 | 0 6064 | 0 6481 |
| 72 | 0 6271 | 0 5612 | 0 6518 | 0 6766 |
| 100 | 0 1807 | 0 6931 | 0 6650 | 0 5604 |

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| D-Control | 0 6411 | 1 0000 | 0 6411 | 0 5803 | 0 6881 | 7 826 | 4 | | | |
| 10 98 | 0 6285 | 0 9803 | 0 6285 | 0 5913 | 0 6710 | 5.201 | 4 | 0 171 | 2 410 | 0 1781 |
| 22 | 0 6665 | 1 0397 | 0 6665 | 0 6236 | 0 7065 | 6 643 | 4 | -0 344 | 2 410 | 0 1781 |
| 43 9 | 0 5956 | 0 9291 | 0 5956 | 0 5600 | 0 6481 | 6 787 | 4 | 0 615 | 2 410 | 0 1781 |
| 72 | 0 6292 | 0 9814 | 0 6292 | 0 5612 | 0 6766 | 7 886 | 4 | 0 161 | 2 410 | 0 1781 |
| 100 | 0.5248 | 0 8186 | 0 5248 | 0 1807 | 0 6931 | 45 045 | 4 | 1.574 | 2 410 | 0 1781 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates non-normal distribution (p <= 0 01) | 0 785154283 | 0 884 | -2.002403144 | 8.376526546 |
| Bartlett's Test indicates unequal variances (p = 1 69E-03) | 19 2979126 | 15 08631706 | | |

| Hypothesis Test (1-tail, 0 05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 100 | >100 | | 1 | 0 178087756 | 0 277794306 | 0 00978056 | 0 01092104 | 0.504879713 | 5, 18 |

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Larval Fish Growth and Survival Test-7 Day Growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID | PpFRCR | Sample ID | TVA, Sequoyah Nuclear Plant, Intake |
| End Date | 1/21/03 | Lab ID | ETS-Env Testing Solutions | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date | | Protocol | EPAF 91-EPA Freshwater | Test Species | PP-Pimephales promelas |
| Comments | | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 0 6881 | 0 5803 | 0 6201 | 0 6758 |
| 100 | 0.5083 | 0 5795 | 0 7181 | 0 6814 |

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| D-Control | 0 6411 | 1 0000 | 0 6411 | 0 5803 | 0 6881 | 7 826 | 4 | | | |
| 100 | 0 6218 | 0 9700 | 0 6218 | 0 5083 | 0 7181 | 15.396 | 4 | 0.356 | 1 943 | 0 1050 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0 01) | 0 963339746 | 0 749 | -0 281148021 | -0 961324515 |
| F-Test indicates equal variances (p = 0 32) | 3 641183138 | 47 46834564 | | |

**Hypothesis Test (1-tail, 0 05)**

Homoscedastic t Test indicates no significant differences

# Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013 Method 1002.0)
## Species: *Ceriodaphnia dubia*

Client: TVA
Facility: SEQUOYAH NUCLEAR PLANT
NPDES #: TN 0026450
Project #: 571

| *Dilution preparation information:* | | | | | | *Comments:* |
|---|---|---|---|---|---|---|
| Dilution prep (%) | 10.98 | 22 | 43.9 | 72 | 100 | |
| Effluent volume (mL) | 164.7 | 330 | 658.5 | 1080 | 1500 | |
| Diluent volume (mL) | 1335.3 | 1170 | 841.5 | 420 | 0 | |
| Total volume (mL) | 1500 | 1500 | 1500 | 1500 | 1500 | |

| *Test organism information:* | | *Test information:* | |
|---|---|---|---|
| Organism age: | <24-HOURS OLD | Randomizing template: | GREEN |
| Date and times organisms were born between: | 01-14-03 0816 TO 1209 | Incubator number and shelf location: | 3 C |
| Organism source: | 01-07-03 A-D | YCT batch: ABS | 12-24-02 |
| Transfer bowl information: | pH = 8.21 Temperature = 25.1°C | *Selenastrum* batch: ABS | 12-24-02 |

*Daily renewal information:*

| Day | Date | Test initiation, renewal, or termination time | Control water batch used | Sample numbers used 101 | INTAKE | Analyst |
|---|---|---|---|---|---|---|
| 0 | 01-14-03 | 1517 | MHS 01-12-03 | 030114.01 | 030114.02 | JJ |
| 1 | 01-15-03 | 1500 | MHS 01-12-03 | 030114.01 | 030114.02 | JJ |
| 2 | 01-16-03 | 1426 | MHS 01-12-03 | 030116.01 | 030116.02 | JJ |
| 3 | 01-17-03 | 1431 | MHS 01-15-03 | 030116.01 | 030116.02 | JJ |
| 4 | 01-18-03 | 1430 | MHS 01-15-03 | 030118.01 | 030118.02 | JJ |
| 5 | 01-19-03 | 1502 | MHS 01-18-03 | 030118.01 | 030118.02 | JJ |
| 6 | 01-20-03 | 1436 | MHS 01-18-03 | 030118.01 | 030118.02 | JJ |
| 7 | 01-21-03 | 1504 | — | — | JJ | JJ |

| *Control information:* | | Acceptance criteria | *Summary of test endpoints:* | |
|---|---|---|---|---|
| % of Male Adults: | 0% | ≤ 20% | 7-day LC50 | > 100% |
| % Adults having 3rd Broods: | 100% | ≥ 80% | NOEC | 100% |
| % Mortality: | 0% | ≤ 20% | LOEC | >100% |
| Mean Offspring/Female: | ~~34.35~~ 34.1 | ≥ 15.0 offspring/female | ChV | >100% |
| % CV: | ~~7.1%~~ 7.5% | < 40.0 % | IC25 | > 100% |

Corrected 01-28-03 JJ

Species: *Ceriodaphnia dubia*

Client: SEQUOYAH - NONTREATED

Date: 01-14-03

**CONTROL**

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 5 | 5 | 5 | 4 | 6 | 5 | 4 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 11 | 12 | 9 | 13 | 10 | 13 | 12 | 13 | 12 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 14 | 0 | 0 | 16 | 0 | 19 | 0 | 0 | 0 | 1* |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 0 | 18 | 18 | 2* | 19 | 0 | 20 | 17 | 16 | 18 |
| Total young produced | | 29 | 35 | 32 | 36 | 34 | 36 | 38 | 35 | ~~34~~ 32 | 34 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |
| X for 3rd Broods | | X | X | X | X | X | X | X | X | X | X |

* Carry over

* Carry over

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | ~~34.3~~ 34.1 |

Corrected 01-28-03 JS

CONCENTRATION: 10.98%

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 5 | 5 | 5 | 6 | 4 | 6 | 5 | 4 | 6 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 13 | 10 | 11 | 10 | 12 | 15 | 12 | 13 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 19 | 18 | 18 | 21 | 19 | 0 | 21 | 17 | 22 | 19 |
| Total young produced | | 36 | 36 | 33 | 37 | 35 | 34 | 42 | 34 | 39 | 37 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 36.3 |
| % Reduction from Control: | 5.8% |

**Species:** *Ceriodaphnia dubia*

**Client:** SEQUOYAH - NONTREATED **Date:** 01-14-03

**CONCENTRATION:** 22%

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 5 | 6 | 6 | 4 | 5 | 5 | 4 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 12 | 13 | 15 | 14 | 13 | 10 | 14 | 14 | 13 | 11 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 17 | 22 | 20 | 19 | 19 | 22 | 20 | 20 | 0 | 16 |
| Total young produced | | 33 | 40 | 40 | 39 | 38 | 36 | 39 | 39 | 35 | 32 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 37.1 |
| % Reduction from Control: | -8.2% |

**CONCENTRATION:** 43.9%

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 6 | 5 | 5 | 4 | 5 | 5 | 6 | 4 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 13 | 11 | 12 | 14 | 13 | 15 | 11 | 13 | 13 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 23 | 20 | 17 | 22 | 23 | 1* | 22 | 20 | 16 | 19 |
| Total young produced | | 41 | 37 | 34 | 41 | 40 | 40 | 38 | 39 | 33 | 36 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

* carryover

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 37.9 |
| % Reduction from Control: | -10.5% |

**Species:** *Ceriodaphnia dubia*

**Client:** SEQUOYAH - NONTREATED **Date:** 01-14-03

**CONCENTRATION:** 72%

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 5 | 6 | 5 | 5 | 5 | 7 | 6 | 5 | 6 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 13 | 14 | 16 | 13 | 11 | 14 | 15 | 13 | 16 | 14 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 18 | 18 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 22 | 19 | 22 | 21 | 20 | 0 | 0 | 23 | 24 | 20 |
| Total young produced | | 40 | 38 | 44 | 39 | 36 | 37 | 40 | 42 | 45 | 40 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 40.1 |
| % Reduction from Control: | -16.9% |

**CONCENTRATION:** 100%

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 5 | 5 | 4 | 6 | 5 | 6 | 6 | 5 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 15 | 13 | 14 | 16 | 11 | 13 | 14 | 12 | 16 | 15 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 20 | 19 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 22 | 24 | 21 | 18 | 23 | 0 | 0 | 19 | 22 | 20 |
| Total young produced | | 42 | 42 | 40 | 38 | 40 | 38 | 39 | 37 | 43 | 39 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 39.8 |
| % Reduction from Control: | -16.0% |

**Species:** *Ceriodaphnia dubia*

**Client:** SEQUOYAH - NONTREATED **Date:** 01-14-03

CONCENTRATION: 100% INTAKE

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 5 | 5 | 6 | 4 | 4 | 4 | 5 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 13 | 14 | 16 | 15 | 16 | 13 | 13 | 11 | 15 | 13 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1* | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 20 | 15 | 19 | 20 | 18 | 17 | 17 | 21 | 17 | 22 |
| Total young produced | | 37 | 34 | 40 | 40 | 40 | 34 | 34 | 37 | 37 | 39 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

* CARRY OVER

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 37.2 |
| % Reduction from Control: | -8.5% |

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1002.0)
## Species: Ceriodaphnia dubia

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

**Client:** TVA Sequoyah Nuclear Plant, Nontreated
**Test dates:** January 14 - 21, 2003
**Project number:** 571

**Reveiwed by:** [illegible]

| Concentration (%) | Replicate number | | | | | | | | | | Survival (%) | Average reproduction (offspring/female) | Coefficient of variation (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| Control | 29 | 35 | 32 | 36 | 34 | 36 | 38 | 35 | 32 | 34 | 100 | 34.1 | 7.5 | Not applicable |
| 10.98% | 36 | 36 | 33 | 37 | 35 | 34 | 42 | 34 | 39 | 37 | 100 | 36.3 | 7.4 | -6.5 |
| 22% | 33 | 40 | 40 | 39 | 38 | 36 | 39 | 39 | 35 | 32 | 100 | 37.1 | 7.9 | -8.8 |
| 43.9% | 41 | 37 | 34 | 41 | 40 | 40 | 38 | 39 | 33 | 36 | 100 | 37.9 | 7.5 | -11.1 |
| 72% | 40 | 38 | 44 | 39 | 36 | 37 | 40 | 42 | 45 | 40 | 100 | 40.1 | 7.2 | -17.6 |
| 100% | 42 | 42 | 40 | 38 | 40 | 38 | 39 | 37 | 43 | 39 | 100 | 39.8 | 5.0 | -16.7 |
| 100% Intake | 37 | 34 | 40 | 40 | 40 | 34 | 34 | 37 | 37 | 39 | 100 | 37.2 | 6.8 | -9.1 |

**Outfall 101:**
**Dunnett's MSD value:** 2.725
**PMSD:** 8.0

**Intake:**
**Dunnett's MSD value:** 1.932
**PMSD:** 5.7

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. On average, a significant difference occurs for Environmental Testing Solutions, LLC chronic toxicity tests when a toxicant reduces Ceriodaphnia reproduction by 10.8% from the control.
Lower PMSD bound determined by USEPA ($10^{th}$ percentile) = 11%.
Upper PMSD bound determined by USEPA ($90^{th}$ percentile) = 37%.
The lower and upper bounds were calculated by the USEPA using 393 tests conducted from 33 laboratories for *Ceriodaphnia* reproduction in chronic reference toxicant tests

USEPA 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID: | CdFRCR | Sample ID | TVA Sequoyah Nuclear Plant |
| End Date: | 1/21/03 | Lab ID: | ETS-Env Testing Solutions | Sample Type: | DMR-Discharge Monitoring Report |
| Sample Date | | Protocol: | EPAF 91-EPA Freshwater | Test Species | CD-Cenodaphnia dubia |
| Comments | | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| D-Control | 29 000 | 35 000 | 32.000 | 36.000 | 34 000 | 36 000 | 38 000 | 35 000 | 32.000 | 34 000 |
| 10 98 | 36 000 | 36 000 | 33 000 | 37 000 | 35 000 | 34 000 | 42 000 | 34 000 | 39 000 | 37 000 |
| 22 | 33 000 | 40 000 | 40 000 | 39 000 | 38 000 | 36 000 | 39 000 | 39 000 | 35 000 | 32 000 |
| 43 9 | 41 000 | 37 000 | 34 000 | 41 000 | 40 000 | 40 000 | 38 000 | 39 000 | 33 000 | 36 000 |
| 72 | 40 000 | 38 000 | 44 000 | 39 000 | 36 000 | 37 000 | 40 000 | 42 000 | 45 000 | 40 000 |
| 100 | 42 000 | 42 000 | 40 000 | 38 000 | 40 000 | 38 000 | 39 000 | 37 000 | 43 000 | 39 000 |

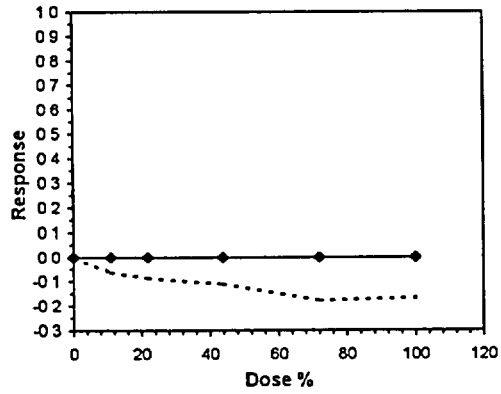
| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 34 100 | 1 0000 | 34 100 | 29 000 | 38 000 | 7.502 | 10 | | | | 37 550 | 1 0000 |
| 10 98 | 36 300 | 1 0645 | 36.300 | 33 000 | 42 000 | 7 352 | 10 | -1 846 | 2.287 | 2 725 | 37 550 | 1 0000 |
| 22 | 37 100 | 1 0880 | 37 100 | 32 000 | 40 000 | 7 879 | 10 | -2 518 | 2.287 | 2 725 | 37 550 | 1 0000 |
| 43 9 | 37 900 | 1 1114 | 37 900 | 33 000 | 41 000 | 7 509 | 10 | -3 189 | 2 287 | 2 725 | 37 550 | 1 0000 |
| 72 | 40 100 | 1 1760 | 40 100 | 36 000 | 45 000 | 7 194 | 10 | -5 036 | 2 287 | 2 725 | 37.550 | 1 0000 |
| 100 | 39 800 | 1 1672 | 39 800 | 37 000 | 43 000 | 4 997 | 10 | -4 784 | 2 287 | 2 725 | 37.550 | 1 0000 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates normal distribution (p > 0 01) | 0 826381624 | 1 035 | -0 091994285 | -0.52674914 |
| Bartlett's Test indicates equal variances (p = 0 90) | 1 604416966 | 15 08631706 | | |

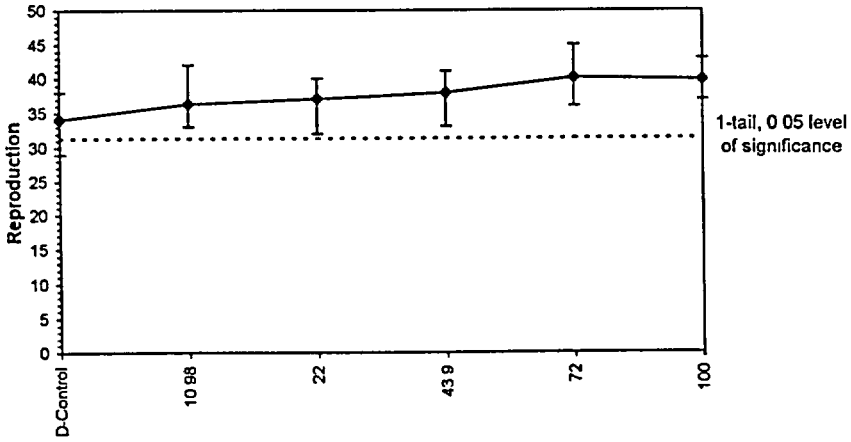
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test - | 100 | >100 | | 1 | 2 724522394 | 0 079898017 | 50 71 | 7 098148148 | 3 4E-05 | 5, 54 |

**Linear Interpolation (80 Resamples)**

| Point | % | SD | 95% CL | Skew |
|---|---|---|---|---|
| IC05 | >100 | | | |
| IC10 | >100 | | | |
| IC15 | >100 | | | |
| IC20 | >100 | | | |
| IC25 | >100 | | | |
| IC40 | >100 | | | |
| IC50 | >100 | | | |




**Dose-Response Plot**




*sqn_01-14-03data xls*

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID | CdFRCR | Sample ID | TVA, Sequoyah Nuclear Plant, Intake |
| End Date | 1/21/03 | Lab ID | ETS-Env Testing Solutions | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date | | Protocol | EPAF 91-EPA Freshwater | Test Species | CD-Cenodaphnia dubia |
| Comments | | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| D-Control | 29 000 | 35 000 | 32 000 | 36 000 | 34 000 | 36 000 | 38 000 | 35 000 | 34 000 | 34 000 |
| 100 | 37 000 | 34 000 | 40 000 | 40 000 | 40 000 | 34 000 | 34 000 | 37 000 | 37 000 | 39 000 |

| | | | Transform: Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| D-Control | 34 300 | 1 0000 | 34 300 | 29 000 | 38 000 | 7 148 | 10 | | | |
| 100 | 37 200 | 1 0845 | 37.200 | 34 000 | 40 000 | 6 801 | 10 | -2.603 | 1 734 | 1 932 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0 01) | 0 940015912 | 0 868 | -0 519383606 | -0.338943619 |
| F-Test indicates equal variances (p = 0 93) | 1 064695001 | 6 54108572 | | |

**Hypothesis Test (1-tail, 0 05)**

Homoscedastic t Test indicates no significant differences

# Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013 Method 1000.0)
## Species: *Pimephales promelas*

Client: TVA
Facility: SEQUOYAH NUCLEAR PLANT
NPDES #: TN 0026450
Project #: 571
UV-TREATED

| *Dilution preparation information:* | | | | | | *Comments:* |
|---|---|---|---|---|---|---|
| Dilution prep (%) | 10.98 | 22 | 43.9 | 72 | 100 | |
| Effluent volume (mL) | 164.7 | 330 | 658.5 | 1080 | 1500 | |
| Diluent volume (mL) | 1335.3 | 1170 | 841.5 | 420 | 0 | |
| Total volume (mL) | 1500 | 1500 | 1500 | 1500 | 1500 | |

| *Test organism information:* | | *Test information:* | |
|---|---|---|---|
| Organism age: | 24-HOURS OLD | Randomizing template: | PURPLE |
| Date and times organisms were born between: | 01-13-03 1230 TO 1530 HDT | Incubator number. | 2 |
| Organism source: | ABS BATCH 01-13-03 | *Artemia* lot number: | B61809Q |
| Transfer bowl information: | pH = 7.96 Temperature = 24.2 °C | Total drying time: | 17.25-HOURS |
| | | Date / Time in: | 01-21-03 1450 |
| Average transfer volume: | 10.4 ml | Date / Time out: | 01-22-03 0800 |
| | | Oven temperature: | 101°C |

*Daily feeding and renewal information:*

| Day | Date | Morning feeding time | Afternoon feeding time | Test initiation, renewal, or termination time | Control water batch used | Sample numbers used 101 | INTAKE | Analyst |
|---|---|---|---|---|---|---|---|---|
| 0 | 01-14-03 | — | 1530 | 1420 | MHS 01-12-03 | 030114.01 | 030114.02 | JH |
| 1 | 01-15-03 | 0914 | 1520 | 1404 | MHS 01-12-03 | 030114.01 | 030114.02 | JH |
| 2 | 01-16-03 | 0841 | 1450 | 1341 | MHS 01-12-03 | 030116.01 | 030116.02 | JH |
| 3 | 01-17-03 | 0908 | 1510 | 1332 | MHS 01-15-03 | 030116.01 | 030116.02 | JH |
| 4 | 01-18-03 | 0910 | 1517 | 1330 | MHS 01-15-03 | 030118.01 | 030118.02 | JH |
| 5 | 01-19-03 | 0918 | 1520 | 1343 | MHS 01-18-03 | 030118.01 | 030118.02 | JH |
| 6 | 01-20-03 | 0837 | 1500 | 1328 | MHS 01-18-03 | 030118.01 | 030118.02 | JH |
| 7 | 01-21-03 | | | 1423 | — | — | JH | JH |

| *Control information:* | | Acceptance criteria |
|---|---|---|
| % Mortality: | 0% | ≤ 20% |
| Average weight per initial larvae: | 0.6354 | |
| Average weight per surviving larvae: | 0.6354 | ≥ 0.25 mg/larvae |

| *Summary of test endpoints:* | |
|---|---|
| 7-day $LC_{50}$ | >100% |
| NOEC | 100% |
| LOEC | >100% |
| ChV | >100% |
| $IC_{25}$ | >100% |

Species: *Pimephales promelas*

Client: SEQUOYAH - UV TREATED

Date: 01-14-03

*Survival and Growth Data*

| Day | CONTROL | | | | 10.98% | | | | 22% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 7 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| A = Pan weight (mg) Porpe | 14.696 | 15.096 | 14.874 | 15.059 | 16.264 | 14.686 | 14.980 | 14.911 | 14.847 | 14.904 | 14.906 | 15.302 |
| B = Pan + Larvae weight (mg) | 20.02 | 21.96 | 22.07 | 21.09 | 23.79 | 20.95 | 21.87 | 21.35 | 21.49 | 21.40 | 21.44 | 21.75 |
| Larvae weight (mg) = A – B | 5.324 | 6.864 | 7.196 | 6.031 | 7.586 | 6.264 | 6.890 | 6.439 | 6.643 | 6.496 | 6.534 | 6.448 |

Calculations and data reviewed

*Comments:*

**Species:** *Pimephales promelas*

**Client:** SEQUOYAH - UV TREATED **Date:** 01-14-03

*Survival and Growth Data*

| Day | 43% | | | | 72% | | | | 100% | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9'd | 10 | 10 | 10 |
| 7 | 1 SMALL 10 | 10 | 10 | 1 SMALL 10 | 10 | 10 | 10 | 1 SMALL 10 | 9 | 10 | 10 | 1 SMALL 10 |
| A = Pan weight (mg) | 14.984 | 14.982 | 14.743 | 14.643 | 14.900 | 14.668 | 14.689 | 14.824 | 15.112 | 15.159 | 15.137 | 14.725 |
| B = Pan + Larvae weight (mg) | 22.00 | 20.74 | 20.30 | 20.98 | 22.31 | 20.75 | 21.30 | 21.42 | 21.35 | 22.22 | 22.00 | 20.91 |
| Larvae weight (mg) = A – B | 7.016 | 5.758 | 5.557 | 6.337 | 7.410 | 6.082 | 6.611 | 6.596 | 6.238 | 7.061 | 6.863 | 6.185 |

Calculations and data reviewed

*Comments:*

**Species:** *Pimephales promelas*

**Client:** SEQUOYAH - UV TREATED **Date:** 01-14-03

*Survival and Growth Data*

| Day | INTAKE | | | |
|---|---|---|---|---|
| | Y | Z | AA | BB |
| 0 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 |
| 3 | 10 | 10 | 10 | 10 |
| 4 | 10 | 10 | 10 | 10 |
| 5 | 10 | 10 | 10 | 10 |
| 6 | 10 | 10 | 10 | 10 |
| 7 | 10 | 10 | 10 | 10 |
| A = Pan weight (mg) | 14.811 | 15.185 | 14.822 | 14.977 |
| B = Pan + Larvae weight (mg) | 21.80 | 21.50 | 21.34 | 21.67 |
| Larvae weight (mg) = A – B | 6.989 | 6.315 | 6.518 | 6.693 |

Calculations and data reviewed: JH

| *Comments:* |
|---|
| |
| |
| |
| |
| |

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1000.0)
## Species: *Pimephales promelas*

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

Client: TVA Sequoyah Nuclear Plant, UV Treated

Test dates: January 14 -21, 2003

Project number: 571

Reveiwed by: [signature]

| Concentration (%) | Replicate | Initial number of larvae | Final number of larvae | A = Pan weight (mg) | B = Pan + Larvae weight (mg) | Larvae weight (mg) = A - B | Weight / Initial number of larvae (mg) | Mean survival (%) | Mean weight (mg) | Coefficient of variation (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | A | 10 | 10 | 14 696 | 20 020 | 5 324 | 0 5324 | 100 0 | 0 6354 | 13.3 | Not applicable |
| | B | 10 | 10 | 15 096 | 21 960 | 6 864 | 0 6864 | | | | |
| | C | 10 | 10 | 14 874 | 22 070 | 7.196 | 0 7196 | | | | |
| | D | 10 | 10 | 15.059 | 21 090 | 6 031 | 0 6031 | | | | |
| 10 98% | E | 10 | 10 | 16 204 | 23.790 | 7 586 | 0.7586 | 100 0 | 0 6795 | 8.7 | -6.9 |
| | F | 10 | 10 | 14 686 | 20 950 | 6 264 | 0 6264 | | | | |
| | G | 10 | 10 | 14 980 | 21 870 | 6.890 | 0 6890 | | | | |
| | H | 10 | 10 | 14 911 | 21.350 | 6 439 | 0 6439 | | | | |
| 22% | I | 10 | 10 | 14 847 | 21.490 | 6 643 | 0 6643 | 100 0 | 0 6530 | 1.3 | -2.8 |
| | J | 10 | 10 | 14 904 | 21.400 | 6 496 | 0 6496 | | | | |
| | K | 10 | 10 | 14 906 | 21 440 | 6 534 | 0 6534 | | | | |
| | L | 10 | 10 | 15 302 | 21.750 | 6 448 | 0.6448 | | | | |
| 43.9% | M | 10 | 10 | 14 984 | 22 000 | 7 016 | 0 7016 | 100 0 | 0 6167 | 10 6 | 2.9 |
| | N | 10 | 10 | 14 982 | 20 740 | 5.758 | 0 5758 | | | | |
| | O | 10 | 10 | 14.743 | 20 300 | 5.557 | 0 5557 | | | | |
| | P | 10 | 10 | 14 643 | 20 980 | 6 337 | 0 6337 | | | | |
| 72% | Q | 10 | 10 | 14 900 | 22 310 | 7 410 | 0.7410 | 100 0 | 0 6675 | 8.2 | -5.1 |
| | R | 10 | 10 | 14 668 | 20 750 | 6 082 | 0 6082 | | | | |
| | S | 10 | 10 | 14 689 | 21.300 | 6 611 | 0 6611 | | | | |
| | T | 10 | 10 | 14.824 | 21 420 | 6 596 | 0 6596 | | | | |
| 100% | U | 10 | 9 | 15.112 | 21 350 | 6.238 | 0 6238 | 97.5 | 0 6587 | 6.7 | -3.7 |
| | V | 10 | 10 | 15.159 | 22 220 | 7.061 | 0 7061 | | | | |
| | W | 10 | 10 | 15.137 | 22 000 | 6.863 | 0 6863 | | | | |
| | X | 10 | 10 | 14.725 | 20 910 | 6.185 | 0 6185 | | | | |
| 100% Intake | Y | 10 | 10 | 14 811 | 21 800 | 6.989 | 0 6989 | 100 0 | 0 6629 | 4 3 | -4.3 |
| | Z | 10 | 10 | 15 185 | 21 500 | 6 315 | 0 6315 | | | | |
| | AA | 10 | 10 | 14 822 | 21.340 | 6 518 | 0 6518 | | | | |
| | BB | 10 | 10 | 14 977 | 21.670 | 6 693 | 0 6693 | | | | |

**Outfall 101:**
Dunnett's MSD value: 0 0982
PMSD: 15 5

**Intake:**
Dunnett's MSD value. 0 0865
PMSD: 13 6

MSD = Minimum Significant Difference
PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. On average, a significant difference occurs for Environmental Testing Solutions, LLC chronic toxicity tests when a toxicant reduces Pimephales growth by 17.9% from the control (determined through Lower PMSD bound determined by USEPA (10th percentile) = 9.4%.
Upper PMSD bound determined by USEPA (90th percentile) = 35%
The lower and upper bounds were calculated by the USEPA using 205 tests conducted from 19 laboratories for Pimephales growth in chronic reference toxicant tests.

USEPA. 2000 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Larval Fish Growth and Survival Test-7 Day Growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID: | PpFRCR | Sample ID | TVA, Sequoyah Nuclear Plant, UV-Treated |
| End Date | 1/21/03 | Lab ID: | ETS-Env Testing Solutions | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date: | | Protocol: | EPAF 91-EPA Freshwater | Test Species | PP-Pimephales promelas |
| Comments | | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 0.5324 | 0 6864 | 0 7196 | 0 6031 |
| 10 98 | 0 7586 | 0 6264 | 0 6890 | 0 6439 |
| 22 | 0 6643 | 0 6496 | 0 6534 | 0 6448 |
| 43 9 | 0 7016 | 0 5758 | 0.5557 | 0 6337 |
| 72 | 0 7410 | 0 6082 | 0 6611 | 0 6596 |
| 100 | 0 6238 | 0 7061 | 0 6863 | 0 6185 |

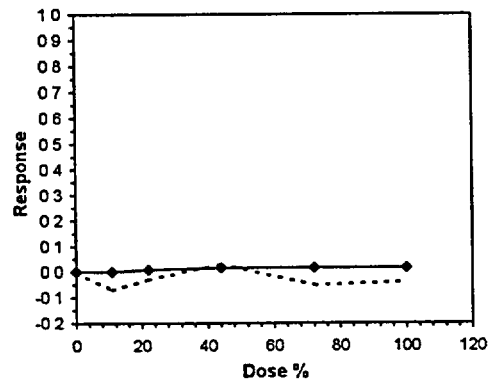
| | | | Transform Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 0 6354 | 1 0000 | 0 6354 | 0 5324 | 0 7196 | 13.275 | 4 | | | | 0 6574 | 1 0000 |
| 10 98 | 0 6795 | 1 0694 | 0 6795 | 0 6264 | 0 7586 | 8 679 | 4 | -1 083 | 2.410 | 0 0982 | 0 6574 | 1 0000 |
| 22 | 0 6530 | 1 0278 | 0 6530 | 0 6448 | 0 6643 | 1 271 | 4 | -0 433 | 2 410 | 0 0982 | 0 6530 | 0.9933 |
| 43 9 | 0 6167 | 0 9706 | 0 6167 | 0.5557 | 0 7016 | 10 629 | 4 | 0 458 | 2 410 | 0 0982 | 0 6476 | 0 9851 |
| 72 | 0 6675 | 1 0505 | 0 6675 | 0 6082 | 0 7410 | 8 216 | 4 | -0 788 | 2 410 | 0 0982 | 0 6476 | 0 9851 |
| 100 | 0 6587 | 1 0367 | 0 6587 | 0 6185 | 0 7061 | 6 700 | 4 | -0 572 | 2 410 | 0 0982 | 0 6476 | 0 9851 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0 01) | 0 959238648 | 0 884 | 0 156850123 | -0 594541763 |
| Bartlett's Test indicates equal variances (p = 0 10) | 9 132943153 | 15 08631706 | | |

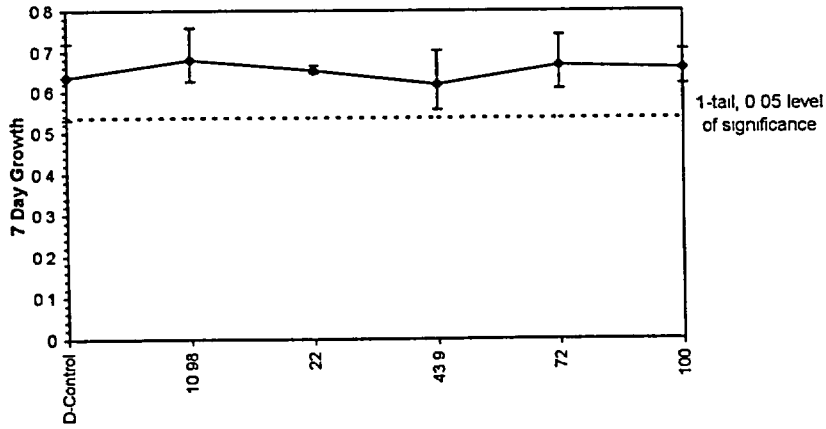
| Hypothesis Test (1-tail, 0 05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 100 | >100 | | 1 | 0 098173695 | 0 154512996 | 0 002049734 | 0 003318839 | 0 68807584 | 5, 18 |

**Linear Interpolation (80 Resamples)**

| Point | % | SD | 95% CL(Exp) | Skew |
|---|---|---|---|---|
| IC05 | >100 | | | |
| IC10 | >100 | | | |
| IC15 | >100 | | | |
| IC20 | >100 | | | |
| IC25 | >100 | | | |
| IC40 | >100 | | | |
| IC50 | >100 | | | |




**Dose-Response Plot**



# Environmental Testing Solutions, LLC

## Statistical Analyses

| Larval Fish Growth and Survival Test-7 Day Growth | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID | PpFRCR | Sample ID | TVA, Sequoyah Nuclear Plant, Intake UV-Treated |
| End Date | 1/21/03 | Lab ID | ETS-Env Testing Solutions | Sample Type | DMR-Discharge Monitoring Report |
| Sample Date | | Protocol | EPAF 91-EPA Freshwater | Test Species | PP-Pimephales promelas |
| Comments | | | | | |

| Conc-% | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 0 5324 | 0 6864 | 0 7196 | 0 6031 |
| 100 | 0 6989 | 0 6315 | 0 6518 | 0 6693 |

| | | | Transform Untransformed | | | | | | 1-Tailed | |
|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD |
| D-Control | 0 6354 | 1 0000 | 0 6354 | 0.5324 | 0 7196 | 13.275 | 4 | | | |
| 100 | 0 6629 | 1 0433 | 0 6629 | 0 6315 | 0 6989 | 4.308 | 4 | -0 618 | 1 943 | 0 0865 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0 01) | 0 976053894 | 0 749 | -0.365780657 | 0 244562207 |
| F-Test indicates equal variances (p = 0 11) | 8 725190163 | 47 46834564 | | |

**Hypothesis Test (1-tail, 0 05)**

Homoscedastic t Test indicates no significant differences

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1000.0)
Species: *Pimephales promelas*

### Daily Chemical Analyses

**Client:** Sequoyah Nuclear Plant Non-Treated
**Test dates:** January 14 - 21, 2003
**Project number:** 571

Mean Temp: 24.8
Min: 24.4
Max: 25.4

**Reviewed by:** Cw

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Control | pH (SU) | 8.06 | 8 07 | 8 19 | 8.11 | 8 15 | 7.74 | 8 18 | 7 66 | 8 09 | 7 62 | 8.16 | 7.68 | 8.11 | 8 00 |
| | DO (mg/L) | 7.9 | 8.1 | 7.7 | 8 0 | 7.9 | 6 9 | 8 0 | 6 6 | 7 8 | 6 4 | 7.7 | 7 6 | 7.6 | 7.9 |
| | Conductivity (µmhos/cm) | 308 | | 301 | | 303 | | 278 | | 286 | | 284 | | 303 | |
| | Alkalinity (mg/L $CaCO_3$) | 64 | | | | | | 60 | | | | 62 | | | |
| | Hardness (mg/L $CaCO_3$) | 84 | | | | | | 86 | | | | 86 | | | |
| | Temperature (°C) | 24 4 | 25.1 | 24 4 | 25.1 | 24 5 | 24 7 | 24 4 | 24 9 | 24 4 | 25.3 | 24 6 | 24.6 | 24.7 | 24 8 |
| 10.98% | pH (SU) | 8 05 | 8 02 | 8 21 | 8 03 | 8.17 | 7.72 | 8 22 | 7.61 | 8 10 | 7 58 | 8 17 | 7 70 | 8 12 | 8 03 |
| | DO (mg/L) | 8 5 | 8 0 | 8 1 | 8 0 | 7.9 | 6.9 | 8 0 | 6 3 | 7 6 | 6 2 | 7.5 | 7 5 | 7 8 | 7.8 |
| | Conductivity (µmhos/cm) | 294 | | 298 | | 288 | | 278 | | 286 | | 279 | | 297 | |
| | Temperature (°C) | 24 4 | 25.0 | 24.5 | 25.1 | 24 6 | 24.7 | 24 5 | 24 9 | 24 5 | 25.3 | 24 6 | 24 6 | 24.8 | 24.8 |
| 22% | pH (SU) | 8 07 | 8 03 | 8 20 | 8 04 | 8.16 | 7.66 | 8 20 | 7 61 | 8 10 | 7 63 | 8 17 | 7 65 | 8 13 | 8 08 |
| | DO (mg/L) | 8 5 | 8 0 | 8 1 | 8.1 | 8.1 | 6.5 | 8 0 | 6 3 | 7.7 | 6 7 | 7.5 | 7.3 | 7.8 | 7.8 |
| | Conductivity (µmhos/cm) | 288 | | 286 | | 278 | | 266 | | 278 | | 272 | | 286 | |
| | Temperature (°C) | 24 4 | 25.0 | 24.5 | 25.1 | 24 6 | 24 7 | 24 5 | 24 9 | 24 5 | 25 4 | 24 6 | 24.6 | 24 8 | 24 8 |
| 43.9% | pH (SU) | 8 07 | 8 03 | 8 19 | 8 02 | 8.17 | 7.72 | 8.18 | 7 65 | 8 09 | 7 64 | 8 15 | 7 72 | 8 14 | 8 09 |
| | DO (mg/L) | 8 6 | 8 0 | 8 1 | 8.1 | 8 1 | 6 9 | 8.3 | 6 3 | 7 8 | 6 7 | 7 5 | 7.3 | 8 0 | 8 2 |
| | Conductivity (µmhos/cm) | 266 | | 263 | | 257 | | 246 | | 259 | | 254 | | 266 | |
| | Temperature (°C) | 24.4 | 25.0 | 24 5 | 25 1 | 24 7 | 24.7 | 24 5 | 24.9 | 24.8 | 25.4 | 24 6 | 24 6 | 24 8 | 24 8 |
| 72% | pH (SU) | 8.08 | 8 03 | 8 19 | 8 01 | 8.16 | 7.74 | 8 16 | 7.69 | 8 11 | 7 67 | 8 13 | 7.72 | 8.14 | 8.16 |
| | DO (mg/L) | 8.6 | 8 0 | 8 1 | 8 1 | 8 1 | 6 8 | 8.6 | 6 6 | 7.9 | 6 5 | 8 0 | 7.5 | 8 1 | 8.1 |
| | Conductivity (µmhos/cm) | 235 | | 231 | | 228 | | 220 | | 235 | | 227 | | 240 | |
| | Temperature (°C) | 24.4 | 25 0 | 24.5 | 25.1 | 24 8 | 24 7 | 24 5 | 24 9 | 25 0 | 25.4 | 24.6 | 24 6 | 24 8 | 24 8 |
| 100% | pH (SU) | 8.07 | 8.06 | 8 18 | 7 99 | 8 18 | 7.79 | 8.14 | 7.80 | 8 12 | 7.75 | 8 15 | 7.88 | 8.15 | 8.22 |
| | DO (mg/L) | 8 5 | 8.0 | 8.3 | 8 0 | 8 3 | 7.1 | 8 6 | 7.1 | 8.2 | 6.8 | 7.8 | 7 8 | 8 2 | 8 0 |
| | Conductivity (µmhos/cm) | 202 | | 201 | | 200 | | 196 | | 208 | | 204 | | 213 | |
| | Alkalinity (mg/L $CaCO_3$) | 68 | | | | 73 | | | | 71 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 86 | | | | 89 | | | | 89 | | | | | |
| | Total Residual Chlorine (mg/L) | <0.10 | | | | <0 10 | | | | <0 10 | | | | | |
| | Temperature (°C) | 24 4 | 25.1 | 24.5 | 25.1 | 24 8 | 24 7 | 24 5 | 24.9 | 25 2 | 25.4 | 24 6 | 24 6 | 24 8 | 24 8 |
| 100% Intake | pH (SU) | 8 08 | 8 06 | 8 19 | 8 04 | 8 17 | 7.79 | 8 11 | 7.72 | 8 11 | 7.72 | 8 01 | 7 86 | 8 14 | 8.18 |
| | DO (mg/L) | 8.6 | 7.9 | 8 2 | 8 1 | 8 2 | 7 0 | 8.8 | 6.9 | 8.5 | 6 9 | 8 6 | 8 0 | 8 4 | 8.3 |
| | Conductivity (µmhos/cm) | 204 | | 200 | | 200 | | 193 | | 209 | | 202 | | 214 | |
| | Alkalinity (mg/L $CaCO_3$) | 70 | | | | 72 | | | | 74 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 80 | | | | 82 | | | | 82 | | | | | |
| | Total Residual Chlorine (mg/L) | <0.10 | | | | <0.10 | | | | <0.10 | | | | | |
| | Temperature (°C) | 24 6 | 25.1 | 24.5 | 25.1 | 24 5 | 24 7 | 24 4 | 24.9 | 24.8 | 25.4 | 24 9 | 24 6 | 24 8 | 24 8 |

Species: *Pimephales promelas*
Client: SEQUOIAH - NONTREATED

Date: 01-14-03

*Daily Chemistry:*

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | |
|---|---|---|---|---|---|---|---|
| CONTROL | pH (S U.) | 8.06 | 8.07 | 8.19 | 8.11 | 8.15 | 7.74 |
| | DO (mg/L) | 7.9 | 8.1 | 7.7 | 8.0 | 7.9 | 6.9 |
| | Conductivity (µmhos/cm) | 308 | | 301 | | 303 | |
| | Alkalinity (mg $CaCO_3$/L) | 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | 84 | | | | | |
| | Temperature (°C) | 24.4 | 25.1 | 24.4 | 25.1 | 24.5 | 24.7 |
| 10.98% | pH (S.U.) | 8.05 | 8.02 | 8.21 | 8.03 | 8.17 | 7.72 |
| | DO (mg/L) | 8.5 | 8.0 | 8.1 | 8.0 | 7.9 | 6.9 |
| | Conductivity (µmhos/cm) | 294 | | 298 | | 280 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.5 | 25.1 | 24.6 | 24.7 |
| 22% | pH (S.U.) | 8.07 | 8.03 | 8.20 | 8.04 | 8.16 | 7.66 |
| | DO (mg/L) | 8.5 | 8.0 | 8.1 | 8.1 | 8.1 | 6.5 |
| | Conductivity (µmhos/cm) | 288 | | 286 | | 278 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.5 | 25.1 | 24.6 | 24.7 |
| 43.97% | pH (S.U.) | 8.07 | 8.03 | 8.19 | 8.02 | 8.17 | 7.72 |
| | DO (mg/L) | 8.6 | 8.0 | 8.1 | 8.1 | 8.1 | 6.9 |
| | Conductivity (µmhos/cm) | 266 | | 263 | | 257 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.5 | 25.1 | 24.7 | 24.7 |
| 72% | pH (S.U.) | 8.08 | 8.03 | 8.19 | 8.01 | 8.16 | 7.74 |
| | DO (mg/L) | 8.6 | 8.0 | 8.1 | 8.1 | 8.1 | 6.8 |
| | Conductivity (µmhos/cm) | 235 | | 231 | | 228 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.5 | 25.1 | 24.8 | 24.7 |
| 100% | pH (S U.) | 8.07 | 8.06 | 8.18 | 7.99 | 8.18 | 7.79 |
| | DO (mg/L) | 8.5 | 8.0 | 8.3 | 8.0 | 8.3 | 7.1 |
| | Conductivity (µmhos/cm) | 202 | | 201 | | 200 | |
| | Alkalinity (mg $CaCO_3$/L) | 68 | | | | 73 | |
| | Hardness (mg $CaCO_3$/L) | 86 | | | | 89 | |
| | TR chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 24.4 | 25.1 | 24.5 | 25.1 | 24.8 | 24.7 |
| 100% INTAKE | pH (S U.) | 8.08 | 8.06 | 8.19 | 8.04 | 8.17 | 7.79 |
| | DO (mg/L) | 8.6 | 7.9 | 8.2 | 8.1 | 8.2 | 7.0 |
| | Conductivity (µmhos/cm) | 204 | | 200 | | 200 | |
| | Alkalinity (mg $CaCO_3$/L) | 70 | | | | 72 | |
| | Hardness (mg $CaCO_3$/L) | 80 | | | | 82 | |
| | TR chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 24.6 | 25.1 | 24.5 | 25.1 | 24.5 | 24.7 |
| | | Initial | Final | Initial | Final | Initial | Final |

**Species:** *Pimephales promelas*
**Client:** SEQUOYAH - NONTREATED

**Date:** 01-14-03

| Concentration | Parameter | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|
| CONTROL | pH (S U.) | 8.18 | 7.66 | 8.09 | 7.62 | 8.16 | 7.68 | 8.11 | 8.00 |
| | DO (mg/L) | 8.0 | 6.6 | 7.8 | 6.4 | 7.7 | 7.6 | 7.6 | 7.9 |
| | Conductivity (µmhos/cm) | 278 | | 286 | | 284 | | 303 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | | | 62 | | | |
| | Hardness (mg $CaCO_3$/L) | 86 | | | | 86 | | | |
| | Temperature (°C) | 24.4 | 24.9 | 24.4 | 25.3 | 24.6 | 24.6 | 24.7 | 24.8 |
| 10.98% | pH (S.U.) | 8.22 | 7.61 | 8.10 | 7.58 | 8.17 | 7.70 | 8.12 | 8.03 |
| | DO (mg/L) | 8.0 | 6.3 | 7.6 | 6.2 | 7.5 | 7.5 | 7.8 | 7.8 |
| | Conductivity (µmhos/cm) | 278 | | 286 | | 279 | | 297 | |
| | Temperature (°C) | 24.5 | 24.9 | 24.5 | 25.3 | 24.6 | 24.6 | 24.8 | 24.8 |
| 22% | pH (S U.) | 8.20 | 7.61 | 8.10 | 7.63 | 8.17 | 7.65 | 8.13 | 8.08 |
| | DO (mg/L) | 8.0 | 6.3 | 7.7 | 6.7 | 7.5 | 7.3 | 7.8 | 7.9 |
| | Conductivity (µmhos/cm) | 266 | | 278 | | 272 | | 286 | |
| | Temperature (°C) | 24.5 | 24.9 | 24.5 | 25.4 | 24.6 | 24.6 | 24.8 | 24.8 |
| 43.99% | pH (S.U.) | 8.18 | 7.65 | 8.09 | 7.64 | 8.15 | 7.72 | 8.14 | 8.09 |
| | DO (mg/L) | 8.3 | 6.3 | 7.8 | 6.7 | 7.5 | 7.3 | 8.0 | 8.2 |
| | Conductivity (µmhos/cm) | 246 | | 259 | | 254 | | 266 | |
| | Temperature (°C) | 24.5 | 24.9 | 24.8 | 25.4 | 24.6 | 24.6 | 24.8 | 24.8 |
| 72% | pH (S U.) | 8.16 | 7.69 | 8.11 | 7.67 | 8.13 | 7.72 | 8.14 | 8.16 |
| | DO (mg/L) | 8.6 | 6.6 | 7.9 | 6.5 | 8.0 | 7.5 | 8.1 | 8.1 |
| | Conductivity (µmhos/cm) | 220 | | 235 | | 227 | | 240 | |
| | Temperature (°C) | 24.5 | 24.9 | 25.0 | 25.4 | 24.6 | 24.6 | 24.8 | 24.8 |
| 100% | pH (S.U.) | 8.14 | 7.80 | 8.12 | 7.75 | 8.15 | 7.88 | 8.15 | 8.22 |
| | DO (mg/L) | 8.6 | 7.1 | 8.2 | 6.8 | 7.8 | 7.8 | 8.2 | 8.0 |
| | Conductivity (µmhos/cm) | 196 | | 208 | | 204 | | 213 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 71 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 89 | | | | | |
| | TR Chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 24.5 | 24.9 | 25.2 | 25.4 | 24.6 | 24.6 | 24.8 | 24.8 |
| 100% INTAKE | pH (S.U.) | 8.11 | 7.72 | 8.11 | 7.72 | 8.01 | 7.86 | 8.14 | 8.18 |
| | DO (mg/L) | 8.8 | 6.9 | 8.5 | 6.9 | 8.6 | 8.0 | 8.4 | 8.3 |
| | Conductivity (µmhos/cm) | 193 | | 209 | | 202 | | 214 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 74 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 82 | | | | | |
| | TR chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 24.4 | 24.9 | 24.8 | 25.4 | 24.9 | 24.6 | 24.8 | 24.8 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1002.0)
## Species: *Ceriodaphnia dubia*

### Daily Chemical Analyses

**Client:** Sequoyah Nuclear Plant Non-Treated
**Test dates:** January 14 - 21, 2003
**Project number:** 571

Mean Temp: 24.8
Min: 24.3
Max: 25.6

**Reviewed by:** Cur

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Control | pH (SU) | 8 06 | 8 23 | 8.19 | 8 19 | 8 15 | 8.21 | 8 18 | 8.18 | 8 09 | 8.16 | 8.16 | 8 14 | 8.11 | 8 16 |
| | DO (mg/L) | 7 9 | 8.1 | 7 7 | 8.3 | 7 9 | 8 2 | 8 0 | 8 0 | 7.8 | 8.1 | 7.7 | 8 2 | 7 6 | 7.8 |
| | Conductivity (µmhos/cm) | 308 | | 301 | | 303 | | 278 | | 286 | | 284 | | 303 | |
| | Alkalinity (mg/L $CaCO_3$) | 64 | | | | | | 60 | | | | 62 | | | |
| | Hardness (mg/L $CaCO_3$) | 84 | | | | | | 86 | | | | 86 | | | |
| | Temperature (°C) | 24 6 | 24 8 | 24 3 | 25 2 | 24 6 | 24 9 | 24.3 | 24 9 | 24 5 | 25 3 | 24 6 | 24.8 | 24 7 | 25.1 |
| 10.98% | pH (SU) | 8 05 | 8 23 | 8 21 | 8 20 | 8.17 | 8.22 | 8 22 | 8.19 | 8 10 | 8 17 | 8.17 | 8.16 | 8.12 | 8.19 |
| | DO (mg/L) | 8 5 | 8 2 | 8 1 | 8.3 | 7 9 | 8.2 | 8 0 | 8 0 | 7 6 | 8 1 | 7 5 | 8 2 | 7 8 | 7.9 |
| | Conductivity (µmhos/cm) | 294 | | 298 | | 288 | | 278 | | 286 | | 279 | | 297 | |
| | Temperature (°C) | 24 6 | 24.8 | 24 5 | 25.2 | 24 6 | 24 9 | 24 5 | 24 9 | 24 6 | 25 3 | 24.6 | 24 8 | 24 6 | 25 0 |
| 22% | pH (SU) | 8 07 | 8 24 | 8 20 | 8 20 | 8 16 | 8 26 | 8.20 | 8 22 | 8 10 | 8 20 | 8 17 | 8.16 | 8 13 | 8 20 |
| | DO (mg/L) | 8 5 | 8 2 | 8.1 | 8 3 | 8.1 | 8 2 | 8 0 | 8 0 | 7.7 | 8 2 | 7 5 | 8 2 | 7 8 | 8 0 |
| | Conductivity (µmhos/cm) | 288 | | 286 | | 278 | | 266 | | 278 | | 272 | | 286 | |
| | Temperature (°C) | 24 6 | 24 8 | 24.5 | 25 2 | 24 6 | 24 9 | 24 5 | 24 9 | 24 6 | 25 3 | 24 7 | 24 8 | 24 6 | 25 0 |
| 43.9% | pH (SU) | 8 07 | 8 24 | 8 19 | 8.19 | 8 17 | 8 24 | 8 18 | 8.21 | 8 09 | 8.20 | 8 15 | 8.16 | 8.14 | 8 21 |
| | DO (mg/L) | 8 6 | 8 3 | 8.1 | 8 2 | 8 1 | 8.2 | 8 3 | 8.1 | 7 8 | 8.3 | 7.5 | 8.1 | 8 0 | 8 0 |
| | Conductivity (µmhos/cm) | 266 | | 263 | | 257 | | 246 | | 259 | | 254 | | 264 | |
| | Temperature (°C) | 24 6 | 24 8 | 24 5 | 25.2 | 24 6 | 24 9 | 24 7 | 24 9 | 24 8 | 25 4 | 24.9 | 24 8 | 24 6 | 25 0 |
| 72% | pH (SU) | 8 08 | 8 28 | 8.19 | 8 21 | 8.16 | 8 31 | 8.16 | 8 27 | 8 11 | 8 26 | 8 13 | 8 21 | 8 14 | 8 27 |
| | DO (mg/L) | 8 6 | 8 3 | 8 1 | 8 2 | 8 1 | 8 1 | 8 6 | 8.2 | 7.9 | 8.3 | 8 0 | 8.1 | 8 1 | 8.0 |
| | Conductivity (µmhos/cm) | 235 | | 231 | | 228 | | 220 | | 235 | | 227 | | 240 | |
| | Temperature (°C) | 24 6 | 24 8 | 24.7 | 25 2 | 24.6 | 24 9 | 25.0 | 24 9 | 24.9 | 25 3 | 25.1 | 24 8 | 24.5 | 25 0 |
| 100% | pH (SU) | 8 07 | 8.30 | 8 18 | 8.22 | 8 18 | 8.30 | 8 14 | 8 30 | 8 12 | 8.28 | 8 15 | 8.25 | 8 15 | 8.30 |
| | DO (mg/L) | 8 5 | 8 4 | 8 3 | 8.3 | 8 3 | 8.3 | 8 6 | 8 2 | 8 2 | 8 3 | 7 8 | 8.2 | 8 2 | 7.8 |
| | Conductivity (µmhos/cm) | 202 | | 201 | | 200 | | 196 | | 208 | | 204 | | 213 | |
| | Alkalinity (mg/L $CaCO_3$) | 68 | | | | 73 | | | | 71 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 86 | | | | 89 | | | | 89 | | | | | |
| | Total Residual Chlorine (mg/L) | <0.10 | | | | <0.10 | | | | <0.10 | | | | | |
| | Temperature (°C) | 24 6 | 24 8 | 24 8 | 25 2 | 24.6 | 24.9 | 25.1 | 25 0 | 24.9 | 25 3 | 25 6 | 24 8 | 24.4 | 25 0 |
| 100% Intake | pH (SU) | 8 08 | 8 29 | 8 19 | 8 22 | 8 17 | 8 27 | 8.11 | 8 27 | 8 11 | 8 23 | 8 01 | 8 25 | 8.14 | 8.30 |
| | DO (mg/L) | 8 6 | 8.1 | 8 2 | 8 0 | 8 2 | 8.1 | 8 8 | 8.2 | 8 5 | 8 2 | 8 6 | 8 3 | 8 4 | 8.1 |
| | Conductivity (µmhos/cm) | 204 | | 200 | | 200 | | 193 | | 209 | | 202 | | 214 | |
| | Alkalinity (mg/L $CaCO_3$) | 70 | | | | 72 | | | | 74 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 80 | | | | 82 | | | | 82 | | | | | |
| | Total Residual Chlorine (mg/L) | <0 10 | | | | <0 10 | | | | <0 10 | | | | | |
| | Temperature (°C) | 24 6 | 24.8 | 24 4 | 25.2 | 24 6 | 24.9 | 24.8 | 25 0 | 24 8 | 25 3 | 24 9 | 24.8 | 24 5 | 25 0 |

**Species:** *Ceriodaphnia dubia*
**Client:** SEQUOYAH- NONTREATED

**Date:** 01-14-03

***Daily Chemistry:***

| Concentration | Parameter | Day 0 Initial | Day 0 Final | Day 1 Initial | Day 1 Final | Day 2 Initial | Day 2 Final |
|---|---|---|---|---|---|---|---|
| CONTROL | pH (S U.) | 8.06 | 8.23 | 8.19 | 8.19 | 8.15 | 8.21 |
| | DO (mg/L) | 7.9 | 8.1 | 7.7 | 8.3 | 7.9 | 8.2 |
| | Conductivity (µmhos/cm) | 308 | | 301 | | 303 | |
| | Alkalinity (mg $CaCO_3$/L) | 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | 84 | | | | | |
| | Temperature (°C) | 24.6 | 24.8 | 24.3 | 25.2 | 24.6 | 24.9 |
| 10.98% | pH (S U.) | 8.05 | 8.23 | 8.21 | 8.20 | 8.17 | 8.22 |
| | DO (mg/L) | 8.5 | 8.2 | 8.1 | 8.3 | 7.9 | 8.2 |
| | Conductivity (µmhos/cm) | 294 | | 298 | | 288 | |
| | Temperature (°C) | 24.6 | 24.8 | 24.5 | 25.2 | 24.6 | 24.9 |
| 22% | pH (S U.) | 8.07 | 8.24 | 8.20 | 8.20 | 8.16 | 8.26 |
| | DO (mg/L) | 8.5 | 8.2 | 8.1 | 8.3 | 8.1 | 8.2 |
| | Conductivity (µmhos/cm) | 288 | | 286 | | 278 | |
| | Temperature (°C) | 24.6 | 24.8 | 24.5 | 25.2 | 24.6 | 24.9 |
| 43.9% | pH (S U.) | 8.07 | 8.24 | 8.19 | 8.19 | 8.17 | 8.24 |
| | DO (mg/L) | 8.6 | 8.3 | 8.1 | 8.2 | 8.1 | 8.2 |
| | Conductivity (µmhos/cm) | 266 | | 263 | | 257 | |
| | Temperature (°C) | 24.6 | 24.8 | 24.5 | 25.2 | 24.6 | 24.9 |
| 72% | pH (S U.) | 8.08 | 8.28 | 8.19 | 8.21 | 8.16 | 8.31 |
| | DO (mg/L) | 8.6 | 8.3 | 8.1 | 8.2 | 8.1 | 8.1 |
| | Conductivity (µmhos/cm) | 235 | | 231 | | 228 | |
| | Temperature (°C) | 24.6 | 24.8 | 24.7 | 25.2 | 24.6 | 24.9 |
| 100% | pH (S U.) | 8.07 | 8.30 | 8.18 | 8.22 | 8.18 | 8.30 |
| | DO (mg/L) | 8.5 | 8.4 | 8.3 | 8.3 | 8.3 | 8.3 |
| | Conductivity (µmhos/cm) | 202 | | 201 | | 200 | |
| | Alkalinity (mg $CaCO_3$/L) | 68 | | | | 73 | |
| | Hardness (mg $CaCO_3$/L) | 86 | | | | 89 | |
| | TR chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 24.6 | 24.8 | 24.8 | 25.2 | 24.6 | 24.9 |
| 100% INTAKE | pH (S U.) | 8.08 | 8.29 | 8.19 | 8.22 | 8.17 | 8.27 |
| | DO (mg/L) | 8.6 | 8.1 | 8.2 | 8.0 | 8.2 | 8.1 |
| | Conductivity (µmhos/cm) | 204 | | 200 | | 200 | |
| | Alkalinity (mg $CaCO_3$/L) | 70 | | | | 72 | |
| | Hardness (mg $CaCO_3$/L) | 80 | | | | 82 | |
| | TR chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 24.6 | 24.8 | 24.4 | 25.2 | 24.6 | 24.9 |

Species: *Ceriodaphnia dubia*
Client: SEQUOYAH NONTREATED Date: 01-14-03

| Concentration | Parameter | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|
| CONTROL | pH (S U.) | 8.18 | 8.18 | 8.09 | 8.16 | 8.16 | 8.14 | 8.11 | 8.16 |
| | DO (mg/L) | 9.0 | 8.0 | 7.8 | 8.1 | 7.7 | 8.2 | 7.6 | 7.8 |
| | Conductivity (µmhos/cm) | 278 | | 286 | | 284 | | 303 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | | | 62 | | | |
| | Hardness (mg $CaCO_3$/L) | 86 | | | | 86 | | | |
| | Temperature (°C) | 24.3 | 24.9 | 24.5 | 25.3 | 24.6 | 24.8 | 24.7 | 25.1 |
| 10.98% | pH (S.U.) | 8.22 | 8.19 | 8.10 | 8.17 | 8.17 | 8.16 | 8.12 | 8.19 |
| | DO (mg/L) | 8.0 | 8.0 | 7.6 | 8.1 | 7.5 | 8.2 | 7.8 | 7.9 |
| | Conductivity (µmhos/cm) | 278 | | 286 | | 279 | | 297 | |
| | Temperature (°C) | 24.5 | 24.9 | 24.6 | 25.3 | 24.6 | 24.8 | 24.6 | 25.0 |
| 22% | pH (S U.) | 8.20 | 8.22 | 8.10 | 8.20 | 8.17 | 8.16 | 8.13 | 8.20 |
| | DO (mg/L) | 8.0 | 8.0 | 7.7 | 8.2 | 7.5 | 8.2 | 7.8 | 8.0 |
| | Conductivity (µmhos/cm) | 266 | | 278 | | 272 | | 286 | |
| | Temperature (°C) | 24.5 | 24.9 | 24.6 | 25.3 | 24.7 | 24.8 | 24.6 | 25.0 |
| 43.9% | pH (S U.) | 8.18 | 8.21 | 8.09 | 8.20 | 8.15 | 8.16 | 8.14 | 8.21 |
| | DO (mg/L) | 8.3 | 8.1 | 7.8 | 8.3 | 7.5 | 8.1 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 246 | | 259 | | 254 | | 264 ~~286~~ | |
| | Temperature (°C) | 24.7 | 24.9 | 24.8 | 25.4 | 24.9 | 24.8 | 24.6 | 25.0 |
| 72% | pH (S U.) | 8.16 | 8.27 | 8.11 | 8.26 | 8.13 | 8.21 | 8.14 | 8.27 |
| | DO (mg/L) | 8.6 | 8.2 | 7.9 | 8.3 | 8.0 | 8.1 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 220 | | 235 | | 227 | | 240 ~~266~~ | |
| | Temperature (°C) | 25.0 | 24.9 | 24.9 | 25.3 | 25.1 | 24.8 | 24.5 | 25.0 |
| 100% | pH (S.U.) | 8.14 | 8.30 | 8.12 | 8.28 | 8.15 | 8.25 | 8.15 | 8.30 |
| | DO (mg/L) | 8.6 | 8.2 | 8.2 | 8.3 | 7.8 | 8.2 | 8.2 | 7.8 |
| | Conductivity (µmhos/cm) | 196 | | 208 | | 204 | | 213 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 71 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 89 | | | | | |
| | TR Chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 25.1 | 25.0 | 24.9 | 25.3 | 25.6 | 24.8 | 24.4 | 25.0 |
| 100% INTAKE | pH (S U.) | 8.11 | 8.27 | 8.11 | 8.23 | 8.01 | 8.25 | 8.14 | 8.30 |
| | DO (mg/L) | 8.8 | 8.2 | 8.5 | 8.2 | 8.6 | 8.3 | 8.4 | 8.1 |
| | Conductivity (µmhos/cm) | 193 | | 209 | | 202 | | 214 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 74 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 82 | | | | | |
| | TR chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 24.8 | 25.0 | 24.8 | 25.3 | 24.9 | 24.8 | 24.5 | 25.0 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1000.0)
## Species: *Pimephales promelas*

### Daily Chemical Analyses

**Client:** Sequoyah Nuclear Plant UV-Treated
**Test dates:** January 14 - 21, 2003
**Project number:** 571

Mean Temp: 24.7
Min: 24.3
Max: 25.3

**Reviewed by:** Cen

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Control | pH (SU) | 8 03 | 8 08 | 8 17 | 8 06 | 8.14 | 7.72 | 8 14 | 7 76 | 8.11 | 7 66 | 8 09 | 7.77 | 8 10 | 8 01 |
| | DO (mg/L) | 8.1 | 8.2 | 8 6 | 8 4 | 8.3 | 6 9 | 8 2 | 7 4 | 8.1 | 6 9 | 8 5 | 7 8 | 8 4 | 8.0 |
| | Conductivity (µmhos/cm) | 306 | | 309 | | 299 | | 286 | | 299 | | 292 | | 302 | |
| | Alkalinity (mg/L $CaCO_3$) | 64 | | | | | | 60 | | | | 62 | | | |
| | Hardness (mg/L $CaCO_3$) | 84 | | | | | | 86 | | | | 86 | | | |
| | Temperature (°C) | 24 4 | 24.5 | 24.3 | 24 9 | 24 6 | 24 7 | 24.5 | 25 0 | 24 6 | 25.1 | 24 6 | 24 6 | 24.8 | 24 8 |
| 10.98% | pH (SU) | 8 02 | 8 07 | 8.18 | 8 02 | 8.14 | 7.75 | 8 12 | 7.69 | 8 10 | 7 63 | 8 07 | 7 78 | 8 11 | 7 98 |
| | DO (mg/L) | 8 2 | 8 2 | 8 4 | 8.2 | 8 2 | 6 9 | 8 2 | 6 9 | 8.1 | 6 5 | 8 6 | 7 6 | 8 3 | 7.7 |
| | Conductivity (µmhos/cm) | 300 | | 296 | | 291 | | 283 | | 295 | | 290 | | 295 | |
| | Temperature (°C) | 24 5 | 24 5 | 24 4 | 24.9 | 24 6 | 24 7 | 24 5 | 25 0 | 24.7 | 25.1 | 24 6 | 24 6 | 24 8 | 24 8 |
| 22% | pH (SU) | 8 04 | 8 07 | 8 18 | 8 04 | 8.13 | 7.71 | 8 11 | 7.71 | 8.10 | 7 66 | 8 07 | 7 85 | 8.10 | 8 01 |
| | DO (mg/L) | 8 0 | 8 2 | 8 5 | 8 2 | 8 2 | 7 0 | 8 2 | 6 9 | 7.9 | 6.8 | 8 6 | 7 6 | 8.3 | 7.7 |
| | Conductivity (µmhos/cm) | 286 | | 283 | | 278 | | 273 | | 282 | | 282 | | 280 | |
| | Temperature (°C) | 24 5 | 24 4 | 24 3 | 24 9 | 24 6 | 24.7 | 24 5 | 25 0 | 24 7 | 25.1 | 24 6 | 24 6 | 24 8 | 24 8 |
| 43.9% | pH (SU) | 8 04 | 8 07 | 8 18 | 8 04 | 8 14 | 7.74 | 8 09 | 7 71 | 8.11 | 7.66 | 8.07 | 7 83 | 8 09 | 8 01 |
| | DO (mg/L) | 7 9 | 8 2 | 8 4 | 8.3 | 8.3 | 6 9 | 8 0 | 7 0 | 7 8 | 6.9 | 8.5 | 7 9 | 8 2 | 8 0 |
| | Conductivity (µmhos/cm) | 264 | | 260 | | 256 | | 254 | | 261 | | 261 | | 261 | |
| | Temperature (°C) | 24.5 | 24.4 | 24.4 | 24 9 | 24 6 | 24 8 | 24 5 | 25.0 | 24.9 | 25 1 | 24 6 | 24 6 | 24 8 | 24 8 |
| 72% | pH (SU) | 8.05 | 8 09 | 8 18 | 8 06 | 8 15 | 7.75 | 8.08 | 7.70 | 8 11 | 7 67 | 8 08 | 7 82 | 8 10 | 8 01 |
| | DO (mg/L) | 7.9 | 8 2 | 8 4 | 8 3 | 8 3 | 6 9 | 8 0 | 6 9 | 7 8 | 6 9 | 8 5 | 7 8 | 8.2 | 8 0 |
| | Conductivity (µmhos/cm) | 230 | | 231 | | 228 | | 226 | | 235 | | 234 | | 233 | |
| | Temperature (°C) | 24.5 | 24 4 | 24 6 | 24 9 | 24 6 | 24 8 | 24 5 | 25 0 | 25.1 | 25.1 | 24 6 | 24 6 | 24 8 | 24 8 |
| 100% | pH (SU) | 8 04 | 8.13 | 8 18 | 8 06 | 8 16 | 7.76 | 8 06 | 7.75 | 8 10 | 7 70 | 8 07 | 7 86 | 8 13 | 8 05 |
| | DO (mg/L) | 7.9 | 8 2 | 8 5 | 8 4 | 8 3 | 7.0 | 7.9 | 6 9 | 7 8 | 6 9 | 8 5 | 7.7 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 201 | | 201 | | 199 | | 198 | | 208 | | 209 | | 207 | |
| | Alkalinity (mg/L $CaCO_3$) | 68 | | | | 73 | | | | 71 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 86 | | | | 89 | | | | 89 | | | | | |
| | Total Residual Chlorine (mg/L) | <0 10 | | | | <0 10 | | | | <0 10 | | | | | |
| | Temperature (°C) | 24.5 | 24 4 | 24 6 | 24 9 | 24 7 | 24 8 | 24 5 | 25 0 | 25 3 | 25 1 | 24 6 | 24 6 | 24 8 | 24 8 |
| 100% Intake | pH (SU) | 8 03 | 8 10 | 8 16 | 8 09 | 8 18 | 7 82 | 8 04 | 7.76 | 8.12 | 7.71 | 8 03 | 7 83 | 8 13 | 8 05 |
| | DO (mg/L) | 8 0 | 8 2 | 8 4 | 8 6 | 8 6 | 7 1 | 7 9 | 7.1 | 7 8 | 6 9 | 8 4 | 7.7 | 8 3 | 8.1 |
| | Conductivity (µmhos/cm) | 199 | | 198 | | 201 | | 197 | | 207 | | 207 | | 207 | |
| | Alkalinity (mg/L $CaCO_3$) | 70 | | | | 72 | | | | 74 | | | | | |
| | Hardness (mg/L $CaCO_3$) | 80 | | | | 82 | | | | 82 | | | | | |
| | Total Residual Chlorine (mg/L) | <0 10 | | | | <0 10 | | | | <0 10 | | | | | |
| | Temperature (°C) | 24 6 | 24 4 | 24 4 | 24.9 | 24.5 | 24 8 | 24 5 | 25 0 | 24 9 | 25 1 | 24 6 | 24 6 | 24 8 | 24 8 |

Species: *Pimephales promelas*

Client: SEQUOYAH UV-TREATED

Date: 01-14-03

*Daily Chemistry:*

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | |
|---|---|---|---|---|---|---|---|
| CONTROL | pH (S.U.) | 8.03 | 8.08 | 8.17 | 8.06 | 8.14 | 7.72 |
| | DO (mg/L) | 8.1 | 8.2 | 8.6 | 8.4 | 8.3 | 6.9 |
| | Conductivity (µmhos/cm) | 306 | | 309 | | 299 | |
| | Alkalinity (mg $CaCO_3$/L) | 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | 84 | | | | | |
| | Temperature (°C) | 24.4 | 24.5 | 24.3 | 24.9 | 24.6 | 24.7 |
| 10.98% | pH (S.U.) | 8.02 | 8.07 | 8.18 | 8.02 | 8.14 | 7.75 |
| | DO (mg/L) | 8.2 | 8.2 | 8.4 | 8.2 | 8.2 | 6.9 |
| | Conductivity (µmhos/cm) | 300 | | 296 | | 291 | |
| | Temperature (°C) | 24.5 | 24.5 | 24.4 | 24.9 | 24.6 | 24.7 |
| 22% | pH (S.U.) | 8.04 | 8.07 | 8.18 | 8.04 | 8.13 | 7.71 |
| | DO (mg/L) | 8.0 | 8.2 | 8.5 | 8.2 | 8.2 | 7.0 |
| | Conductivity (µmhos/cm) | 286 | | 283 | | 278 | |
| | Temperature (°C) | 24.5 | 24.4 | 24.3 | 24.9 | 24.6 | 24.7 |
| 43.9% | pH (S.U.) | 8.04 | 8.07 | 8.18 | 8.04 | 8.14 | 7.74 |
| | DO (mg/L) | 7.9 | 8.2 | 8.4 | 8.3 | 8.3 | 6.9 |
| | Conductivity (µmhos/cm) | 264 | | 260 | | 256 | |
| | Temperature (°C) | 24.5 | 24.4 | 24.4 | 24.9 | 24.6 | 24.8 |
| 72% | pH (S.U.) | 8.05 | 8.09 | 8.18 | 8.06 | 8.15 | 7.75 |
| | DO (mg/L) | 7.9 | 8.2 | 8.4 | 8.3 | 8.3 | 6.9 |
| | Conductivity (µmhos/cm) | 230 | | 231 | | 228 | |
| | Temperature (°C) | 24.5 | 24.4 | 24.6 | 24.9 | 24.6 | 24.8 |
| 100% | pH (S.U.) | 8.04 | 8.13 | 8.18 | 8.06 | 8.16 | 7.76 |
| | DO (mg/L) | 7.9 | 8.2 | 8.5 | 8.4 | 8.3 | 7.0 |
| | Conductivity (µmhos/cm) | 201 | | 201 | | 199 | |
| | Alkalinity (mg $CaCO_3$/L) | 68 | | | | 73 | |
| | Hardness (mg $CaCO_3$/L) | 86 | | | | 89 | |
| | TR chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 24.5 | 24.4 | 24.6 | 24.9 | 24.7 | 24.8 |
| 100% INTAKE | pH (S.U.) | 8.03 | 8.10 | 8.16 | 8.09 | 8.18 | 7.82 |
| | DO (mg/L) | 8.0 | 8.2 | 8.4 | 8.6 | 8.6 | 7.1 |
| | Conductivity (µmhos/cm) | 199 | | 198 | | 201 | |
| | Alkalinity (mg $CaCO_3$/L) | 70 | | | | 72 | |
| | Hardness (mg $CaCO_3$/L) | ~~84~~ 80 | | | | ~~89~~ 82 | |
| | TR chlorine (mg/L) | <0.10 | | | | <0.10 | |
| | Temperature (°C) | 24.6 | 24.4 | 24.4 | 24.9 | 24.5 | 24.8 |
| | | Initial | Final | Initial | Final | Initial | Final |

Species: *Pimephales promelas*
Client: SEQUOYAH UV TREATED Date: 01-14-03

| Concentration | Parameter | Day 3 Initial | Day 3 Final | Day 4 Initial | Day 4 Final | Day 5 Initial | Day 5 Final | Day 6 Initial | Day 6 Final |
|---|---|---|---|---|---|---|---|---|---|
| CONTROL | pH (S.U.) | 8.14 | 7.76 | 8.11 | 7.66 | 8.09 | 7.77 | 8.10 | 8.01 |
| | DO (mg/L) | 8.2 | 7.4 | 8.1 | 6.9 | 8.5 | 7.8 | 8.4 | 8.0 |
| | Conductivity (µmhos/cm) | 286 | | 299 | | 292 | | 302 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | | | 62 | | | |
| | Hardness (mg $CaCO_3$/L) | 86 | | | | 86 | | | |
| | Temperature (°C) | 24.5 | 25.0 | 24.6 | 25.1 | 24.6 | 24.6 | 24.8 | 24.8 |
| 10.98% | pH (S.U.) | 8.12 | 7.69 | 8.10 | 7.63 | 8.07 | 7.78 | 8.11 | 7.98 |
| | DO (mg/L) | 8.2 | 6.9 | 8.1 | 6.5 | 8.6 | 7.6 | 8.3 | 7.7 |
| | Conductivity (µmhos/cm) | 283 | | 295 | | 290 | | 295 | |
| | Temperature (°C) | 24.5 | 25.0 | 24.7 | 25.1 | 24.6 | 24.6 | 24.8 | 24.8 |
| 22% | pH (S.U.) | 8.11 | 7.71 | 8.10 | 7.66 | 8.07 | 7.85 | 8.10 | 8.01 |
| | DO (mg/L) | 8.2 | 6.9 | 7.9 | 6.8 | 8.6 | 7.6 | 8.3 | 7.7 |
| | Conductivity (µmhos/cm) | 273 | | 282 | | 282 | | 280 | |
| | Temperature (°C) | 24.5 | 25.0 | 24.7 | 25.1 | 24.6 | 24.6 | 24.8 | 24.8 |
| 43.9% | pH (S.U.) | 8.09 | 7.71 | 8.11 | 7.66 | 8.07 | 7.83 | 8.09 | 8.01 |
| | DO (mg/L) | 8.0 | 7.0 | 7.8 | 6.9 | 8.5 | 7.9 | 8.2 | 8.0 |
| | Conductivity (µmhos/cm) | 254 | | 261 | | 261 | | 261 | |
| | Temperature (°C) | 24.5 | 25.0 | 24.9 | 25.1 | 24.6 | 24.6 | 24.8 | 24.8 |
| 72% | pH (S.U.) | 8.08 | 7.70 | 8.11 | 7.67 | 8.08 | 7.82 | 8.10 | 8.01 |
| | DO (mg/L) | 8.0 | 6.9 | 7.8 | 6.9 | 8.5 | 7.8 | 8.2 | 8.0 |
| | Conductivity (µmhos/cm) | 226 | | 235 | | 234 | | 233 | |
| | Temperature (°C) | 24.5 | 25.0 | 25.1 | 25.1 | 24.6 | 24.6 | 24.8 | 24.8 |
| 100% | pH (S.U.) | 8.06 | 7.75 | 8.10 | 7.70 | 8.07 | 7.86 | 8.13 | 8.05 |
| | DO (mg/L) | 7.9 | 6.9 | 7.8 | 6.9 | 8.5 | 7.7 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 198 | | 208 | | 209 | | 207 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 71 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 89 | | | | | |
| | TR Chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 24.5 | 25.0 | 25.3 | 25.1 | 24.6 | 24.6 | 24.8 | 24.8 |
| 100% INTAKE | pH (S.U.) | 8.04 | 7.76 7.1 | 8.12 | 7.71 | 8.03 | 7.83 | 8.13 | 8.05 |
| | DO (mg/L) | 7.9 | ~~8.1~~ | 7.8 | 6.9 | 8.4 | 7.7 | 8.3 | 8.1 |
| | Conductivity (µmhos/cm) | 197 | | 207 | | 207 | | 207 | |
| | Alkalinity (mg $CaCO_3$/L) | | | 74 | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | 82 | | | | | |
| | TR chlorine (mg/L) | | | <0.10 | | | | | |
| | Temperature (°C) | 24.5 | 25.0 | 24.9 | 25.1 | 24.6 | 24.6 | 24.8 | 24.8 |

# Total Hardness
## (EPA Method 130.2)

Matrix: Water, MDL = 1.0 mg $CaCO_3$/L

| | |
|---|---|
| Analyst | KLW |
| Date analyzed | 01-27-03 |

*Titrant normality and multiplier determination:*

| Titrant reference number | Normality check standard number | Begin ml | End ml | Total ml (E) | Normality (N) of EDTA = 0.2/E (acceptable range = 0.018 - 0.022) | pH Factor or Multiplier = (N x 50000)/ 50 ml sample = N x 1000 |
|---|---|---|---|---|---|---|
| | | | | | | |

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Hardness (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS101 | 40 | 50 | 19.6 | 21.6 | 2.0 | 20.6 | 41.2 | 103% |

*Duplicate sample precision:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Hardness (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 030114.02 | SQN INT 1 | 50 | 21.6 | 25.5 | 3.9 | 20.6 | S 80 | |
| ↓ | Duplicate | 50 | 25.5 | 29.5 | 4.0 | 20.6 | D 82 | 2.5% |

*Matrix spike recovery:*

| Reference standard number | Spike value (SV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Spike hardness (A) (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| INSS101 | 40 | ~~25.5~~ 50 | 25.5 | 31.5 | 6.0 | 20.6 | 120 |

| Sample hardness (B) (mg $CaCO_3$/L) | Measured spike value (MV) MV = A - B (mg $CaCO_3$/L) | % R = MV / SV x 100 (acceptable range = 75 to 125%) |
|---|---|---|
| 82 | 38 | 95% |

*Sample measurements:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Hardness (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| | Blank (should be = 0 mg $CaCO_3$/L) | | | | | | |
| 030116.02 | SQN INT 2 | 50 | 31.5 | 35.5 | 4.0 | 20.6 | 82 |
| 030118.02 | ↓ 3 | 50 | 35.5 | 39.5 | 4.0 | | 82 |
| 030124.02 | FAP | 5 | 25.4 | 28.5 | 3.1 | (10) | 640 |
| 030124.01 | FGD | 2 | 39.5 | 45.1 | 5.6 | (25) | 2900 |
| 030125.09 | FGD 1 FAP | 2 | 28.5 | 30.2 | 1.7 | (25) | 890 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

Note: If >15ml of titrant is used, sample must be diluted. Reviewed by: KLW Date reviewed 01-28-03

# Total Residual Chlorine
## (EPA Method 330.5)

Matrix: Water, MDL = 0.10 mg/L
Meter: Accumet Model 25 pH/Ion Meter

| | |
|---|---|
| Analyst | JKK |
| Date analyzed | 01.14.03 |

| | |
|---|---|
| Iodide reagent: | INR060 |
| Acid reagent: | INR059 |
| Slope: | -47.3% |

*Calibration:*

| | 0.10 mg/L | 1.00 mg/L |
|---|---|---|
| Reference standard number | INSS091 | INSS091 |

*Note:* For samples with a residual chlorine of > 1 0 mg/L, the calibration range must be adjusted to bracket the chlorine levels of the samples

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS091 | 0.50 | 0.511 | 102.2% |

*Duplicate sample precision:*

| Sample number | Sample ID | Sample characteristics | Residual chlorine (mg/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|
| 030114.01 | SQN 101 | CLEAR, NO COLOR | S < 0.000213 | |
| ↓ | Duplicate | | D < 0.000208 | — |

*Sample measurements:*

| Sample number | Sample ID | Sample characteristics | Residual chlorine (mg/L) |
|---|---|---|---|
| | Blank (should be = < 0 10 mg/L) | | < 0.000318 |
| 030114.02 | SQN INTAKE | CLEAR, NO COLOR | < 0.000152 |
| 030114.04 | G.Town DRY Scale | NO COLOR, BLACK PARTICLES | < 0.000621 |
| 030114.03 | G.Town Scale Auto | NO COLOR, BLACK PARTICLES | < 0.000345 |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted.

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS 091 | 0.50 | 0.486 | 97.2% |

| | |
|---|---|
| Reviewed by | JP |
| Date reviewed | 01-28-03 |

# Total Residual Chlorine
## (EPA Method 330.5)

Matrix: Water, MDL = 0.10 mg/L
Meter: Accumet Model 25 pH/Ion Meter

| | |
|---|---|
| Analyst | KEW |
| Date analyzed | 01.16.03 |

| | |
|---|---|
| Iodide reagent: | INR060 |
| Acid reagent: | INR059 |
| Slope: | 50.87. |

***Calibration:***

| | 0.10 mg/L | 1.00 mg/L |
|---|---|---|
| Reference standard number | INS5091 | INS5091 |

*Note:* For samples with a residual chlorine of > 1.0 mg/L, the calibration range must be adjusted to bracket the chlorine levels of the samples.

***Laboratory control standard:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INS5091 | 0.50 | 0.508 | 101.6% |

***Duplicate sample precision:***

| Sample number | Sample ID | Sample characteristics | Residual chlorine (mg/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|
| 030116.01 | SQN 101 | no color, clear | S <0.00114 | |
| ↓ | Duplicate | | D <0.00853 | — |

***Sample measurements:***

| Sample number | Sample ID | Sample characteristics | Residual chlorine (mg/L) |
|---|---|---|---|
| | Blank (should be = < 0.10 mg/L) | | <0.00497 |
| 030116.02 | SQN INT | no color, floating particles | <0.00743 |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

KEW

*Note* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted

***Laboratory control standard:***

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INS5091 | 0.50 | 0.502 | 100.4% |

| | |
|---|---|
| Reviewed by | [illegible] |
| Date reviewed | 01-28-03 |

# Total Residual Chlorine
## (EPA Method 330.5)

Matrix: Water, MDL = 0.10 mg/L
Meter: Accumet Model 25 pH/Ion Meter

| | |
|---|---|
| Analyst | KW |
| Date analyzed | 01.10.03 |

| | |
|---|---|
| Iodide reagent: | INR060 |
| Acid reagent: | INR059 |
| Slope: | 48.0% |

*Calibration:*

| | 0.10 mg/L | 1.00 mg/L |
|---|---|---|
| Reference standard number | INSS091 | INSS091 |

*Note:* For samples with a residual chlorine of > 1.0 mg/L, the calibration range must be adjusted to bracket the chlorine levels of the samples.

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS091 | 0.50 | 0.537 | 107.4% |

*Duplicate sample precision:*

| Sample number | Sample ID | Sample characteristics | Residual chlorine (mg/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|
| 030118.01 | SQN 101 | no color, clear | S <0.0113 | |
| ↓ | Duplicate | | D <0.00793 | — |

*Sample measurements:*

| Sample number | Sample ID | Sample characteristics | Residual chlorine (mg/L) |
|---|---|---|---|
| | Blank (should be = < 0.10 mg/L) | | <0.0446 |
| 030118.02 | SQN INT | no color, clear | <0.00753 |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |

KK

*Note:* All samples were analyzed in excess of EPA recommended holding time (15 minutes) unless otherwise noted.

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg/L) | Measured value (MV) (mg/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|
| INSS091 | 0.50 | 0.533 | 106.6% |

| | |
|---|---|
| Reviewed by | JJ |
| Date reviewed | 01-28-03 |

# Alkalinity
## (EPA Method 310.1)

Matrix: Water, MDL = 1.0 mg $CaCO_3$/L

Analyst: KEL
Date analyzed: 01-27-03

**Titrate samples to pH = 4.50 S.U.**

*Titrant normality and multiplier determination:*

| pH of Deionized water = 4.5 S.U. | Titrant reference number | Normality check standard number | Begin ml | End ml | Total ml (E) | Normality (N) of $H_2SO_4$ = (5 ml $Na_2CO_3$ x 0.05)/E = 0.25/E (acceptable range = 0.018 - 0.022) | pH Factor or Multiplier = (N x 50000)/ 100 ml sample = N x 500 |
|---|---|---|---|---|---|---|---|
| 5.2 | INR055 ~~HSR038~~ | INOS110 | 0.1 | 12.5 | 12.4 | 0.0202 | 10.1 |

blk correction 0.0 - 0.1 = -0.1 ml

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INR065 | 100 | 100 | 12.4 | 22.5 | 10.1 | 10.1 | 102 | 102% |

*Duplicate sample precision:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Alkalinity (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 01-18-03A | MHS $H_2O$ | 100 | 22.5 | 28.6 | 6.1 | 10.1 | S 62 | |
| 01-18-03A | Duplicate | 100 | 28.6 | 35.0 | 6.4 | 10.1 | D 65 | 4.7 |

not enough to recheck - initial value within limits

*Matrix spike recovery:*

| Reference standard number | Spike value (SV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Spike alkalinity (A) (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| INR065 | 50 | 100 | 28.6 | 40.1 | 11.5 | 10.1 | 120 |

| Sample alkalinity (B) (mg $CaCO_3$/L) | Measured spike value (MV) MV = A - B (mg $CaCO_3$/L) | % R = MV / SV x 100 (acceptable range = 75 to 125%) |
|---|---|---|
| 65 | 55 | 110% |

*Sample measurements:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Alkalinity (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| 01-15-03 | MHS $H_2O$ | 100 | 40.7 | 46.6 | 5.9 | 10.1 | 60 |
| 01-18-038 | ↓ | ↓ | 0.5 | 6.5 | 6.0 | ↓ | 61 |
| 01-22-03 | ↓ | ↓ | 6.5 | 12.8 | 6.3 | ↓ | 64 |
| 01-23-03 | ↓ | ↓ | 12.8 | 19.1 | 6.3 | ↓ | 64 |
| 01-18-03 | SSW $H_2O$ | ↓ | 19.1 | 22.4 | 3.3 | ↓ | 33 |
| 030114.03 | G.Town Scale A | ↓ | 22.4 | 25.4 | 3.0 | ↓ | 30 |
| 030114.01 | SQN 101 1 | ↓ | 25.4 | 32.1 | 6.7 | ↓ | 68 |
| 030116.01 | ↓ 2 | ↓ | 32.1 | 39.3 | 7.2 | ↓ | 73 |
| 030118.01 | ↓ 3 | ↓ | 39.4 | 46.4 | 7.0 | ↓ | 71 |

Reviewed by: KH Date reviewed: 01-28-03

# Alkalinity
## (EPA Method 310.1)
Matrix: Water, MDL = 1.0 mg $CaCO_3$/L

Analyst: KLW
Date analyzed: 01.27.03

**Titrate samples to pH = 4.50 S.U.**

*Titrant normality and multiplier determination:*

| pH of Deionized water = 4.5 S.U. | Titrant reference number | Normality check standard number | Begin ml | End ml | Total ml (E) | Normality (N) of $H_2SO_4$ = (5 ml $Na_2CO_3$ x 0.05)/E = 0.25/E (acceptable range = 0.018 - 0.022) | pH Factor or Multiplier = (N x 50000)/ 100 ml sample = N x 500 |
|---|---|---|---|---|---|---|---|
| | | | | | | | |

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Alkalinity (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INSS101 | 100 | 100 | 0.1 | 10.0 | 9.9 | 10.1 | 100 | 100 % |

*Duplicate sample precision:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Alkalinity (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 030116.02 | SQN INT 2 | 100 | 10.0 | 17.1 | ~~17~~ 7.1 | 10.1 | S 72 | |
| ↓ | Duplicate | 100 | 17.2 | 24.3 | 7.1 | 10.1 | D 72 | – |

*Matrix spike recovery:*

| Reference standard number | Spike value (SV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Spike alkalinity (A) (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| INSS101 | 50 | 100 | 17.2 | 29.3 | 12.1 | 10.1 | 120 |

| Sample alkalinity (B) (mg $CaCO_3$/L) | Measured spike value (MV) MV = A - B (mg $CaCO_3$/L) | % R = MV / SV x 100 (acceptable range = 75 to 125%) |
|---|---|---|
| 72 | 48 | 96 % |

*Sample measurements:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Alkalinity (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| 030114.02 | SQN INT 1 | 100 | 29.3 ~~24.3~~ | 36.2 ~~29.3~~ | 6.9 | 10.1 | 70 |
| 030118.02 | ↓ 3 | ↓ | 36.6 | 43.9 | 7.3 | ↓ | 74 |
| 030124.02 | FAP | | 6.9 | 15.9 | 9.0 | | 91 |
| 030124.01 | FGD | | 15.9 | 21.1 | 5.2 | | 52 |
| 030125.09 | FAP \| FGD | | 21.1 | 29.7 | 8.6 | | 87 |
| 01.06.03 | Salt $H_2O$ | ↓ | 30.0 | 47.0 | 17.0 | ↓ | 170 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

Reviewed by: KLW Date reviewed: 01.28.03

# Total Hardness
## (EPA Method 130.2)
Matrix: Water, MDL = 1.0 mg $CaCO_3$/L

| | |
|---|---|
| Analyst | KBL |
| Date analyzed | 01.27.03 |

*Titrant normality and multiplier determination:*

| Titrant reference number | Normality check standard number | Begin ml | End ml | Total ml (E) | Normality (N) of EDTA = 0.2/E (acceptable range = 0.018 - 0.022) | pH Factor or Multiplier = (N x 50000)/ 50 ml sample = N x 1000 |
|---|---|---|---|---|---|---|
| INR050 | INS092 | 1.0 | ~~10.7~~ 10.7 | 9.7 | 0.0206 | 20.6 |

*Laboratory control standard:*

| Reference standard number | True value (TV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Hardness (MV) (mg $CaCO_3$/L) | % RS = MV / TV x 100 (acceptable range = 90 to 110%) |
|---|---|---|---|---|---|---|---|---|
| INS101 | 40 | 50 | 10.7 | 12.7 | 2.0 | 20.6 | 41.2 | 103% |

*Duplicate sample precision:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Hardness (mg $CaCO_3$/L) | %RPD = {(S - D) /[(S+D)/2]} x 100 (acceptable range = ± 10%) |
|---|---|---|---|---|---|---|---|---|
| 01.15.03 | MHS H20 | 50 | 12.7 | 16.9 | 4.2 | 20.6 | S 86 | |
| 01.15D3 | Duplicate | 50 | 16.9 | 21.1 | 4.2 | 20.6 | D 86 | — |

*Matrix spike recovery:*

| Reference standard number | Spike value (SV) (mg $CaCO_3$/L) | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Spike hardness (A) (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| INS901 | 40 | 50 | 16.9 | 23.0 | 6.1 | 20.6 | 130 |

| Sample hardness (B) (mg $CaCO_3$/L) | Measured spike value (MV) MV = A - B (mg $CaCO_3$/L) | % R = MV / SV x 100 (acceptable range = 75 to 125%) |
|---|---|---|
| 86 | 44 | 110% |

*Sample measurements:*

| Sample number | Sample ID | Sample volume (ml) | Begin ml | End ml | Total ml | Multiplier | Hardness (mg $CaCO_3$/L) |
|---|---|---|---|---|---|---|---|
| TV=ND | Blank (should be = 0 mg $CaCO_3$/L) | 50 | 1.0 | 1.0 | 0.0 | 20.6 | ND |
| 01.18.03A | MHS H2O | | 23.0 | 27.2 | 4.2 | | 86 |
| 01.18.03B | ↓ | | 27.2 | 31.1 | 3.9 | | 80 |
| 01.22.03 | | | 31.1 | 35.4 | 4.3 | | 89 |
| 01.25.03 | ↓ | | 35.4 | 39.3 | 3.9 | | 80 |
| 01.18.03 | SSW H2O | | 39.3 | 41.5 | 2.2 | | 45 |
| 030114.03 | G.Town Scale A | | 41.5 | 43.6 | 2.1 | | 43 |
| 030114.01 | SAN 101 1 | | 6.8 | 11.0 | 4.2 | | 86 |
| 030116.01 | ↓ 2 | | 11.0 | 15.3 | 4.3 | | 89 |
| 030118.01 | ↓ 3 | | 15.3 | 19.6 | 4.3 | | 89 |

Note If >15ml of titrant is used, sample must be diluted. Reviewed by: KBL Date reviewed 01.28.03

Sequoyah Nuclear Plant Biomonitoring
January 14 - 21, 2003

Appendix D

Reference Toxicant Test and
Control Chart

# Environmental Testing Solutions, LLC

## Potassium Chloride Chronic Reference Toxicant Control Chart
## for *Pimephales promelas*
## using Moderately Hard Synthetic Water

| Test number | Test date | 7-day $IC_{25}$ (g KCl/L) | CT (g/L KCl) | S | Control Limits CT - 2S | Control Limits CT + 2S | $S_{A.10}$ | Warning Limits CT - $S_{A.10}$ | Warning Limits CT + $S_{A.10}$ | $S_{A.25}$ | Control Limits CT - $S_{A.25}$ | Control Limits CT + $S_{A.25}$ | CV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 05-07-02 | 0 61 | | | | | | | | | | | |
| 2 | 05-23-02 | 0.76 | 0 69 | 0.11 | 0 47 | 0 90 | 0 08 | 0.57 | 0 81 | 0.14 | 0 48 | 0.90 | 0.15 |
| 3 | 05-24-02 | 0 66 | 0 68 | 0 08 | 0.52 | 0 83 | 0 08 | 0 56 | 0.80 | 0.14 | 0 47 | 0 89 | 0.11 |
| 4 | 06-04-02 | 0 67 | 0 68 | 0 06 | 0.55 | 0 80 | 0 08 | 0 56 | 0 80 | 0.14 | 0.47 | 0 89 | 0 09 |
| 5 | 06-11-02 | 0 70 | 0 68 | 0 06 | 0.57 | 0.79 | 0 08 | 0.56 | 0 80 | 0.14 | 0.47 | 0.89 | 0.08 |
| 6 | 08-06-02 | 0.55 | 0.66 | 0 07 | 0 52 | 0 80 | 0 08 | 0.54 | 0.78 | 0 14 | 0 45 | 0 87 | 0.11 |
| 7 | 08-13-02 | 0 58 | 0 65 | 0 07 | 0.50 | 0 79 | 0 08 | 0 53 | 0.77 | 0.14 | 0.44 | 0 86 | 0.11 |
| 8 | 08-20-02 | 0 63 | 0 65 | 0 07 | 0.51 | 0.78 | 0 08 | 0 53 | 0.77 | 0 14 | 0.44 | 0.86 | 0.10 |
| 9 | 09-10-02 | 0 61 | 0 64 | 0 06 | 0.51 | 0.77 | 0 08 | 0 52 | 0 76 | 0 13 | 0.43 | 0 85 | 0.10 |
| 10 | 09-17-02 | 0 64 | 0 64 | 0 06 | 0 52 | 0.76 | 0 08 | 0 52 | 0.76 | 0.13 | 0 43 | 0 85 | 0 09 |
| 11 | 10-01-02 | 0.60 | 0 64 | 0 06 | 0 52 | 0.75 | 0 08 | 0.52 | 0.76 | 0.13 | 0 43 | 0 85 | 0 09 |
| 12 | 10-01-02 | 0 47 | 0 62 | 0 07 | 0 48 | 0.77 | 0 07 | 0.50 | 0.74 | 0.13 | 0.41 | 0 83 | 0.12 |
| 13 | 10-08-02 | 0 53 | 0 62 | 0 08 | 0 47 | 0.77 | 0 07 | 0 50 | 0.74 | 0.13 | 0.41 | 0.83 | 0 12 |
| 14 | 10-15-02 | 0 66 | 0 62 | 0.07 | 0.47 | 0.77 | 0 07 | 0 50 | 0.74 | 0.13 | 0.41 | 0 83 | 0 12 |
| 15 | 10-22-02 | 0 64 | 0 62 | 0 07 | 0 48 | 0.76 | 0 07 | 0.50 | 0.74 | 0.13 | 0.41 | 0 83 | 0.11 |
| 16 | 11-05-02 | 0 59 | 0 62 | 0 07 | 0 48 | 0.76 | 0 07 | 0 50 | 0.74 | 0 13 | 0.41 | 0.83 | 0 11 |
| 17 | 12-03-02 | 0 61 | 0 62 | 0 07 | 0.48 | 0 75 | 0 07 | 0 50 | 0 74 | 0 13 | 0 41 | 0.83 | 0.11 |
| 18 | 12-03-02 | 0.52 | 0 61 | 0 07 | 0 47 | 0.75 | 0 07 | 0 49 | 0.73 | 0 13 | 0.40 | 0 82 | 0.11 |
| 19 | 01-07-03 | 0 64 | 0 61 | 0 07 | 0 48 | 0.75 | 0 07 | 0.49 | 0.73 | 0.13 | 0 40 | 0 82 | 0.11 |
| 20 | 01-14-03 | 0.61 | 0 61 | 0 07 | 0 48 | 0 74 | 0 07 | 0 49 | 0.73 | 0.13 | 0.40 | 0 82 | 0.11 |

*Note·* 7-d $IC_{25}$ = 7-day 25% inhibition concentration. An estimation of the concentration of potassium chloride that would cause a 25% reduction in Pimephales growth for the test population.

CT = Central tendency (mean $IC_{25}$)

S = Standard deviation of the $IC_{25}$ values

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile CV. $S_{A.10}$ = 0.12, as determined by USEPA for the method and endpoint.

$S_{A.25}$ = Standard deviation corresponding to the 25th percentile CV. $S_{A.25}$ = 0 21, as determined by the USEPA for the method and endpoint.

CV = Coefficient of variation of the IC25 values

Control and warning limits were established using the standard deviation of the $IC_{25}$ values corresponding to the 10th and 25th percentile CVs. These ranges are more stringent than the control and warning limits recommended by USEPA for the test method and endpoint

USEPA Recommended Control and Warning Limits,

Warning Limit = Standard deviation corresponding to the 75th percentile CV. $S_{A.75}$ = 0 38.

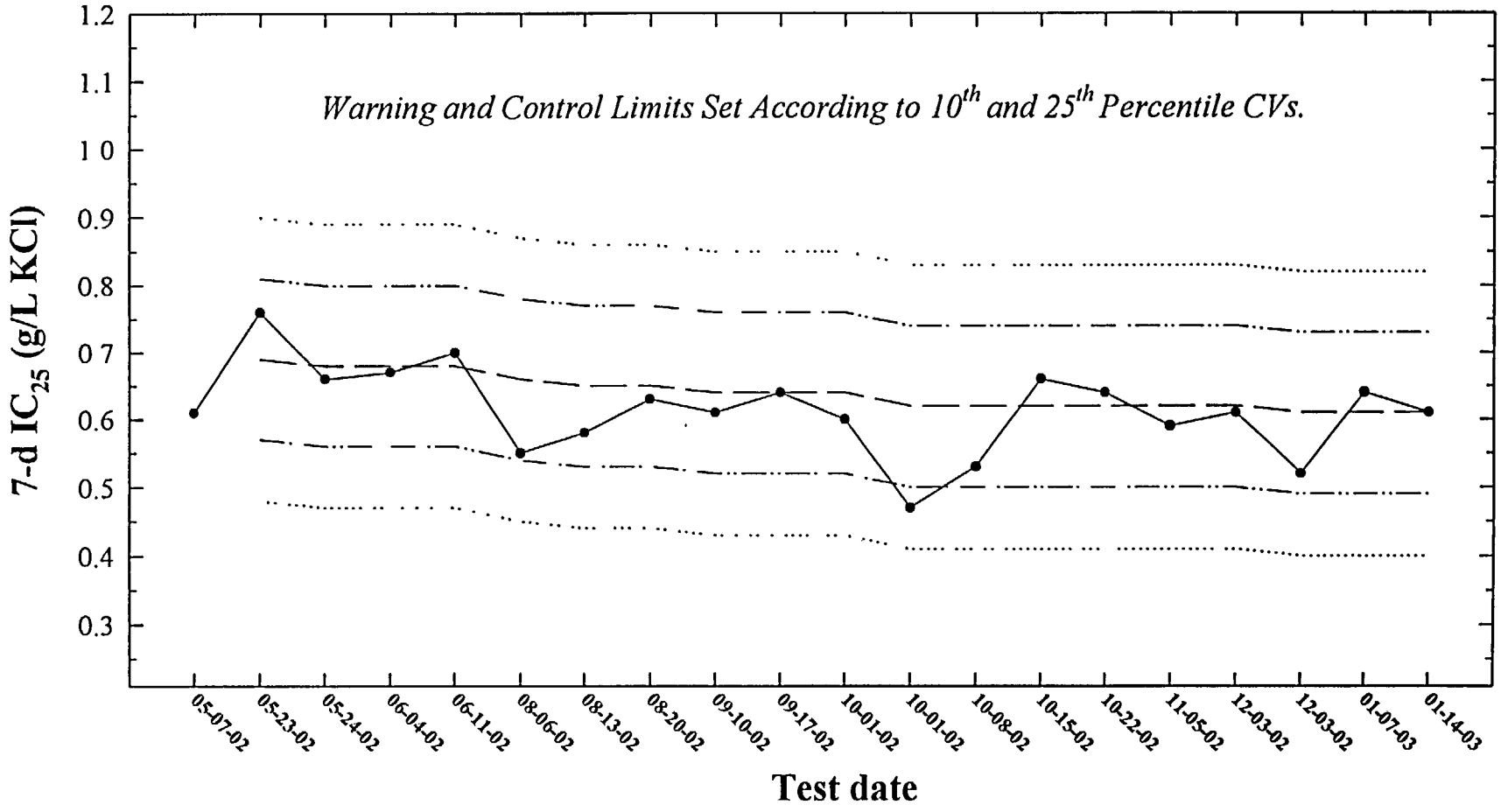
Control Limit = Standard deviation corresponding to the 90th percentile CV. $S_{A.90}$ = 0 45.

USEPA 2000 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH

*Organisms obtained from Aquatic BioSystems, Inc*

# Environmental Testing Solutions, LLC

## Potassium Chloride Chronic Reference Toxicant Control Chart for *Pimephales promelas* using Moderately Hard Synthetic Water




—•— **7-day $IC_{25}$** = 25% inhibition concentration. An estimation of the concentration of potassium chloride that would cause a 25% reduction in *Pimephales* growth for the test population.

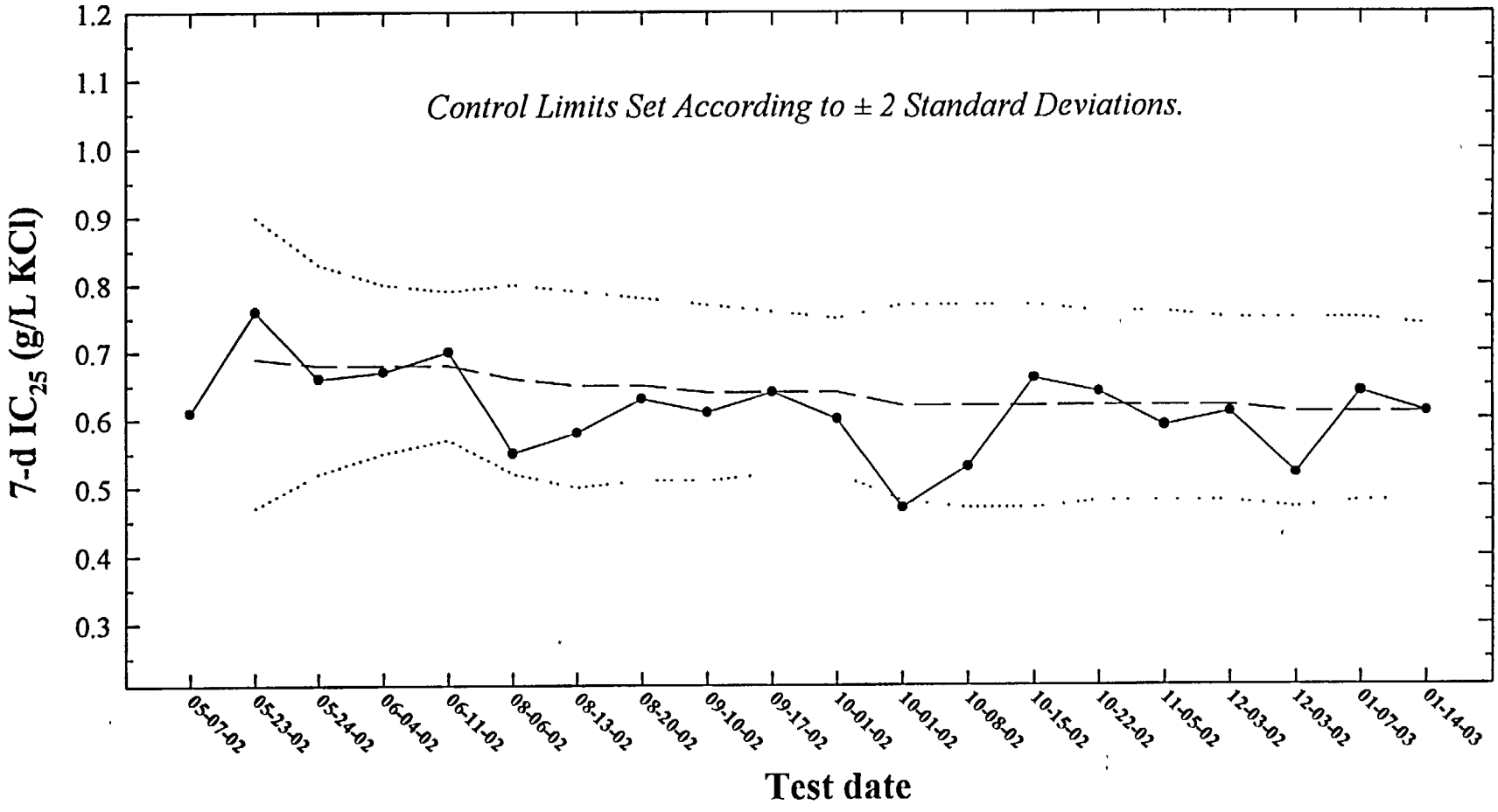
— – **Central Tendency** (mean $IC_{25}$)

— · — **Warning Limits** (mean $IC_{25} \pm S_{A.10}$, $S_{A.10} = 0.12$, as determined by USEPA for the method and endpoint)

·····  **Control Limits** (mean $IC_{25} \pm S_{A\,25}$, $S_{A\,25} = 0.21$, as determined by USEPA for the method and endpoint)

# Environmental Testing Solutions, LLC

## Potassium Chloride Chronic Reference Toxicant Control Chart for *Pimephales promelas* using Moderately Hard Synthetic Water




—•— **7-day $IC_{25}$** = 25% inhibition concentration. An estimation of the concentration of potassium chloride that would cause a 25% reduction in *Pimephales* growth for the test population.

— – **Central Tendency** (mean $IC_{25}$)

. . .. **Control Limits** (mean $IC_{25}$ ± 2 Standard Deviations)

# Environmental Testing Solutions, LLC

## Precision of Endpoint Measurements

### Potassium Chloride Chronic Reference Toxicant Data for *Pimephales promelas* using Moderately Hard Synthetic Water

| Test number | Test date | Control Survival (%) | Control Mean Growth (mg/larvae) | CV (%) | CT for Control Growth CV (%) | MSD | PMSD (%) | CT for PMSD (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 05-07-02 | 97.5 | 0.458 | 12.3 | | 0 07 | 15.4 | |
| 2 | 05-23-02 | 100 | 0 378 | 9.9 | 11.1 | 0 09 | 22.5 | 19.0 |
| 3 | 05-24-02 | 97.5 | 0.444 | 21.3 | 14.5 | 0.11 | 25.0 | 21.0 |
| 4 | 06-04-02 | 100 | 0.682 | 14 0 | 14.4 | 0.15 | 22.1 | 21.3 |
| 5 | 06-11-02 | 100 | 0 650 | 3.7 | 12.2 | 0.11 | 16.6 | 20 3 |
| 6 | 08-06-02 | 97.5 | 0 694 | 22.2 | 13.9 | 0.16 | 23.5 | 20 9 |
| 7 | 08-13-02 | 100 | 0 659 | 13.6 | 13.8 | 0 12 | 17.5 | 20 4 |
| 8 | 08-20-02 | 100 | 0 765 | 8.5 | 13.2 | 0 12 | 16.3 | 19 9 |
| 9 | 09-10-02 | 100 | 0 854 | 1.2 | 11.8 | 0 11 | 12.5 | 19.1 |
| 10 | 09-17-02 | 100 | 0.824 | 13.4 | 12.0 | 0.12 | 14 6 | 18 6 |
| 11 | 10-01-02 | 97.5 | 0.750 | 18.4 | 12.6 | 0 19 | 25 4 | 19.2 |
| 12 | 10-01-02 | 100 | 0.975 | 12 7 | 12.6 | 0 13 | 12.8 | 18 7 |
| 13 | 10-08-02 | 97.5 | 0 929 | 8.0 | 12.2 | 0 18 | 19.5 | 18 8 |
| 14 | 10-15-02 | 100 | 1.037 | 16 9 | 12.6 | 0 23 | 21.9 | 19 0 |
| 15 | 10-22-02 | 100 | 0 822 | 10 6 | 12.4 | 0.13 | 15 6 | 18 7 |
| 16 | 11-05-02 | 100 | 0 874 | 2 8 | 11.8 | 0.12 | 13.8 | 18 4 |
| 17 | 12-03-02 | 100 | 0 852 | 9.1 | 11.7 | 0.12 | 13.7 | 18 2 |
| 18 | 12-03-02 | 100 | 0 668 | 10 4 | 11.6 | 0.15 | 22 4 | 18 4 |
| 19 | 01-07-03 | 100 | 0 886 | 4 1 | 11.2 | 0.14 | 15.7 | 18 3 |
| 20 | 01-14-03 | 100 | 0 677 | 3 0 | 10 8 | 0.07 | 11 0 | 17 9 |

*Note·* Average control growth (mg/larvae) = **0.744**

CV = Coefficient of variation for control survival
On average, the CV for control growth is **10.8%** in Environmental Testing Solutions, LLC Pimephales chronic toxicity tests
Lower CV bound determined by USEPA (10th percentile) = 3 5%
Upper CV bound determined by USEPA (90th percentile) = 20%

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. On average, a significant difference occurs for Environmental Testing Solutions, LLC chronic toxicity tests when a toxicant reduces Pimephales growth by **17.9%** from the control
Lower PMSD bound determined by USEPA (10th percentile) = 9 4%
Upper PMSD bound determined by USEPA (90th percentile) = 35%

CT = Central Tendancy (mean Control Growth CV or mean PMSD)

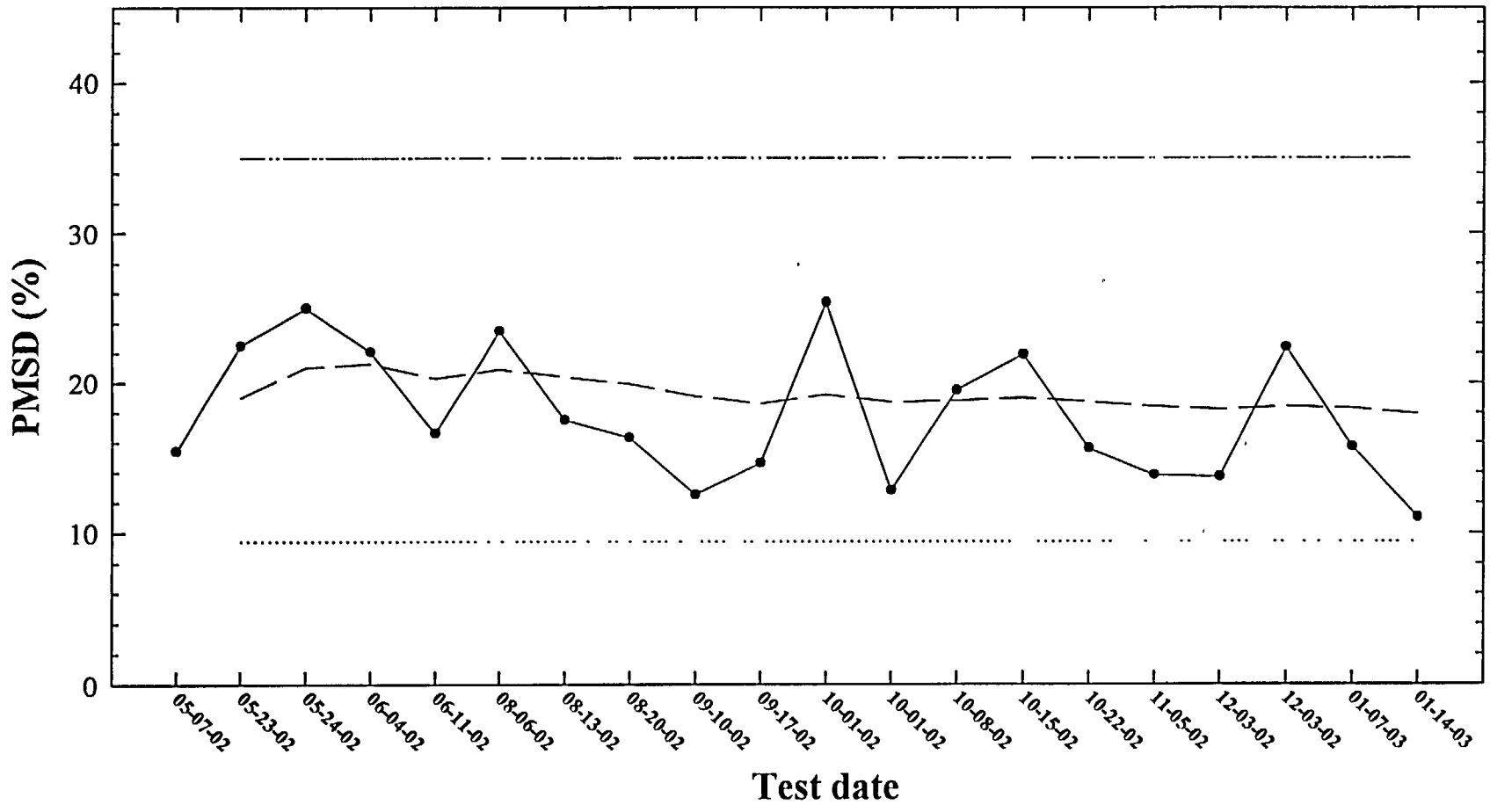
The lower and upper bounds were calculated by the USEPA using 205 tests conducted from 19 laboratories for Pimephales growth in chronic reference toxicant tests

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program EPA-833-R-00-003 US Environmental Protection Agency, Cincinnati, OH.

# Environmental Testing Solutions, LLC

## Precision of Endpoint Measurements

### Potassium Chloride Chronic Reference Toxicant Control Chart for *Pimephales promelas* using Moderately Hard Synthetic Water




—●— **PMSD** = percent minimum significant difference. PMSD is the minimum significant difference between the control and treatment that can be declared statistically significant

— · **Central Tendency** (mean PMSD)

····· **Lower and Upper PMSD Bounds**
Lower PMSD Bound (10th percentile) = 9 4%, Upper PMSD Bound (90th percentile) = 35%
(Lower and upper PMSD bounds were determined by USEPA for the method and endpoint )

# Potassium Chloride Chronic Reference Toxicant Test
## (EPA-821-R-02-013 Method 1000.0)
## Species: *Pimephales promelas*

PpKCICR Test Number: 3

| *Dilution preparation information:* | | | | | | *Comments:* |
|---|---|---|---|---|---|---|
| KCl CHM number. | | CHM067 | | | | |
| Stock preparation: | | 50 g KCl/L· Dissolve 50 g KCl in 1-L Deionized water | | | | |
| Dilution prep (mg/L) | 300 | 450 | 600 | 750 | 900 | |
| Stock volume (mL) | 6 | 9 | 12 | 15 | 18 | |
| Diluent volume (mL) | 994 | 991 | 988 | 985 | 982 | |
| Total volume (mL) | 1000 | 1000 | 1000 | 1000 | 1000 | |

| *Test organism information:* | | *Test information:* | |
|---|---|---|---|
| Organism age. | 24-HOURS OLD | Randomizing template. | RED |
| Date and times organisms were born between: | 01-13-03 1230 TO 1530 HST | Incubator number: | 2 |
| Organism source: | ABS BATCH 01-13-03 | *Artemia* lot number: | BG1809Q |
| Transfer bowl information· | pH = 7.96 Temperature = 24.3 °C | Total drying time· | 11.25-HOURS |
| Average transfer volume: | 10.4 mL | Date / Time in· | 01-21-03 1450 |
| | | Date / Time out: | 01-22-03 0800 |
| | | Oven temperature: | 101°C |

*Daily feeding and renewal information:*

| Day | Date | Morning feeding time | Afternoon feeding time | Test initiation, renewal, or termination time | Control water batch used | Analyst |
|---|---|---|---|---|---|---|
| 0 | 01-14-03 | —— | 1530 | 1400 | MHS 01-12-03 | JJ |
| 1 | 01-15-03 | 0914 | 1520 | 1342 | MHS 01-12-03 | JJ |
| 2 | 01-16-03 | 0841 | 1450 | 1311 | MHS 01-12-03 | JJ |
| 3 | 01-17-03 | 0908 | 1510 | 1317 | MHS 01-15-03 | JJ |
| 4 | 01-18-03 | 0910 | 1517 | 1308 | MHS 01-15-03 | JJ |
| 5 | 01-19-03 | 0918 | 1520 | 1320 | MHS 01-18-03 | JJ |
| 6 | 01-20-03 | 0837 | 1500 | 1309 | MHS 01-18-03 | JJ |
| 7 | 01-21-03 | | | 1352 | —— | JJ |

| *Control information:* | | Acceptance criteria |
|---|---|---|
| % Mortality: | 0% | ≤ 20% |
| Average weight per initial larvae: | 0.6772 | |
| Average weight per surviving larvae: | 0.6772 | ≥ 0.25 mg/larvae |

| *Summary of test endpoints:* | |
|---|---|
| 7-day $LC_{50}$ | 766.2 |
| NOEC | ~~600~~ 450 |
| LOEC | ~~750~~ 600 |
| ChV | ~~670.8~~ 519.6 |
| $IC_{25}$ | 607.9 |

PpKClCR Test Number: 3

*Survival and Growth Data*

| Day | CONTROL | | | | 300 mg KCl/L | | | | 450 mg KCl/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J | K | L |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 2 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 d | 10 | 9 d |
| 3 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 d | 9 | 10 | 9 |
| 4 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 10 | 9 |
| 5 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 10 | 9 |
| 6 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 10 | 9 |
| 7 | 10 | 10 | 1 SMALL 10 | 10 | 10 | 10 | 10 | 10 | 9 | 9 | 10 | 9 |
| A = Pan weight (mg) Red | 15.051 | 15.100 | 14.935 | 14.983 | 14.592 | 15.040 | 14.658 | 14.733 | 15.001 | 15.006 | 15.000 | 14.946 |
| B = Pan + Larvae weight (mg) | 21.72 | 21.99 | 21.92 | 21.53 | 21.14 | 21.44 | 20.69 | 20.90 | 20.21 | 21.73 | 21.51 | 20.75 |
| Larvae weight (mg) = A – B | 6.669 | 6.890 | 6.985 | 6.545 | 6.548 | 6.400 | 6.032 | 6.167 | 5.209 | 6.724 | 6.510 | 6.104 |

Calculations and data reviewed: JL

| *Comments:* |
|---|
| |
| |
| |
| |
| |

PpKClCR Test Number. 3

*Survival and Growth Data*

| Day | 600 mg KCl/L | | | | 750 mg KCl/L | | | | 900 mg KCl/L | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | M | N | O | P | Q | R | S | T | U | V | W | X |
| 0 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| 1 | 10 | 10 | 10 | 9 1d | 8 2d | 9 1d | 10 | 10 | 8 2d | 6 4d | 4 6d | 7 3d |
| 2 | 9 1d | 10 | 10 | 9 | 6 2d | 9 | 7 3d | 9 1d | 3 5d | 4 2d | 3 1d | 5 2d |
| 3 | 9 | 10 | 10 | 8 1d | 6 | 9 | 4 3d | 7 2d | 3 | 4 | 3 | 5 |
| 4 | 9 | 10 | 10 | 8 | 6 | 8 1d | 4 | 7 | 3 | 3 1d | 2 1d | 5 |
| 5 | 8 1d | 10 | 9 1d | 8 | 6 | 7 1d | 4 | 7 | 3 | 3 | 2 | 5 |
| 6 | 8 | 10 | 9 | 8 | 6 | 7 | 4 | 7 | 2 1d | 2 1d | 2 | 5 |
| 7 | 8 | 10 | 9 | 8 | 6 | 7 | 3 1d | 7 | 2 | 2 | 1 1d | 4 1d |
| A = Pan weight (mg) | 14.763 | 15.114 | 15.184 | 15.219 | 15.087 | 15.125 | 14.685 | 14.704 | 14.979 | 15.172 | 14.996 | 15.120 |
| B = Pan + Larvae weight (mg) | 19.72 | 20.34 | 21.15 | 19.89 | 18.24 | 18.32 | 16.35 | 17.92 | 15.88 | 16.00 | 15.43 | 17.40 |
| Larvae weight (mg) = A – B | 4.957 | 5.226 | 5.966 | 4.671 | 3.153 | 3.195 | 1.665 | 3.211 | 0.901 | 0.828 | 0.434 | 2.280 |

Calculations and data reviewed. JH

| *Comments:* |
|---|
| |
| |
| |
| |
| |

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA/600/4-91/002, Method 1000.0)
## Species: *Pimephales promelas*

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

**Test number:** PpKClCR # 43

**Test dates:** January 14 - 21, 2003

**Reveiwed by:**

| Concentration (mg/L KCl) | Replicate | Initial number of larvae | Final number of larvae | A = Pan weight (mg) | B = Pan + Larvae weight (mg) | Larvae weight (mg) = A - B | Weight / Initial number of larvae (mg) | Mean survival (%) | Mean weight (mg) | Coefficient of variation (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | A | 10 | 10 | 15 051 | 21 720 | 6 669 | 0 6669 | 100 0 | 0 6772 | 3 0 | Not applicable |
| | B | 10 | 10 | 15 100 | 21 990 | 6 890 | 0 6890 | | | | |
| | C | 10 | 10 | 14 935 | 21 920 | 6 985 | 0 6985 | | | | |
| | D | 10 | 10 | 14 985 | 21 530 | 6 545 | 0 6545 | | | | |
| 300 | E | 10 | 10 | 14 592 | 21 140 | 6 548 | 0 6548 | 100 0 | 0 6287 | 3.7 | 7.2 |
| | F | 10 | 10 | 15 060 | 21 460 | 6 400 | 0 6400 | | | | |
| | G | 10 | 10 | 14 658 | 20 690 | 6 032 | 0 6032 | | | | |
| | H | 10 | 10 | 14 733 | 20 900 | 6 167 | 0 6167 | | | | |
| 450 | I | 10 | 9 | 15 001 | 20 210 | 5 209 | 0 5209 | 92 5 | 0 6137 | 10 9 | 9 4 |
| | J | 10 | 9 | 15 006 | 21 730 | 6 724 | 0 6724 | | | | |
| | K | 10 | 10 | 15 000 | 21 510 | 6 510 | 0 6510 | | | | |
| | L | 10 | 9 | 14 646 | 20 750 | 6 104 | 0 6104 | | | | |
| 600 | M | 10 | 8 | 14 763 | 19 720 | 4 957 | 0 4957 | 87 5 | 0 5205 | 10.7 | 23.1 |
| | N | 10 | 10 | 15 114 | 20 340 | 5 226 | 0 5226 | | | | |
| | O | 10 | 9 | 15 184 | 21 150 | 5 966 | 0 5966 | | | | |
| | P | 10 | 8 | 15 219 | 19 890 | 4 671 | 0 4671 | | | | |
| 750 | Q | 10 | 6 | 15 087 | 18 240 | 3 153 | 0 3153 | 57 5 | 0 2806 | 27.1 | 58 6 |
| | R | 10 | 7 | 15 125 | 18 320 | 3 195 | 0 3195 | | | | |
| | S | 10 | 3 | 14 685 | 16 350 | 1 665 | 0 1665 | | | | |
| | T | 10 | 7 | 14 709 | 17 920 | 3 211 | 0 3211 | | | | |
| 900 | U | 10 | 2 | 14 979 | 15 880 | 0 901 | 0 0901 | 22 5 | 0.1111 | 72 6 | 83 6 |
| | V | 10 | 2 | 15 172 | 16 000 | 0 828 | 0 0828 | | | | |
| | W | 10 | 1 | 14 996 | 15 430 | 0 434 | 0 0434 | | | | |
| | X | 10 | 4 | 15 120 | 17 400 | 2 280 | 0 2280 | | | | |

**Dunnett's MSD value.** 0 0747

**PMSD** 11.0

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test On average, a significant difference occurs for Environmental Testing Solutions, LLC chronic toxicity tests when a toxicant reduces Pimephales growth by 17 9% from the control (determined through reference toxicant testing)

Lower PMSD bound determined by USEPA (10th percentile) = 9 4%.

Upper PMSD bound determined by USEPA (90th percentile) = 35%.

The lower and upper bounds were calculated by the USEPA using 205 tests conducted from 19 laboratories for Pimephales growth in chronic reference toxicant tests

USEPA 2000 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program EPA-833-R-00-003 US Environmental Protection Agency, Cincinnati, OH

*Organisms obtained from Aquatic BioSystems, Inc*

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Larval Fish Growth and Survival Test-7 Day Growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID: | PpKClCR | Sample ID | REF-Ref Toxicant |
| End Date | 1/21/03 | Lab ID: | ETS-Env Testing Solutions | Sample Type | KCL-Potassium chloride |
| Sample Date | | Protocol: | EPAF 91-EPA Freshwater | Test Species: | PP-Pimephales promelas |
| Comments | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 0 6669 | 0 6890 | 0 6985 | 0 6545 |
| 300 | 0.6548 | 0 6400 | 0 6034 | 0 6167 |
| 450 | 0.5209 | 0 6724 | 0 6510 | 0 6104 |
| 600 | 0.4957 | 0.5226 | 0 5966 | 0 4671 |
| 750 | 0 3153 | 0.3195 | 0 1665 | 0 3211 |
| 900 | 0 0901 | 0 0828 | 0 0434 | 0 2280 |

| | | | Transform Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| D-Control | 0 6772 | 1 0000 | 0 6772 | 0 6545 | 0 6985 | 2.971 | 4 | | | | 0 6772 | 1 0000 |
| 300 | 0 6287 | 0 9284 | 0 6287 | 0 6034 | 0 6548 | 3 664 | 4 | 1 487 | 2 290 | 0 0747 | 0 6287 | 0 9284 |
| 450 | 0 6137 | 0 9062 | 0 6137 | 0 5209 | 0 6724 | 10 915 | 4 | 1 949 | 2 290 | 0 0747 | 0 6137 | 0 9062 |
| *600 | 0 5205 | 0 7686 | 0 5205 | 0 4671 | 0 5966 | 10 675 | 4 | 4 805 | 2 290 | 0 0747 | 0 5205 | 0 7686 |
| 750 | 0 2806 | 0 4143 | 0 2806 | 0 1665 | 0 3211 | 27 123 | 4 | | | | 0 2806 | 0 4143 |
| 900 | 0 1111 | 0 1640 | 0 1111 | 0 0434 | 0 2280 | 72 567 | 4 | | | | 0 1111 | 0 1640 |

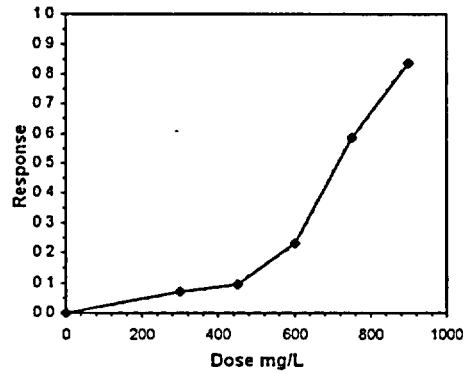
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0 01) | 0 978783071 | 0 844 | -0 294340189 | 0 81047745 |
| Bartlett's Test indicates equal variances (p = 0 17) | 5 08170414 | 11 34488201 | | |

| Hypothesis Test (1-tail, 0 05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 450 | 600 | 519 6152423 | | 0 074686143 | 0 110282614 | 0 017191687 | 0 002127351 | 0 003268048 | 3, 12 |

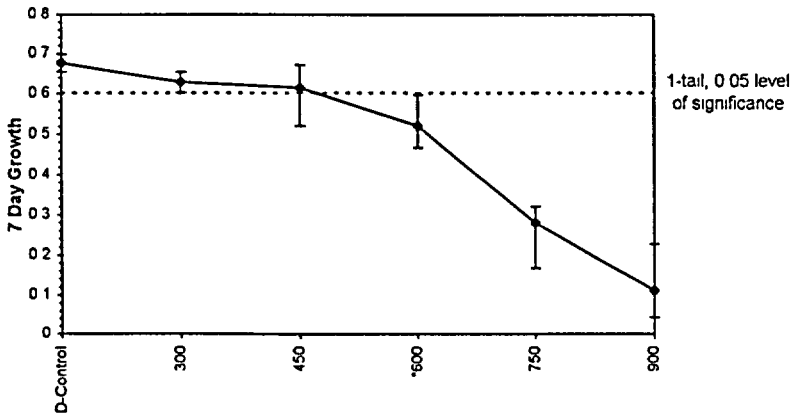
**Linear Interpolation (80 Resamples)**

| Point | mg/L | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05* | 209 47 | 99 35 | 100 51 | 615 49 | 0 9262 |
| IC10 | 456 72 | 68 67 | 178 35 | 533 06 | -0 8708 |
| IC15 | 511 23 | 36 88 | 385 26 | 646 37 | -0 0122 |
| IC20 | 565 74 | 28.33 | 483 20 | 644 00 | 0 2266 |
| IC25 | 607 87 | 18 18 | 538 08 | 644 29 | -0 3037 |
| IC40 | 671.38 | 12.57 | 634 30 | 707 60 | 0 2316 |
| IC50 | 713 73 | 16 39 | 666 92 | 757 68 | 0 1007 |

* indicates IC estimate less than the lowest concentration




**Dose-Response Plot**




*Organisms obtained from Aquatic BioSystems, Inc*

01-14-03 xls

# Environmental Testing Solutions, LLC
## Statistical Analyses

**Larval Fish Growth and Survival Test-7 Day Survival**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 1/14/03 | Test ID | PpKCICR | Sample ID | REF-Ref Toxicant |
| End Date | 1/21/03 | Lab ID- | ETS-Env Testing Solutions | Sample Type | KCL-Potassium chloride |
| Sample Date | | Protocol | EPAF 91-EPA Freshwater | Test Species | PP-Pimephales promelas |
| Comments | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| D-Control | 1 0000 | 1 0000 | 1 0000 | 1 0000 |
| 300 | 1 0000 | 1 0000 | 1 0000 | 1 0000 |
| 450 | 0 9000 | 0 9000 | 1 0000 | 0 9000 |
| 600 | 0.8000 | 1 0000 | 0 9000 | 0 8000 |
| 750 | 0 6000 | 0 7000 | 0 3000 | 0 7000 |
| 900 | 0 2000 | 0 2000 | 0 1000 | 0 4000 |

| | | | Transform· Arcsin Square Root | | | | | Rank | 1-Tailed | Number | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Resp | Number |
| D-Control | 1 0000 | 1 0000 | 1 4120 | 1 4120 | 1 4120 | 0 000 | 4 | | | 0 | 40 |
| 300 | 1 0000 | 1 0000 | 1 4120 | 1 4120 | 1 4120 | 0 000 | 4 | 18 00 | 10 00 | 0 | 40 |
| 450 | 0 9250 | 0 9250 | 1 2898 | 1 2490 | 1 4120 | 6.318 | 4 | 12 00 | 10 00 | 3 | 40 |
| 600 | 0 8750 | 0 8750 | 1 2188 | 1 1071 | 1 4120 | 11.906 | 4 | 12 00 | 10 00 | 5 | 40 |
| *750 | 0 5750 | 0 5750 | 0 8620 | 0 5796 | 0 9912 | 22.581 | 4 | 10 00 | 10 00 | 17 | 40 |
| *900 | 0 2250 | 0 2250 | 0 4834 | 0.3218 | 0 6847 | 31 014 | 4 | 10 00 | 10 00 | 31 | 40 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0 01) | 0 91671133 | 0 884 | -0 316165842 | 1.346744523 |
| Equality of variance cannot be confirmed | | | | |

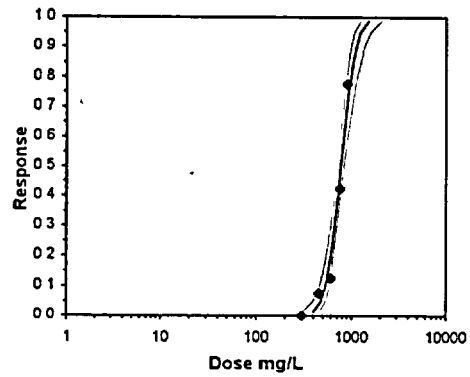
| Hypothesis Test (1-tail, 0 05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 600 | 750 | 670 8203932 | |

**Maximum Likelihood-Probit**

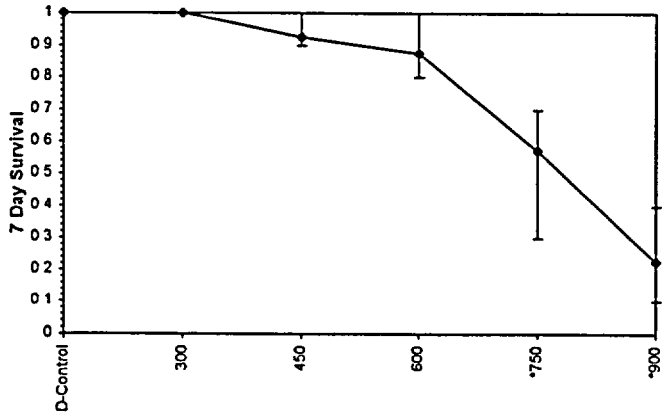
| Parameter | Value | SE | 95% Fiducial Limits | |
|---|---|---|---|---|
| Slope | 7 973053748 | 1 226780987 | 5 568562967 | 10 37754453 |
| Intercept | -17 99699151 | 3 501004343 | -24 85896016 | -11 13502287 |
| TSCR | | | | |

| Control | Chi-Sq | Critical | P-value | Mu | Sigma | Iter |
|---|---|---|---|---|---|---|
| 0 | 4 775674923 | 7 814724922 | 0 19 | 2 884339205 | 0 125422458 | 3 |

| Point | Probits | mg/L | 95% Fiducial Limits | |
|---|---|---|---|---|
| EC01 | 2 674 | 391.3478345 | 298 8202155 | 454 7289305 |
| EC05 | 3 355 | 476 4722581 | 394 0635725 | 531 6781157 |
| EC10 | 3 718 | 529 180533 | 455 680513 | 579 1682188 |
| EC15 | 3 964 | 567 9984976 | 501 707135 | 614 6857919 |
| EC20 | 4 158 | 600 8706682 | 540 6378554 | 645 5854668 |
| EC25 | 4.326 | 630 5841393 | 575 3650197 | 674 5827642 |
| EC40 | 4 747 | 712 1373932 | 664 9097825 | 762 8281132 |
| EC50 | 5 000 | 766 1948124 | 717 5883266 | 830 2770165 |
| EC60 | 5 253 | 824 3556709 | 768 9792198 | 910 1076376 |
| EC75 | 5 674 | 930 969317 | 854 5525702 | 1070 235307 |
| EC80 | 5 842 | 977 0064101 | 889 455908 | 1143 436754 |
| EC85 | 6 036 | 1033 549401 | 931 2294474 | 1236 051701 |
| EC90 | 6 282 | 1109 365193 | 985 7885374 | 1364 419056 |
| EC95 | 6 645 | 1232 085304 | 1071 437863 | 1581.301415 |
| EC99 | 7 326 | 1500 083795 | 1250 293331 | 2089 403779 |




**Dose-Response Plot**




*Organisms obtained from Aquatic BioSystems, Inc*

PpKClCR Test Number: 3

*Daily Chemistry:*

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final | Initial | Final |
| CONTROL | pH (S.U.) | 8.06 | 8.03 | 8.19 | 8.11 | 8.15 | 7.67 |
| | DO (mg/L) | 7.9 | 7.7 | 7.7 | 8.4 | 7.9 | 6.6 |
| | Conductivity (µmhos/cm) | 308 | | 301 | | 303 | |
| | Alkalinity (mg $CaCO_3$/L) | 64 | | | | | |
| | Hardness (mg $CaCO_3$/L) | 84 | | | | | |
| | Temperature (°C) | 24.5 | 24.8 | 24.3 | 24.4 | 24.2 | 24.7 |
| 300 mg KCl/L | pH (S.U.) | 8.05 | 7.96 | 8.17 | 8.05 | 8.14 | 7.63 |
| | DO (mg/L) | 7.8 | 7.6 | 7.8 | 8.2 | 8.6 | 6.8 |
| | Conductivity (µmhos/cm) | 858 | | 839 | | 857 | |
| | Temperature (°C) | 24.5 | 24.8 | 24.3 | 24.4 | 24.3 | 24.7 |
| 450 mg KCl/L | pH (S.U.) | 8.05 | 7.95 | 8.16 | 8.10 | 8.15 | 7.65 |
| | DO (mg/L) | 8.0 | 7.7 | 7.9 | 8.4 | 8.6 | 6.8 |
| | Conductivity (µmhos/cm) | 1126 | | 1113 | | 1124 | |
| | Temperature (°C) | 24.5 | 24.8 | 24.3 | 24.4 | 24.3 | 24.7 |
| 600 mg KCl/L | pH (S U.) | 8.04 | 7.90 | 8.15 | 8.06 | 8.13 | 7.63 |
| | DO (mg/L) | 8.0 | 7.7 | 7.9 | 8.4 | 8.6 | 6.8 |
| | Conductivity (µmhos/cm) | 1402 | | 1376 | | 1385 | |
| | Temperature (°C) | 24.5 | 24.8 | 24.3 | 24.4 | 24.2 | 24.7 |
| 750 mg KCl/L | pH (S U.) | 8.03 | 7.93 | 8.14 | 8.04 | 8.10 | 7.63 |
| | DO (mg/L) | 8.2 | 7.7 | 7.8 | 8.4 | 8.6 | 6.9 |
| | Conductivity (µmhos/cm) | 1664 | | 1627 | | 1641 | |
| | Temperature (°C) | 24.5 | 24.8 | 24.3 | 24.4 | 24.2 | 24.7 |
| 900 mg KCl/L | pH (S.U.) | 8.02 | 7.91 | 8.14 | 8.05 | 8.14 | 7.68 |
| | DO (mg/L) | 8.1 | 7.7 | 7.9 | 8.4 | 8.6 | 7.3 |
| | Conductivity (µmhos/cm) | 1894 | | 1873 | | 1873 | |
| | Temperature (°C) | 24.5 | 24.8 | 24.3 | 24.4 | 24.2 | 24.7 |

PpKClCR Test Number: 3

| Concentration | Parameter | Day 3 Initial | Day 3 Final | Day 4 Initial | Day 4 Final | Day 5 Initial | Day 5 Final | Day 6 Initial | Day 6 Final |
|---|---|---|---|---|---|---|---|---|---|
| CONTROL | pH (S.U.) | 8.18 | 7.81 | 8.09 | 7.82 | 8.16 | 7.72 | 8.11 | 7.90 |
| | DO (mg/L) | 8.0 | 7.4 | 7.8 | 7.6 | 7.7 | 7.4 | 7.6 | 7.9 |
| | Conductivity (µmhos/cm) | 278 | | 296 | | 284 | | 303 | |
| | Alkalinity (mg $CaCO_3$/L) | 60 | | | | 62 | | | |
| | Hardness (mg $CaCO_3$/L) | 86 | | | | 86 | | | |
| | Temperature (°C) | 24.4 | 25.1 | 24.3 | 24.6 | 24.5 | 24.6 | 24.3 | 24.5 |
| 300 mg KCl/L | pH (S.U.) | 8.17 | 7.81 | 8.16 | 7.71 | 8.15 | 7.68 | 8.15 | 7.89 |
| | DO (mg/L) | 8.3 | 7.4 | 8.4 | 7.2 | 8.6 | 7.4 | 8.4 | 7.7 |
| | Conductivity (µmhos/cm) | 787 | | 859 | | 834 | | 873 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.4 | 24.6 | 24.5 | 24.6 | 24.4 | 24.7 |
| 450 mg KCl/L | pH (S.U.) | 8.14 | 7.81 | 8.15 | 7.71 | 8.15 | 7.70 | 8.16 | 7.90 |
| | DO (mg/L) | 8.5 | 7.4 | 8.6 | 7.2 | 8.6 | 7.4 | 8.4 | 7.6 |
| | Conductivity (µmhos/cm) | 1046 | | 1137 | | 1104 | | 1154 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.4 | 24.6 | 24.5 | 24.6 | 24.4 | 24.7 |
| 600 mg KCl/L | pH (S.U.) | 8.13 | 7.77 | 8.14 | 7.78 | 8.15 | 7.65 | 8.15 | 7.86 |
| | DO (mg/L) | 8.3 | 7.4 | 8.6 | 7.6 | 8.6 | 7.4 | 8.2 | 7.6 |
| | Conductivity (µmhos/cm) | 1291 | | 1404 | | 1374 | | 1435 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.4 | 24.6 | 24.5 | 24.6 | 24.4 | 24.5 |
| 750 mg KCl/L | pH (S.U.) | 8.10 | 7.83 | 8.12 | 7.77 | 8.14 | 7.64 | 8.13 | 7.85 |
| | DO (mg/L) | 8.3 | 7.4 | 8.5 | 7.6 | 8.5 | 7.3 | 8.4 | 7.5 |
| | Conductivity (µmhos/cm) | 1534 | | 1652 | | 1628 | | 1698 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.4 | 24.6 | 24.5 | 24.6 | 24.4 | 24.5 |
| 900 mg KCl/L | pH (S.U.) | 8.10 | 7.80 | 8.13 | 7.86 | 8.13 | 7.76 | 8.13 | 7.93 |
| | DO (mg/L) | 8.2 | 7.5 | 8.5 | 7.9 | 8.4 | 7.3 | 8.4 | 7.4 |
| | Conductivity (µmhos/cm) | 1758 | | 1860 | | 1857 | | 1924 | |
| | Temperature (°C) | 24.4 | 25.0 | 24.4 | 24.6 | 24.5 | 24.6 | 24.4 | 24.5 |

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# Environmental Testing Solutions, LLC

## Sodium Chloride Chronic Reference Toxicant Control Chart
## for *Ceriodaphnia dubia*
## using Moderately Hard Synthetic Water

| Test number | Test date | 7-d $IC_{25}$ (g/L NaCl) | CT (g/L NaCl) | S | Control Limits CT - 2S | Control Limits CT + 2S | $S_{A.10}$ | Warning Limits CT - $S_{A.10}$ | Warning Limits CT + $S_{A.10}$ | $S_{A.25}$ | Control Limits CT - $S_{A.25}$ | Control Limits CT + $S_{A.25}$ | CV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 11-01-01 | 1.00 | | | | | | | | | | | |
| 2 | 12-05-01 | 1.05 | 1.03 | 0 04 | 0 96 | 1 10 | 0 08 | 0 95 | 1 11 | 0.17 | 0 86 | 1 20 | 0 04 |
| 3 | 01-09-02 | 0.91 | 0.99 | 0 07 | 0 84 | 1 13 | 0 08 | 0 91 | 1 07 | 0.17 | 0 82 | 1.16 | 0 07 |
| 4 | 02-05-02 | 1.07 | 1.01 | 0 07 | 0 86 | 1.16 | 0 08 | 0 93 | 1 09 | 0 17 | 0 84 | 1.18 | 0 07 |
| 5 | 03-19-02 | 1.03 | 1.01 | 0 06 | 0 89 | 1.14 | 0 08 | 0.93 | 1 09 | 0.17 | 0 84 | 1.18 | 0 06 |
| 6 | 04-09-02 | 1.03 | 1.02 | 0.06 | 0.90 | 1.13 | 0 08 | 0.94 | 1.10 | 0 17 | 0 85 | 1.19 | 0 06 |
| 7 | 05-07-02 | 1 05 | 1.02 | 0 05 | 0.91 | 1.13 | 0 08 | 0.94 | 1.10 | 0 17 | 0 85 | 1.19 | 0 05 |
| 8 | 06-04-02 | 1 06 | 1 03 | 0 05 | 0 92 | 1.13 | 0 08 | 0 95 | 1.11 | 0.17 | 0 86 | 1.20 | 0 05 |
| 9 | 07-09-02 | 1.03 | 1.03 | 0 05 | 0 93 | 1.12 | 0.08 | 0 95 | 1.11 | 0 17 | 0 86 | 1 20 | 0 05 |
| 10 | 08-06-02 | 1 05 | 1.03 | 0.05 | 0 94 | 1.12 | 0 08 | 0 95 | 1.11 | 0.18 | 0 86 | 1.20 | 0 05 |
| 11 | 09-04-02 | 1 05 | 1.03 | 0 04 | 0.94 | 1.12 | 0 08 | 0 95 | 1.11 | 0.18 | 0 86 | 1.20 | 0 04 |
| 12 | 10-08-02 | 1.03 | 1 03 | 0 04 | 0 95 | 1.12 | 0 08 | 0 95 | 1.11 | 0.18 | 0 86 | 1 20 | 0 04 |
| 13 | 11-05-02 | 1.03 | 1.03 | 0 04 | 0 95 | 1.11 | 0.08 | 0 95 | 1.11 | 0 18 | 0 86 | 1.20 | 0 04 |
| 14 | 12-03-02 | 1 02 | 1.03 | 0 04 | 0 95 | 1.11 | 0 08 | 0.95 | 1.11 | 0 18 | 0 86 | 1.20 | 0 04 |
| 15 | 12-03-02 | 1 03 | 1.03 | 0 04 | 0.95 | 1.11 | 0 08 | 0 95 | 1.11 | 0.18 | 0 86 | 1 20 | 0.04 |
| 16 | 12-04-02 | 1 02 | 1.03 | 0 04 | 0.96 | 1.10 | 0 08 | 0 95 | 1.11 | 0.18 | 0.86 | 1 20 | 0 04 |
| 17 | 12-06-02 | 1.03 | 1.03 | 0 04 | 0 96 | 1.10 | 0 08 | 0 95 | 1.11 | 0 18 | 0 86 | 1.20 | 0 03 |
| 18 | 12-11-02 | 1.04 | 1.03 | 0 03 | 0 96 | 1.10 | 0 08 | 0.95 | 1.11 | 0.18 | 0 86 | 1.20 | 0 03 |
| 19 | 12-18-02 | 1 04 | 1.03 | 0.03 | 0.96 | 1.10 | 0 08 | 0 95 | 1.11 | 0.18 | 0 86 | 1 20 | 0.03 |
| 20 | 01-07-03 | 0 96 | 1 03 | 0.04 | 0.95 | 1.10 | 0 08 | 0 95 | 1.11 | 0.17 | 0.86 | 1.20 | 0 04 |

*Note:*

7-d $IC_{25}$ = 7-day 25% inhibition concentration. An estimation of the concentration of sodium chloride that would cause a 25% reduction in Ceriodaphnia reproduction for the test population.

CT = Central tendency (mean $IC_{25}$)

S = Standard deviation of the $IC_{25}$ values

$S_{A.10}$ = Standard deviation corresponding to the 10th percentile CV. $S_{A.10}$ = 0 08, as determined by USEPA for the method and endpoint.

$S_{A.25}$ = Standard deviation corresponding to the 25th percentile CV. $S_{A.25}$ = 0.17, as determined by the USEPA for the method and endpoint

CV = Coefficient of variation of the $IC_{25}$ values

Control and warning limits were established using the standard deviation of the $IC_{25}$ values corresponding to the 10th and 25th percentile CVs These ranges are more stringent than the control and warning limits recommended by USEPA for the test method and endpoint.

USEPA Recommended Control and Warning Limits.

Warning Limit = Standard deviation corresponding to the 75th percentile CV $S_{A.75}$ = 0 45

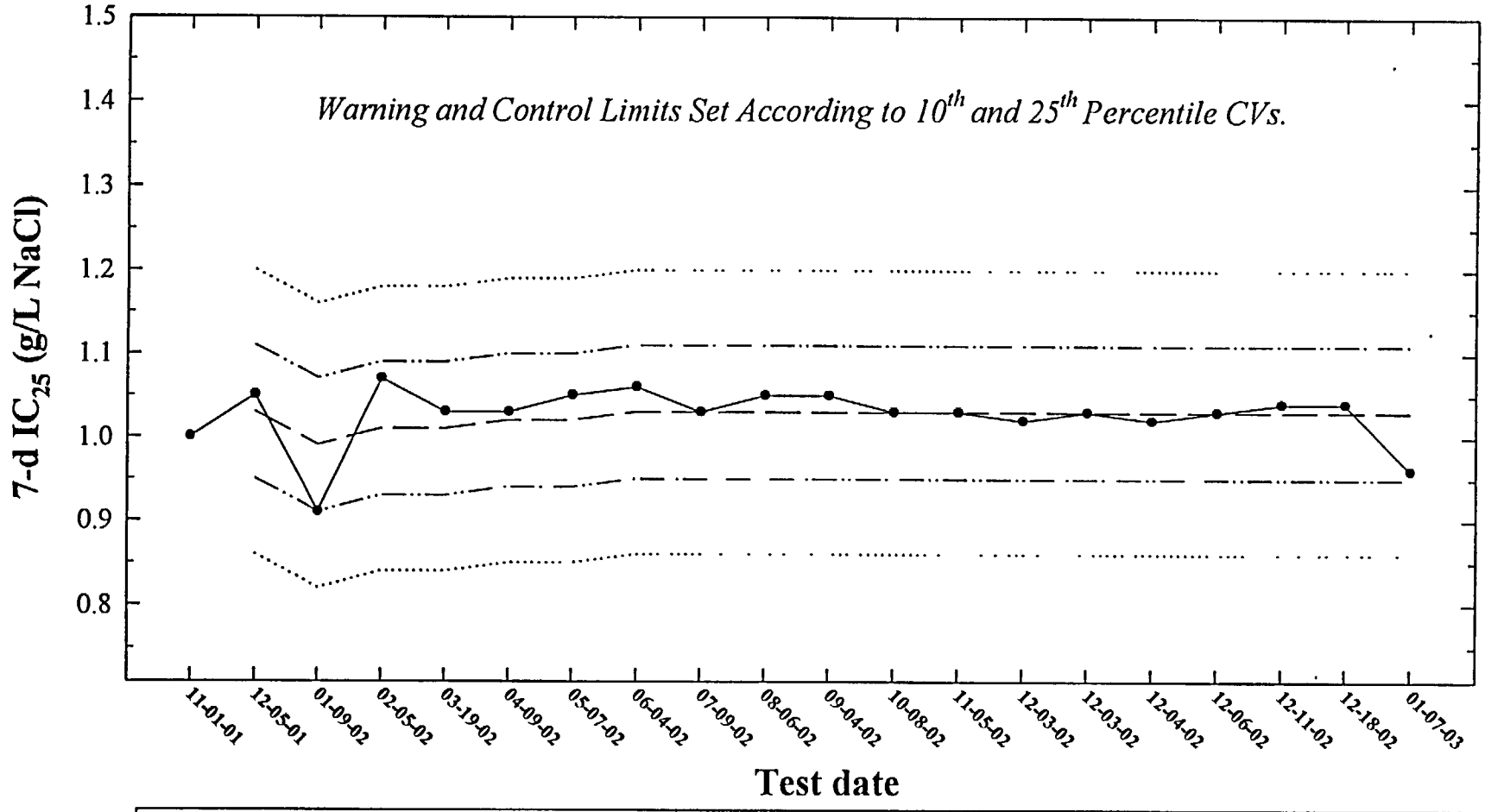
Control Limit = Standard deviation corresponding to the 90th percentile CV $S_{A.90}$ = 0 62

USEPA 2000 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program EPA-833-R-00-003 US Environmental Protection Agency, Cincinnati, OH

*01-07-03 xls*

# Environmental Testing Solutions, LLC

## Sodium Chloride Chronic Reference Toxicant Control Chart for *Ceriodaphnia dubia* using Moderately Hard Synthetic Water




—•— **7-day $IC_{25}$** = 25% inhibition concentration. An estimation of the concentration of sodium chloride that would cause a 25% reduction in *Ceriodaphnia* reproduction for the test population.

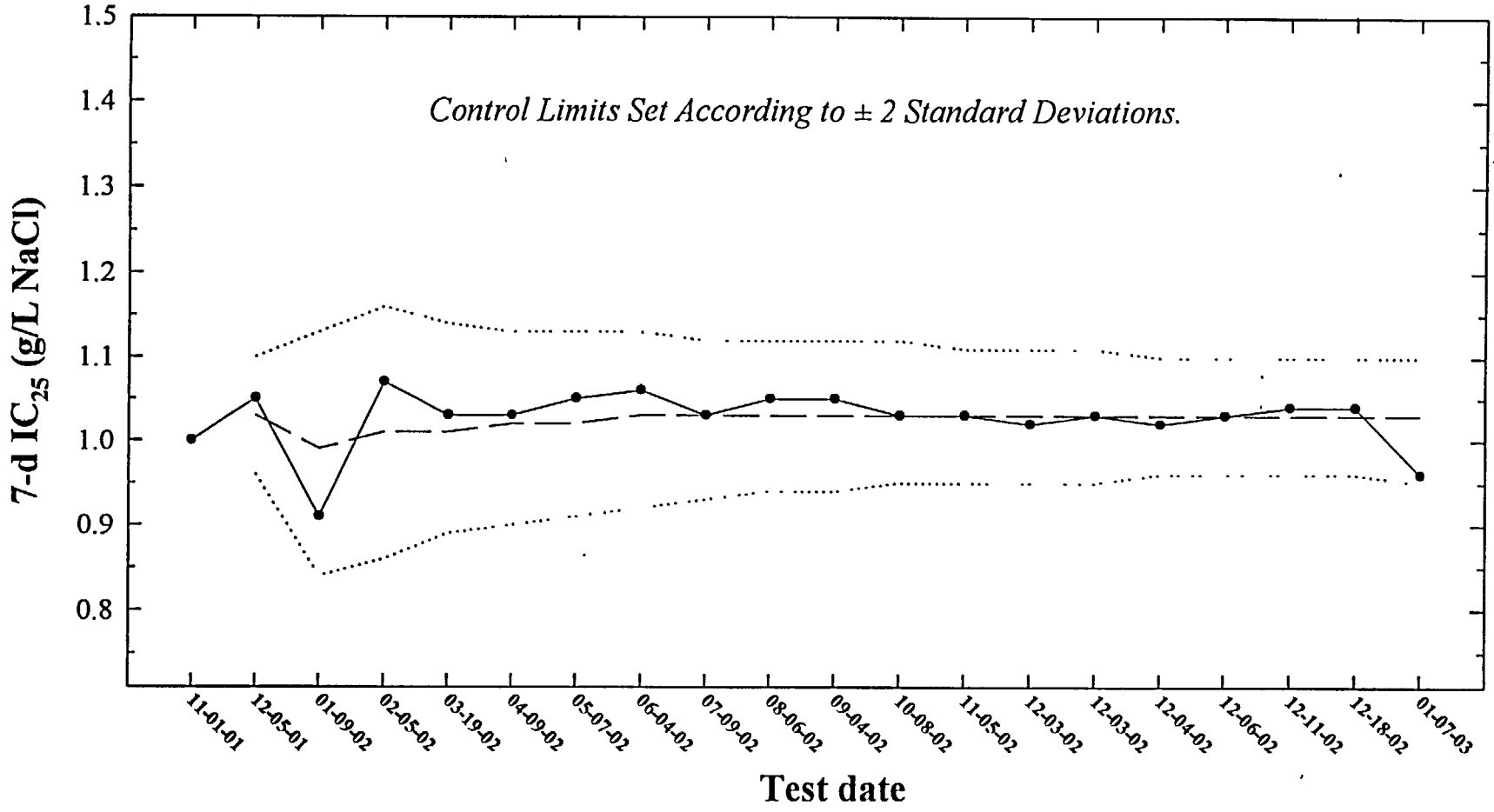
— – **Central Tendency** (mean $IC_{25}$)

—··— **Warning Limits** (mean $IC_{25} \pm S_{A.10}$, $S_{A.10} = 0.08$, as determined by USEPA for the method and endpoint)

····· **Control Limits** (mean $IC_{25} \pm S_{A\,25}$, $S_{A\,25} = 0.17$, as determined by USEPA for the method and endpoint)

# Environmental Testing Solutions, LLC

## Sodium Chloride Chronic Reference Toxicant Control Chart for *Ceriodaphnia dubia* using Moderately Hard Synthetic Water




—•— **7-day $IC_{25}$** = 25% inhibition concentration. An estimation of the concentration of sodium chloride that would cause a 25% reduction in *Ceriodaphnia* reproduction for the test population.

— – **Central Tendency** (mean $IC_{25}$)

. . .... **Control Limits** (mean $IC_{25}$ ± 2 Standard Deviations)

# Environmental Testing Solutions, LLC

## Precision of Endpoint Measurements

### Sodium Chloride Chronic Reference Toxicant Data for *Ceriodaphnia dubia* using Moderately Hard Synthetic Water

| Test number | Test date | Control Survival (%) | Control Mean Reproduction (offspring/female) | CV (%) | CT for Control Reproduction CV (%) | MSD | PMSD (%) | CT for PMSD (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 11-01-01 | 100 | 27.0 | 6.5 | | 3.1 | 11 6 | |
| 2 | 12-05-01 | 100 | 24 8 | 9.1 | 7.8 | 4.8 | 19.5 | 15.5 |
| 3 | 01-09-02 | 100 | 27.5 | 7.5 | 7.7 | 4.5 | 16.3 | 15.8 |
| 4 | 02-05-02 | 100 | 23 1 | 10.1 | 8 3 | 2.6 | 11.1 | 14 6 |
| 5 | 03-19-02 | 100 | 26 3 | 8 6 | 8 4 | 3.0 | 11.2 | 14 0 |
| 6 | 04-09-02 | 100 | 26 2 | 12 3 | 9 0 | 2.8 | 10.7 | 13 4 |
| 7 | 05-07-02 | 100 | 27.3 | 8 1 | 8 9 | 2.3 | 8.4 | 12.7 |
| 8 | 06-04-02 | 100 | 26 0 | 9.9 | 9.0 | 3.8 | 14.7 | 12 9 |
| 9 | 07-09-02 | 100 | 29 5 | 8 0 | 8.9 | 3.5 | 11.7 | 12.8 |
| 10 | 08-06-02 | 100 | 28 4 | 10 4 | 9 1 | 2.7 | 9.5 | 12 5 |
| 11 | 09-04-02 | 100 | 31 4 | 6 7 | 8 8 | 3.0 | 9.5 | 12.2 |
| 12 | 10-08-02 | 100 | 31.1 | 9.2 | 8.9 | 2.9 | 9.4 | 12 0 |
| 13 | 11-05-02 | 100 | 29.5 | 8 0 | 8 8 | 2.5 | 8.4 | 11.7 |
| 14 | 12-03-02 | 90 | 34.0 | 6 2 | 8 6 | 2.7 | 8.0 | 11.4 |
| 15 | 12-03-02 | 100 | 33.2 | 6.0 | 8 4 | 3.3 | 9.9 | 11.3 |
| 16 | 12-04-02 | 100 | 32.5 | 11.0 | 8 6 | 3.2 | 9.8 | 11.2 |
| 17 | 12-06-02 | 100 | 29 7 | 13.7 | 8 9 | 3.0 | 10 0 | 11 2 |
| 18 | 12-11-02 | 100 | 33 8 | 7.4 | 8.8 | 2.9 | 8 5 | 11 0 |
| 19 | 12-18-02 | 100 | 30 5 | 7.0 | 8.7 | 2.9 | 9.4 | 10 9 |
| 20 | 01-07-03 | 100 | 33 2 | 8 1 | 8.7 | 2.9 | 8 6 | 10.8 |

*Note·* Average control reproduction (offspring per female) = 29.3

CV = Coefficient of variation for control reproduction.
On average, the CV for control reproduction is **8.7%** in Environmental Testing Solutions, LLC
Lower CV bound determined by USEPA (10th percentile) = 8 9%
Upper CV bound determined by USEPA (90th percentile) = 42%

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference
PMSD is a measure of test precision The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test. On average, a significant difference occurs for Environmental Testing Solutions, LLC chronic toxicity tests when a toxicant reduces Ceriodaphnia reproduction by **10.8%** from the control.
Lower PMSD bound determined by USEPA (10th percentile) = 11%
Upper PMSD bound determined by USEPA (90th percentile) = 37%

CT = Central Tendancy (mean Control Reproduction CV or mean PMSD)

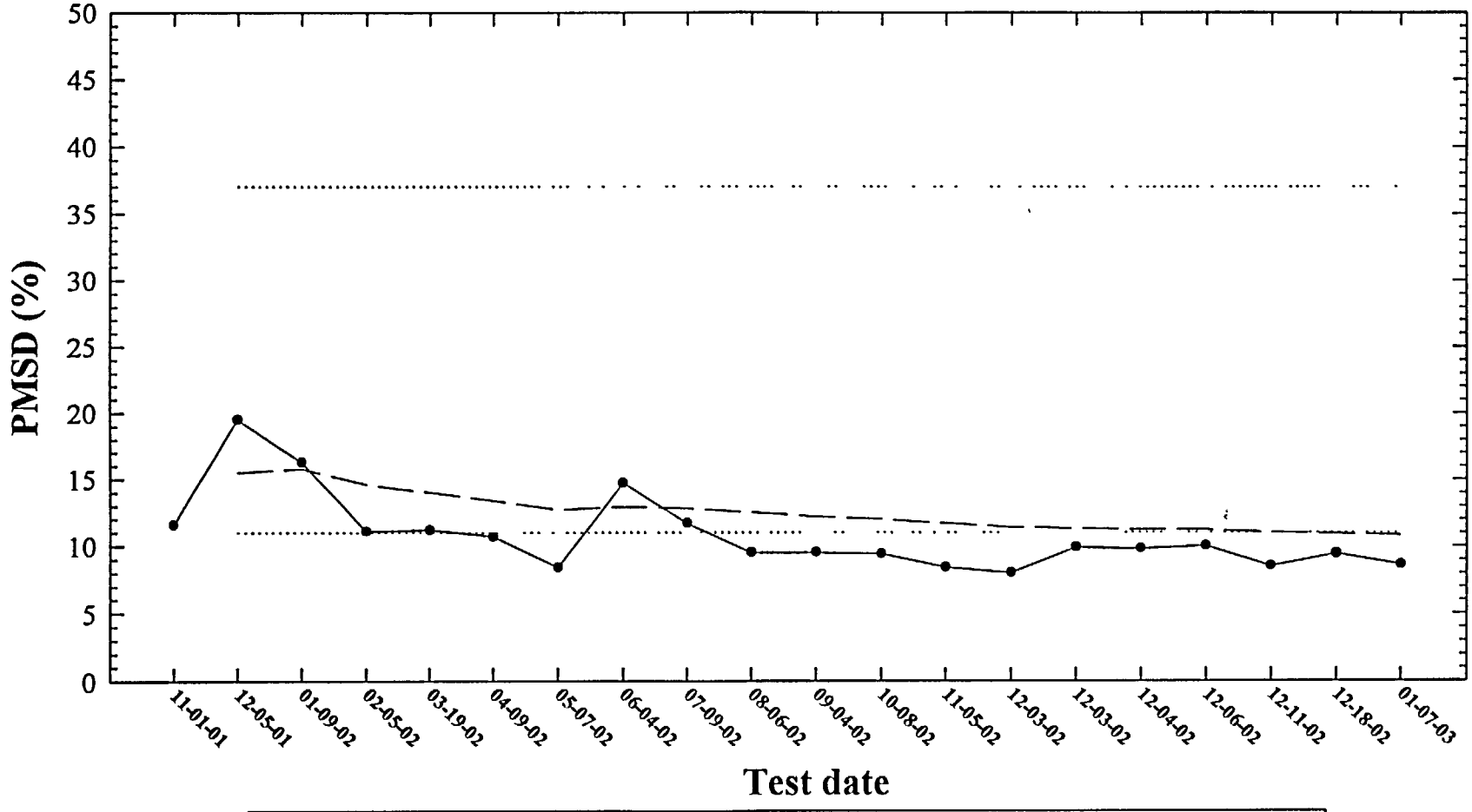
The lower and upper bounds were calculated by the USEPA using 393 tests conducted from 33 laboratories for Ceriodaphnia reproduction in chronic reference toxicant tests

USEPA. 2000 Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH

# Environmental Testing Solutions, LLC

## Precision of Endpoint Measurements

### Sodium Chloride Chronic Reference Toxicant Control Chart for *Ceriodaphnia dubia* using Moderately Hard Synthetic Water




—●— **PMSD** = percent minimum significant difference PMSD is the minimum significant difference between the control and treatment that can be declared statistically significant.

— - **Central Tendency** (mean PMSD)

····· ·· **Lower and Upper PMSD Bounds**
Lower PMSD Bound (10th percentile) = 11%, Upper PMSD Bound (90th percentile) = 37%
(Lower and upper PMSD bounds were determined by USEPA for the method and endpoint.)

# Sodium Chloride Chronic Reference Toxicant Test
## (EPA-821-R-02-013 Method 1002.0)
### Species: *Ceriodaphnia dubia*

CdNaClCR Test Number: 6

| | Date | Time | Analyst |
|---|---|---|---|
| Test start | 01-07-03 | 1200 | dl |
| Test end | 01-14-03 | 1156 | dl |

| *Dilution preparation information:* | | | | | | *Comments:* |
|---|---|---|---|---|---|---|
| NaCl CHM number: | CHM 060 | | | | | |
| Stock preparation: | 100 g NaCl/l (dissolve 50 g NaCl in 500 ml deionized water) | | | | | |
| Dilution prep (mg/L) | 600 | 800 | 1000 | 1200 | 1400 | |
| Stock volume (mL) | 9 | 12 | 15 | 18 | 21 | |
| Diluent volume (mL) | 1491 | 1488 | 1485 | 1482 | 1479 | |
| Total volume (mL) | 1500 | 1500 | 1500 | 1500 | 1500 | |

| *Test organism information:* | | *Test information:* | |
|---|---|---|---|
| Organism age: | < 24 HOURS OLD | Randomizing template: | ORANGE |
| Date and times organisms were born between: | 01-07-03 0811 TO 1014 | Incubator number: | 2 |
| Organism source: | 12-31-02 A+B | YCT batch: ABS | 12-24-02 |
| Transfer bowl information: | pH = 8.00 Temperature (°C)= 25.2 | *Selenastrum* batch: ABS | 12-24-02 |

## CONTROL

### *Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 4 | 4 | 5 | 0 | 5 | 5 | 3 | 6 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 10 | 12 | 14 | 10 | 10 | 15 | 11 | 13 | 11 | 12 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 | 18 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 17 | 18 | 18 | 15 | 0 | 16 | 19 | 15 | 19 | 0 |
| Total young produced | | 31 | 34 | 36 | 30 | 29 | 36 | 35 | 31 | 36 | 34 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |
| X for 3rd Broods | | X | X | X | X | X | X | X | X | X | X |

Test Renewal, Feeding, and Randomization Record Label

| | Mon | Tue | Wed | Thu | Fri | Sat | Sun |
|---|---|---|---|---|---|---|---|
| Date | 01-13 | 01-07 | 01-08 | 01-09 | 01-10 | 01-11 | 01-12 |
| Time | 1206 | 1200 | 1157 | 1207 | 1155 | 1200 | 1146 |
| Analyst | dl | dl | dl | dl | dl | dl | dl |
| Shelf Location | B1 | B1 | B1 | B1 | B1 | B1 | B1 |

| *Control information:* | | Acceptance criteria | *Summary of test endpoints (mg NaCl/L):* | |
|---|---|---|---|---|
| % of Mal- Adults: | 0% | ≤ 20% | 7-day LC50 | >1400 |
| % Adults having 3rd Broods: | 100% | ≥ 80% | NOEC | 800 |
| % Mortality: | 0% | ≤ 20% | LOEC | 1000 |
| Mean Offspring/Female: | 33.2 | ≥ 15 0 offspring/female | ChV | 894.4 |
| % CV: | 8.1% | < 40.0 % | IC25 | 962.4 |

CdNaClCR Test Number: 6

## 600 mg NaCl/L

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 4 | 5 | 6 | 5 | 0 | 3 | 5 | 5 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 14 | 10 | 12 | 12 | 14 | 11 | 11 | 15 | 12 | 13 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 16 | 0 | 0 | 17 | 17 | 0 | 14 | 0 | 15 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 0 | 19 | 15 | 0 | 0 | 18 | 0 | 14 | 0 | 18 |
| Total young produced | | 34 | 34 | 33 | 34 | 35 | 32 | 30 | 34 | 31 | 35 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 33.2 |
| % Reduction from Control: | 0% |

## 800 mg NaCl/L

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 5 | 5 | 4 | 6 | 4 | 4 | 5 | 4 | 4 | 4 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 13 | 10 | 12 | 10 | 12 | 13 | 12 | 10 | 12 | 10 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 16 | 14 | 0 | 18 | 14 | 16 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 13 | 17 | 16 | 16 | 0 | 0 | 14 | 0 | 18 | 0 |
| Total young produced | | 31 | 32 | 32 | 32 | 32 | 31 | 31 | 32 | 35 | 30 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

* CARRY OVER

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 31.8 |
| % Reduction from Control: | 4.2% |

CdNaClCR Test Number: 6

## 1000 mg NaCl/L

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 3 | 4 | 4 | 3 | 5 | 5 | 4 | 4 | 3 | 3 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 8 | 10 | 6 | 5 | 10 | 12 | 6 | 8 | 5 | 5 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 10 | 13 | 9 | 11 | 0 | 12 | 8 | 13 | 16 | 14 |
| Total young produced | | 21 | 27 | 19 | 19 | 29 | 29 | 18 | 25 | 24 | 22 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female: | 23.3 |
| % Reduction from Control: | 29.8% |

## 1200 mg NaCl/L

*Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 3 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 0 | 3 | 3 | 2 | 4 | 5 | 0 | 0 | 4 | 2 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 6 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 5 | 0 | 4 | 7 | 0 | 0 | 0 | 0 | 0 | 3 |
| Total young produced | | 5 | 3 | 7 | 12 | 9 | 5 | 0 | 3 | 10 | 5 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female. | 5.9 |
| % Reduction from Control: | 82.2% |

CdNaClCR Test Number: 6

**1400 mg NaCl/L** *Survival and Reproduction Data*

| Day | | Replicate number 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 2 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 3 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 4 | Young produced | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 5 | Young produced | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 4 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 6 | Young produced | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Adult mortality | L | L | L | L | L | L | L | L | L | L |
| 7 | Young produced | 2 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total young produced | | 2 | 1 | 3 | 0 | 0 | 6 | 0 | 0 | 4 | 0 |
| Final Adult Mortality | | L | L | L | L | L | L | L | L | L | L |

| *Concentration:* | |
|---|---|
| % Mortality: | 0% |
| Mean Offspring/Female | 1.6 |
| % Reduction from Control. | 95.2% |

# Environmental Testing Solutions, LLC

## Chronic Whole Effluent Toxicity Test (EPA-821-R-02-013, Method 1002.0)
## Species: Ceriodaphnia dubia

## Quality Control
## Verification of Data Entry, Calculations, and Statistical Analyses

**Test number:** CdNaClCR #35

**Test dates:** January 7 - 14, 2003

Reviewed by: J Lemon

| Concentration (mg/L NaCl) | Replicate number | | | | | | | | | | Survival (%) | Average reproduction (offspring/female) | Coefficient of variation (%) | Percent reduction from control (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| Control | 31 | 34 | 36 | 30 | 29 | 36 | 35 | 31 | 36 | 34 | 100 | 33.2 | 8 1 | Not applicable |
| 600 | 34 | 34 | 33 | 34 | 35 | 32 | 30 | 34 | 31 | 35 | 100 | 33.2 | 5 1 | 0 0 |
| 800 | 31 | 32 | 32 | 32 | 32 | 31 | 31 | 32 | 35 | 30 | 100 | 31 8 | 4.1 | 4 2 |
| 1000 | 21 | 27 | 19 | 19 | 29 | 29 | 18 | 25 | 24 | 22 | 100 | 23 3 | 17 8 | 29 8 |
| 1200 | 5 | 3 | 7 | 12 | 9 | 5 | 0 | 3 | 10 | 5 | 100 | 5 9 | 61 6 | 82.2 |
| 1400 | 2 | 1 | 3 | 0 | 0 | 6 | 0 | 0 | 4 | 0 | 100 | 1 6 | 132 4 | 95 2 |

**Dunnett's MSD value:** 2 853

**PMSD:** 8 6

MSD = Minimum Significant Difference

PMSD = Percent Minimum Significant Difference

PMSD is a measure of test precision. The PMSD is the minimum percent difference between the control and treatment that can be declared statistically significant in a whole effluent toxicity test On average, a significant difference occurs for Environmental Testing Solutions, LLC chronic toxicity tests when a toxicant reduces Ceriodaphnia reproduction by 10 8% from the control

Lower PMSD bound determined by USEPA (10th percentile) = 11%

Upper PMSD bound determined by USEPA (90th percentile) = 37%.

The lower and upper bounds were calculated by the USEPA using 393 tests conducted from 33 laboratories for *Ceriodaphnia* reproduction in chronic reference toxicant tests.

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

# Environmental Testing Solutions, LLC

## Statistical Analyses

**Ceriodaphnia Survival and Reproduction Test-Reproduction**

| | | | | | |
|---|---|---|---|---|---|
| Start Date | 01-07-03 | Test ID | CdNaClCR | Sample ID | REF-Ref Toxicant |
| End Date | 01-14-03 | Lab ID | ETS-Env Testing Solutions | Sample Type | NACL-Sodium chloride |
| Sample Date | | Protocol | EPAF 91-EPA Freshwater | Test Species | CD-Ceriodaphnia dubia |
| Comments | | | | | |

| Conc-mg/L | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| D-Control | 31 000 | 34 000 | 36 000 | 30 000 | 29 000 | 36 000 | 35 000 | 31 000 | 36 000 | 34 000 |
| 600 | 34 000 | 34 000 | 33 000 | 34 000 | 35 000 | 32.000 | 30 000 | 34 000 | 31 000 | 35 000 |
| 800 | 31 000 | 32 000 | 32 000 | 32 000 | 32 000 | 31.000 | 31 000 | 32 000 | 35 000 | 30 000 |
| 1000 | 21 000 | 27 000 | 19 000 | 19 000 | 29 000 | 29 000 | 18 000 | 25 000 | 24 000 | 22 000 |
| 1200 | 5 000 | 3 000 | 7 000 | 12 000 | 9 000 | 5 000 | 0 000 | 3 000 | 10 000 | 5 000 |
| 1400 | 2 000 | 1 000 | 3 000 | 0 000 | 0 000 | 6 000 | 0 000 | 0 000 | 4 000 | 0 000 |

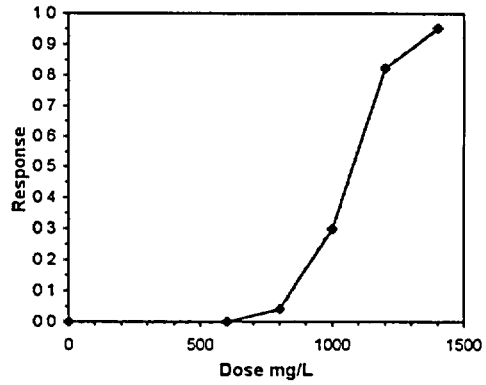
| | | | Transform: Untransformed | | | | | Rank | 1-Tailed | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-mg/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Mean | N-Mean |
| D-Control | 33 200 | 1 0000 | 33 200 | 29 000 | 36 000 | 8.132 | 10 | | | 33 200 | 1 0000 |
| 600 | 33 200 | 1 0000 | 33 200 | 30 000 | 35 000 | 5 080 | 10 | 100 50 | 75 00 | 33 200 | 1 0000 |
| 800 | 31.800 | 0 9578 | 31.800 | 30 000 | 35 000 | 4 140 | 10 | 92 00 | 75 00 | 31 800 | 0 9578 |
| *1000 | 23.300 | 0 7018 | 23 300 | 18 000 | 29 000 | 17.759 | 10 | 56 00 | 75 00 | 23.300 | 0 7018 |
| *1200 | 5 900 | 0 1777 | 5 900 | 0 000 | 12 000 | 61 605 | 10 | 55 00 | 75 00 | 5 900 | 0 1777 |
| *1400 | 1 600 | 0 0482 | 1 600 | 0 000 | 6 000 | 132 419 | 10 | 55 00 | 75 00 | 1 600 | 0 0482 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates normal distribution (p > 0 01) | 0 644731641 | 1 035 | 0 179333511 | -0 088421936 |
| Bartlett's Test indicates unequal variances (p = 8 16E-03) | 15 57795048 | 15 08631706 | | |

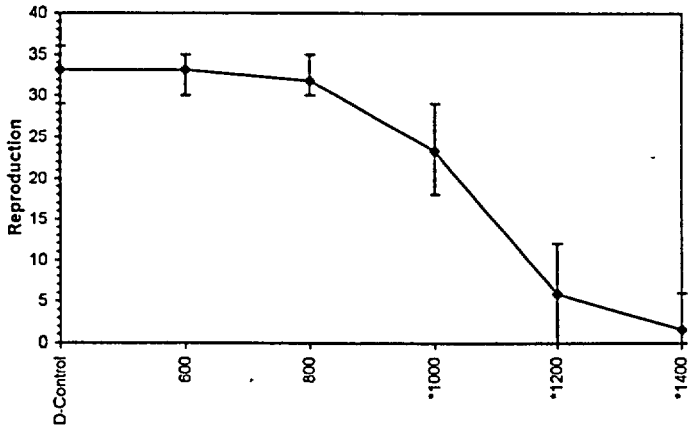
| Hypothesis Test (1-tail, 0 05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 800 | 1000 | 894 427191 | |

**Linear Interpolation (80 Resamples)**

| Point | mg/L | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05 | 806 1176471 | 62 23646999 | 621 4058824 | 831 0523374 | -2 1157 |
| IC10 | 845 1764706 | 16 10286245 | 814 5108696 | 870 8034163 | 0 2593 |
| IC15 | 884 2352941 | 17 9163057 | 850 0187974 | 918 8172779 | 0 6136 |
| IC20 | 923 2941176 | 21 52170053 | 886 7947958 | 967 7859037 | 0 6574 |
| IC25 | 962 3529412 | 23 5811894 | 921 1981225 | 1005 440864 | 0 1587 |
| IC40 | 1038 850575 | 11 70688582 | 1014 698685 | 1060 114799 | -0 2641 |
| IC50 | 1077 011494 | 9 250837919 | 1059 673238 | 1093 762391 | 0 1241 |




**Dose-Response Plot**



CdNaClCR Test Number: 6

*Daily Chemistry:* HHS BATCH : 01-06-03

| Concentration | Parameter | Day 0 | | Day 1 | | Day 2 | |
|---|---|---|---|---|---|---|---|
| CONTROL | pH (S.U.) | 7.98 | 8.25 | 8.10 | 8.27 | 8.18 | 8.14 |
| | DO (mg/L) | ~~7.4~~ 7.3 | 7.6 | 7.4 | 8.1 | 7.9 | 8.0 |
| | Conductivity (µmhos/cm) | 297 | | 297 | | 318 | |
| | Alkalinity (mg $CaCO_3$/L) | 62 | | | | | |
| | Hardness (mg $CaCO_3$/L) | 84 | | | | | |
| | Temperature (°C) | 25.1 | 24.9 | 24.3 | 25.1 | 24.6 | 25.0 |
| 600 mg NaCl/L | pH (S U.) | 8.01 | 8.16 | 8.15 | 8.22 | 8.18 | 8.11 |
| | DO (mg/L) | 7.3 | 7.6 | 7.4 | 8.1 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 1412 | | 1393 | | 1415 | |
| | Temperature (°C) | 25.1 | 24.9 | 24.3 | 25.1 | 24.6 | 25.0 |
| 800 mg NaCl/L | pH (S.U.) | 7.99 | 8.17 | 8.15 | 8.26 | 8.19 | 8.12 |
| | DO (mg/L) | 7.3 | 7.8 | 7.6 | 8.1 | 8.0 | 8.1 |
| | Conductivity (µmhos/cm) | 1733 | | 1740 | | 1734 | |
| | Temperature (°C) | 25.1 | 24.9 | 24.3 | 25.1 | 24.6 | 25.0 |
| 1000 mg NaCl/L | pH (S.U.) | 7.97 | 8.16 | 8.15 | 8.24 | 8.16 | 8.12 |
| | DO (mg/L) | 7.4 | 7.8 | 7.6 | 8.1 | 8.1 | 8.0 |
| | Conductivity (µmhos/cm) | 2026 | | ~~17~~ 2040 | | 2131 | |
| | Temperature (°C) | 25.1 | 24.9 | 24.3 | 25.1 | 24.6 | 25.0 |
| 1200 mg NaCl/L | pH (S.U.) | 7.98 | 8.14 | 8.14 | 8.20 | 8.17 | 8.10 |
| | DO (mg/L) | 7.3 | 7.7 | 7.6 | 8.1 | 8.2 | 8.2 |
| | Conductivity (µmhos/cm) | 2448 | | 2472 | | 2444 | |
| | Temperature (°C) | 25.1 | 24.9 | 24.3 | 25.1 | 24.6 | 25.0 |
| 1400 mg NaCl/L | pH (S.U.) | 7.96 | 8.13 | 8.14 | 8.21 | 8.16 | 8.10 |
| | DO (mg/L) | 7.3 | 7.8 | 7.6 | 8.1 | 8.2 | 8.1 |
| | Conductivity (µmhos/cm) | 2795 ~~29~~ | | 2812 | | 2798 | |
| | Temperature (°C) | 25.1 | 24.9 | 24.3 | 25.1 | 24.6 | 25.0 |
| | | Initial | Final | Initial | Final | Initial | Final |

10000

CdNaClCR Test Number: 6

| Concentration | Parameter | Day 3 | | Day 4 | | Day 5 | | Day 6 | |
|---|---|---|---|---|---|---|---|---|---|
| CONTROL | pH (S.U.) | 8.20 | 8.24 | 8.17 | 8.19 | 8.20 | 8.19 | 8.15 | 8.08 |
| | DO (mg/L) | 8.2 | 8.3 | 8.0 | 8.2 | 7.7 | 8.0 | 7.6 | 7.9 |
| | Conductivity (µmhos/cm) | 297 | | 307 | | 303 | | 294 | |
| | Alkalinity (mg $CaCO_3$/L) | | | | | | | | |
| | Hardness (mg $CaCO_3$/L) | | | | | | | | |
| | Temperature (°C) | 24.5 | 24.8 | 24.7 | 25.1 | 24.2 | 24.4 | 24.2 | 24.7 |
| 600 mg NaCl/L | pH (S U.) | 8.15 | 8.20 | 8.13 | 8.11 | 8.16 | 8.12 | 8.15 | 8.05 |
| | DO (mg/L) | 7.9 | 8.3 | 8.2 | 8.2 | 8.1 | 7.9 | 7.8 | 8.0 |
| | Conductivity (µmhos/cm) | 1426 | | 1435 | | 1395 | | 1447 | |
| | Temperature (°C) | 24.4 | 24.8 | 24.7 | 25.1 | 24.2 | 24.4 | 24.2 | 24.7 |
| 800 mg NaCl/L | pH (S U.) | 8.14 | 8.22 | 8.12 | 8.16 | 8.17 | 8.12 | 8.16 | 8.09 |
| | DO (mg/L) | 8.0 | 8.3 | 8.2 | 8.2 | 8.2 | 8.0 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 1759 | | 1774 | | 1741 | | 1771 | |
| | Temperature (°C) | 24.4 | 24.8 | 24.7 | 25.1 | 24.2 | 24.4 | 24.2 | 24.7 |
| 1000 mg NaCl/L | pH (S.U.) | 8.13 | 8.23 | 8.11 | 8.15 | 8.16 | 8.14 | 8.14 | 8.11 |
| | DO (mg/L) | 8.1 | 8.3 | 8.2 | 8.2 | 8.1 | 7.9 | 8.0 | 8.0 |
| | Conductivity (µmhos/cm) | 2059 | | 2062 | | 2037 | | 2063 | |
| | Temperature (°C) | 24.4 | 24.8 | 24.7 | 25.1 | 24.2 | 24.4 | 24.2 | 24.7 |
| 1200 mg NaCl/L | pH (S.U.) | 8.12 | 8.23 | 8.10 | 8.15 | 8.13 | 8.12 | 8.13 | 8.11 |
| | DO (mg/L) | 8.2 | 8.3 | 8.2 | 8.2 | 8.2 | 7.8 | 8.0 | 8.4 |
| | Conductivity (µmhos/cm) | 2475 | | 2499 | | 2468 | | 2517 | |
| | Temperature (°C) | 24.4 | 24.8 | 24.7 | 25.1 | 24.2 | 24.4 | 24.2 | 24.7 |
| 1400 mg NaCl/L | pH (S.U.) | 8.12 | 8.22 | 8.10 | 8.10 | 8.12 | 8.11 | 8.12 | 8.08 |
| | DO (mg/L) | 8.3 | 8.5 | 8.4 | 8.4 | 8.2 | 7.8 | 8.0 | 8.3 |
| | Conductivity (µmhos/cm) | 2829 | | 2793 | | 2762 | | 2818 | |
| | Temperature (°C) | 24.4 | 24.8 | 24.7 | 25.1 | 24.2 | 24.4 | 24.2 | 24.7 |
| | | Initial | Final | Initial | Final | Initial | Final | Initial | Final |