# Appendix A Exposure durations classified as acute toxicity tests

Appendix Table A.1: Classification of acute toxicity tests for temperate species. Adapted from Warne et al. (2018).

| Test species type   | Life stage <sup>a</sup> | Relevant endpoints <sup>b</sup>  | Test duration |
|---------------------|-------------------------|--|---------------|
| Fish and amphibians | Adults/juveniles        | Allc   | <21 d         |
|                     | Embryos/larvae          | All  | <7 d          |
| Macroinvertebrates  | Adults/juveniles        | All  | <14 d         |
|                     | Embryos/larvae          | All (except fertilisation <sup>f</sup> , larval development/<br>metamorphosis) | <7 d          |
|                     | Embryos/larvae          | Larval development/ metamorphosis  | <48 h         |
| Microinvertebrates  | Adults/juveniles/larvae | All (except fertilisation, larval development/<br>metamorphosis)               | <7 d          |
|                     | Embryos/larvae          | Larval development/ metamorphosis  | <48 h         |
| Macrophytes         | Mature                  | All  | <7 d          |
| Macroalgae          | Mature                  | Lethality and growth   | <7 d          |
| Microalgae          | Not applicable          | All  | ≤24 h         |
| Microorganisms      | Not applicable          | All  | ≤24 h         |

Notes: a The life stage at the start of the toxicity test. b Endpoints need to be ecologically relevant – see Warne et al. (2018). c For acute tests, 'All' refers to all ecologically relevant endpoints for a particular life stage of a particular species. d Macroinvertebrates include invertebrates where full-grown adults are  $\geq 2 \text{ mm} \log($  for example decapods, echinoderms, molluscs, annelids, corals, amphipods, larger cladocerans [such as Daphnia magna, Daphnia carinata and Daphnia pulex] and insect species where larvae are  $\geq 2 \text{ mm} \log$ ). e Microinvertebrates are defined here as invertebrate species where full-grown adults are typically <2 mm long. Examples of invertebrates that meet this criterion are some cladocerans (for example Ceriodaphnia dubia and Moina australiensis), copepods, conchostracans, rotifer, acari, bryozoa and hydra. f Fertilisation tests are typically  $\geq 1$  h and are considered chronic.

# Appendix B Further details of bioavailability models

#### Appendix Table B.I: Regression-based models currently used or proposed for acute copper criteria/guideline value derivation.

|            |                          | Species/group coefficients derived  |   | Predicted       | Slopes |               |          |
|------------|--------------------------|---|---|-----------------|--------|---------------|----------|
| Model type | Key References           | from  | TMFs included                                   | R <sup>2</sup>  | рН     | Log(Hardness) | Log(DOC) |
| Hardness   | US EPA (1984)            | Pooled model based on Daphnia magna,<br>D. pulicaria, Oncorhynchus clarkii, O.<br>mykiss, O. tshawytscha, Pimephales<br>promelas, Poecilia reticulata, Lepomis<br>macrochirus | Hardness  | Not<br>reported |        | 0.9422        |          |
| MLR        | Brix et al. (2017; 2021) | Ceriodaphnia dubia  | Hardness, pH, DOC                               | 0.69            | 0.99   | 0.13          | 0.64     |
| MLR        | Brix et al. (2017; 2021) | D. magna  | Hardness, pH, DOC                               | 0.78            | 0.77   | 0.54          | 0.75     |
| MLR        | Brix et al. (2017; 2021) | D. obtusa   | Hardness, pH, DOC                               | 0.79            | 0.55   | 0.28          | 0.84     |
| MLR        | Brix et al. (2017; 2021) | D. pulex  | Hardness, DOC (pH included but not significant) | 0.71            | NS     | 0.62          | 0.76     |
| MLR        | Brix et al. (2017; 2021) | O. mykiss   | Hardness, DOC (pH included but not significant) | 0.55            | NS     | 0.76          | 0.51     |
| MLR        | Brix et al. (2017; 2021) | P. promelas   | Hardness, pH, DOC                               | 0.76            | 0.96   | 0.99          | 0.67     |
| MLR        | Brix et al. (2017; 2021) | Pooled model, based on all above species  | Hardness, pH, DOC                               |                 | 0.78   | 0.58          | 0.70     |

| Model type            | Key References                       | Species/group<br>coefficients derived from   | TMFs included  | Binding coefficients (logK) |         |              |      |       |       |       |
|-----------------------|--------------------------------------|--|--|-----------------------------|---------|--------------|------|-------|-------|-------|
|                       |                                      |  |  | Cu-BL*                      | CuOH-BL | CuCO₃-<br>BL | H-BL | Ca-BL | Mg-BL | Na-BL |
| Fish BLM              | Di Toro et al. 2001                  | P. promelas and O.<br>mykiss   | Temp., pH, DOC, humic<br>acid, Ca, Mg, Na, K, Cl,<br>SO4, alkalinity | 7.4                         |         |              | 5.4  | 3.6   | 3.6   | 3     |
| Invertebrate BLM      | De Schamphelaere et al. 2002a; 2002b | D. magna   | Temp., pH, DOC, humic<br>acid, Ca, Mg, Na, K, Cl,<br>SO4, alkalinity | 8.02                        | 7.32    | 7.01         | 5.4  | 3.47  | 3.58  | 3.19  |
| Fish BLM              | US EPA (2007)                        | As for Di Toro et al.<br>(2001)  | Temp., pH, DOC, humic<br>acid, Ca, Mg, Na, K, Cl,<br>SO4, alkalinity | 7.4                         | -1.3    |              | 5.4  | 3.6   | 3.6   | 3     |
| Fish/invertebrate BLM | BC (2019) & ECCC (2021)              | Not specifically reported<br>by BC or ECCC but model<br>files state "derived from<br>fathead minnow" and<br>changed based on an<br>"updated database". | Temp., pH, DOC, humic<br>acid, Ca, Mg, Na, K, Cl,<br>SO4, alkalinity | 7.4                         | -0.8    |              | 6.4  | 4.4   | 4.4   | 4     |
| Plant BLM             | ECCC (2021)                          | Not reported by ECCC,<br>but model files suggest<br>adopted from BLM for<br>barley & soils.  | Temp., pH, DOC, humic<br>acid, Ca, Mg, Na, K, Cl,<br>SO4, alkalinity | 5.4                         | 21.2    | -3.3         | 7.2  | 4     | 3.5   |       |

#### Appendix Table B.2: Biotic ligand models currently used or proposed for acute copper criteria/guideline value derivation.

Note: \* BL = Biotic ligand

|          | Key References         | Species/group coefficients derived from   | TMFs included                                   | Adjusted R <sup>2</sup> |          | Slopes | 5     |
|----------|------------------------|---|---|-------------------------|----------|--------|-------|
| Model    |                        |   |   |                         | Hardness | рН     | DOC   |
| Hardness | US EPA (1987)          | D. magna, Physa heterostropha, O. mykiss, P.<br>promelas, Salvelinus fontinalis, P. reticulata,<br>Morone saxatilis, L. macrochirus | Hardness  | Not reported            | 0.8473   | N/A    | N/A   |
| MLR      | CCME (2018)            | D. pulex  | Hardness, DOC (pH included but not significant) | 0.584                   | 0.845    | n/a    | 0.284 |
| MLR      | CCME (2018)            | D. magna  | Hardness, DOC (pH included but not significant) | 0.967                   | 0.865    | n/a    | 0.191 |
| MLR      | CCME (2018)            | Pooled <i>Daphnia</i> (model used by CCME for acute zinc GV)  | Hardness, DOC (pH included but not significant) | 0.811                   | 0.833    | n/a    | 0.24  |
| MLR      | CCME (2018)            | Ceriodaphnia dubia  | Hardness, pH (no DOC data available)            | n/a                     | n/a      | n/a    | n/a   |
| MLR      | CCME (2018)            | O. mykiss   | Hardness, pH (no DOC data available)            | 0.486                   | 1.299    | -0.905 | n/a   |
| MLR      | CCME (2018)            | Salmo trutta  | Hardness, pH (no DOC data available)            | 0.481                   | 0.348    | -0.347 | n/a   |
| MLR      | CCME (2018)            | P. promelas   | Hardness, pH (no DOC data available)            | 0.339                   | n/a      | -1.164 | n/a   |
| MLR      | DeForest et al. (2023) | C. dubia (cladoceran)   | Hardness, pH, DOC                               | 0.729                   | 0.282    | -0.862 | n/a   |
| MLR      | DeForest et al. (2023) | D. magna (cladoceran)   | Hardness, pH, DOC                               | 0.449                   | 0.507    | n/a    | n/a   |
| MLR      | DeForest et al. (2023) | D. pulex (cladoceran)   | Hardness, pH, DOC                               | 0.569                   | 0.837    | n/a    | 0.297 |
| MLR      | DeForest et al. (2023) | O. mykiss (rainbow trout)   | Hardness, pH, DOC                               | 0.501                   | 0.99     | -0.392 | n/a   |
| MLR      | DeForest et al. (2023) | P. promelas (fathead minnow)  | Hardness, pH, DOC                               | 0                       | n/a      | n/a    | n/a   |
| MLR      | DeForest et al. (2023) | Pomacea paludosa (snail)  | Hardness, pH, DOC                               | 0.902                   | 0.808    | 0.106  | 0.233 |
| MLR      | DeForest et al. (2023) | P. paludosa (snail)   | Hardness, DOC                                   | 0.897                   | 0.827    | n/a    | 0.247 |
| MLR      | DeForest et al. (2023) | Pooled fish & invertebrates as listed above   | Hardness, pH, DOC                               | -0.06-0.87*             | 0.6      | -0.12  | 0.127 |

#### Appendix Table B.3: Regression-based models currently used or proposed for acute zinc guideline value derivation.

Note: \* Species-dependent intercepts.

#### Appendix Table B.4:

Biotic ligand models currently used or proposed for acute zinc guideline value derivation, including key binding coefficients.

| Model type             | Key References                    | Species/group coefficients<br>derived from  | TMFs included   |        | Binding coefficients (logK) |                 |       |       |         |
|------------------------|-----------------------------------|---|---|--------|-----------------------------|-----------------|-------|-------|---------|
|                        |                                   |   |   | Zn-BL* | ZnOH-BL                     | H-BL            | Ca-BL | Mg BL | Na-BL   |
| Fish BLM               | Santore et al. (2002)             | P. promelas, O. mykiss  | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.5    |                             | 6.7             | 4.8   |       |         |
| Invertebrate BLM       | Heijerick et al. (2002)           | D. magna  | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.3    |                             |                 | 3.3   | 3.1   |         |
| Fish BLM               | De Schamphelaere et al.<br>(2004) | O. mykiss   | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.5    |                             | 6.7             | 3.8   | 3.5   |         |
| Fish/ invertebrate BLM | HydroQual                         | D. magna, P. promelas, O. mykiss  | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.5    | -3.8                        | 6.6             | 3.8   | 3.6   |         |
| Invertebrate BLM       | Clifford & McGeer (2009)          | D. pulex  | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.6    | -3.8                        | Not<br>reported | 4.9   | 4.4   |         |
| Fish/ invertebrate BLM | DeForest et al. (2012)            | Unified/pooled model based on <i>D.</i><br>magna, <i>D. pulex, P. promelas, O.</i><br>mykiss              | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.4    | -3.8                        | 6.4             | 3.8   | 3.3   |         |
| Fish/ invertebrate BLM | Windward (2019)                   | Not specified, model files state<br>based on pooled data, presumably<br>same as earlier HydroQual version | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.4    | -2.4                        | 6.4             | 3.8   | 3.3   | 0.2-120 |
| Fish/ invertebrate BLM | DeForest et al. (2023)            | 6 fish & invertebrate species (as per MLRs above)   | Temp., pH, DOC, humic acid, Ca, Mg, Na, K,<br>Cl, SO4, alkalinity | 5.4    | -2.6                        | 6.4             | 4.2   | 3.6   |         |

Note: \* BL = Biotic ligand

# Appendix C Details of acute toxicity testing

This appendix contains a copy of the NIWA report on acute toxicity test for copper and zinc.



# Zinc and copper acute toxicity to a New Zealand native daphnid in natural waters

Prepared for Hydrotoxy Research

September 2024

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# Contents

| Execu | Executive summary6 |  |                |  |  |
|-------|--------------------|--|----------------|--|--|
| 1     | Intro              | duction  | .7             |  |  |
| 2     | Meth               | ods  | .8             |  |  |
|       | 2.1                | Water collection and characterisation              | .8             |  |  |
|       | 2.2                | Acute Daphnia thomsoni toxicity testing            | .9             |  |  |
|       | 2.3                | Reference toxicant                                 | 11             |  |  |
|       | 2.4                | Chemical analysis1                                 | 11             |  |  |
|       | 2.5                | Test acceptability criteria                        | 11             |  |  |
|       | 2.6                | Statistical analysis1                              | 11             |  |  |
| 3     | Resul              | ts and discussion1                                 | 12             |  |  |
|       | 3.1                | Characterisation of natural waters1                | 12             |  |  |
|       | 3.2                | Daphnia toxicity testing                           | 12             |  |  |
| 4     | Sumn               | nary2  | 28             |  |  |
| 5     | Ackno              | owledgements                                       | 29             |  |  |
| c     | Class              | any of obbyoutions and torms                       | 20             |  |  |
| 0     | GIOSS              | ary of appreviations and terms                     | su             |  |  |
| 7     | Refer              | ences  | 31             |  |  |
| Арре  | ndix A             | Hill Labs results - Natural water characterisation | 33             |  |  |
| Арре  | ndix B             | Hill Labs results – Zinc                           | 35             |  |  |
| Арре  | ndix C             | Hill Labs results - Copper                         | 37             |  |  |
| Арре  | ndix D             | Hill Labs results – Okutua, zinc and copper        | 39             |  |  |
| Арре  | ndix E             | Toxicity tests raw data4                           | 41             |  |  |
| Арре  | ndix F             | CETIS statistical analyses – Mahurangi             | 47             |  |  |
| Арре  | ndix G             | CETIS statistical analyses – Hoteo                 | 56             |  |  |
| Арре  | ndix H             | CETIS statistical analyses – Okutua (pH adjusted)6 | 65             |  |  |
| Арре  | ndix I             | CETIS statistical analyses – Clutha7               | 75             |  |  |
| Арре  | ndix J             | CETIS statistical analyses – Waihou                | 35             |  |  |
| Арре  | ndix K             | Physico-chemical data for zinc tests               | <del>)</del> 5 |  |  |

| Appendix L | Physico-chemical data for copper tests97         |
|------------|--|
| Appendix M | NIWA's unpublished zinc and copper toxicity data |

#### Tables

| Table E-1:  | Summary of the natural water sample characteristics and toxicity statistics   | 6      |
|-------------|---|--------|
| Table 2-1.  | Summary of natural water sample sites and collection dates  | 0<br>8 |
| Table 2-2:  | Laboratory Danhaia culture water composition from NIWA SOP 11.0   | 0      |
|             | (NIWA 2022).  | 9      |
| Table 2-3:  | Summary of <i>D. thomsoni</i> toxicity test conditions.   | 10     |
| Table 3-1:  | Characteristics of the natural water samples.   | 12     |
| Table 3-2:  | Nominal and measured zinc concentrations at the start $(T_0)$ and end $(T_{48})$ of the Mahurangi Stream <i>Daphnia</i> survival toxicity test.               | 13     |
| Table 3-3:  | Nominal and measured zinc concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Hoteo River <i>Daphnia</i> survival toxicity test.                | 14     |
| Table 3-4:  | Nominal and measured zinc concentrations at the start $(T_0)$ and end $(T_{48})$ of the Okutua Creek <i>Daphnia</i> survival toxicity test.                   | 15     |
| Table 3-5:  | Nominal and measured zinc concentrations at the start $(T_0)$ and end $(T_{48})$ of the Clutha River <i>Daphnia</i> survival toxicity test.                   | 15     |
| Table 3-6:  | Nominal and measured zinc concentrations at the start $(T_0)$ and end $(T_{48})$ of the Waihou River <i>Daphnia</i> survival toxicity test.                   | 16     |
| Table 3-7:  | Toxicity statistics as statistically derived by CETIS <sup>™</sup> (µg/L zinc) for <i>D. thomsoni</i> 48-hour survival in each natural water tested.          | 20     |
| Table 3-8:  | Nominal and measured copper concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Mahurangi Stream <i>Daphnia</i> survival toxicity test.         | 20     |
| Table 3-9:  | Nominal and measured copper concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Hoteo River <i>Daphnia</i> survival toxicity test.              | 21     |
| Table 3-10: | Nominal and measured copper concentrations at the start $(T_0)$ and end $(T_{48})$ of the Okutua Creek <i>Daphnia</i> survival toxicity test.                 | 22     |
| Table 3-11: | Nominal and measured copper concentrations at the start $(T_0)$ and end $(T_{48})$ of the Clutha River <i>Daphnia</i> survival toxicity test.                 | 22     |
| Table 3-12: | Nominal and measured copper concentrations at the start $(T_0)$ and end $(T_{48})$ of the Waihou River <i>Daphnia</i> survival toxicity test.                 | 23     |
| Table 3-13: | Toxicity statistics as statistically derived by CETIS <sup>TM</sup> ( $\mu$ g/L copper) for <i>D. thomsoni</i> 48-hour survival in each natural water tested. | 27     |
| Table 4-1:  | Summary of the natural water sample characteristics and toxicity statistics for <i>D. thomsoni</i> exposed to zinc and copper in 48-hour survival test.       | 28     |
| Table K-1:  | Summary of physico-chemical measures from acute <i>D. thomsoni</i> zinc toxicity testing with Mahurangi Stream water.   | 95     |
| Table K-2:  | Summary of physico-chemical measures from acute <i>D. thomsoni</i> zinc toxicity testing with Hoteo River water.  | 95     |
| Table K-3:  | Summary of physico-chemical measures from acute <i>D. thomsoni</i> zinc toxicity testing with pH adjusted Okutua Creek water.                                 | 95     |
|             |   |        |

| Table K-4: | Summary of physico-chemical measures from acute <i>D. thomsoni</i> zinc toxicity testing with Clutha River water.               | 96  |
|------------|---|-----|
| Table K-5: | Summary of physico-chemical measures from acute <i>D. thomsoni</i> zinc toxicity testing with Waihou River water.               | 96  |
| Table L-1: | Summary of physico-chemical measures from acute <i>D. thomsoni</i> copper toxicity testing with Mahurangi Stream water.         | 97  |
| Table L-2: | Summary of physico-chemical measures from acute <i>D. thomsoni</i> copper toxicity testing with Hoteo River water.              | 97  |
| Table L-3: | Summary of physico-chemical measures from acute <i>D. thomsoni</i> copper toxicity testing with pH adjusted Okutua Creek water. | 97  |
| Table L-4: | Summary of physico-chemical measures from acute <i>D. thomsoni</i> copper toxicity testing with Clutha River water.             | 98  |
| Table L-5: | Summary of physico-chemical measures from acute <i>D. thomsoni</i> copper toxicity testing with Waihou River water.             | 98  |
| Table M-1: | Summary of NIWA's unpublished or publicly unavailable New Zealand native species acute zinc toxicity testing data.              | 99  |
| Table M-2: | Summary of NIWA's unpublished or publicly unavailable New Zealand native species acute copper toxicity testing data.            | 101 |

### Figures

| Figure 3-1:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured zinc concentrations for Mahurangi Stream natural water sample.           | 17 |
|--------------|---|----|
| Figure 3-2:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured zinc concentrations for Hoteo River natural water sample.                | 17 |
| Figure 3-3:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured zinc concentrations for pH adjusted Okutua Creek natural water sample.   | 18 |
| Figure 3-4:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured zinc concentrations for Clutha River natural water sample.               | 19 |
| Figure 3-5:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured zinc concentrations for Waihou River natural water sample.               | 19 |
| Figure 3-6:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured copper concentrations for Mahurangi Stream natural water sample.         | 24 |
| Figure 3-7:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured copper concentrations for Hoteo River natural water sample.              | 24 |
| Figure 3-8:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured copper concentrations for pH adjusted Okutua Creek natural water sample. | 25 |
| Figure 3-9:  | Survival of <i>D. thomsoni</i> (compared to control) versus measured copper concentrations for Clutha River natural water sample.             | 26 |
| Figure 3-10: | Survival of <i>D. thomsoni</i> (compared to control) versus measured copper concentrations for Waihou River natural water sample.             | 26 |

# **Executive summary**

Natural water samples were collected from five rivers and streams across New Zealand, chosen based on their representativeness of the pH, hardness, and dissolved organic carbon (DOC) variability in New Zealand streams, as identified in a previous study (Gadd et al. 2022). These sites were also selected due to the absence of upstream anthropogenic discharges. The water samples exhibited the following ranges: pH from 6.0 to 7.6, hardness between 2.7 and 74 mg/L (as CaCO<sub>3</sub>), and DOC concentrations ranging from <0.3 to 12 mg/L.

Zinc and copper toxicity in these waters was tested with a native NZ freshwater daphnid, *Daphnia thomsoni* (Order Cladocera, commonly called a "water flea") in an acute 48-hour survival test.

The toxicity tests met acceptability criteria based on survival of control organisms ( $\geq$ 90%). The measured concentrations in the lowest positive treatments in the Mahurangi and Hoteo test waters demonstrated potential zinc contamination. Mean measured zinc (excluding those with potential contamination) or copper concentrations were used for statistical analyses. Zinc and copper exposures resulted in reduced survival of *D. thomsoni* in all five natural waters tested in a dose-dependent manner.

The zinc  $EC_{50}$  concentrations for this species ranged from 343 to 826 µg/L. The highest toxicity was observed in the natural water sample with the lowest pH and hardness, and highest DOC concentration. The lowest toxicity was observed in waters with the highest tested pH and hardness. The copper  $EC_{50}$  concentrations for this species ranged from 32 to 272 µg/L. The highest toxicity was observed in the natural water samples with low DOC concentration and the lowest toxicity was observed in waters with environmentally moderate pH and hardness (Table E-1).

The toxicity modifying characteristics of the five natural water samples and their corresponding toxicity testing endpoints for the zinc and copper 48-hour *D. thomsoni* tests are summarised below in Table E-1.

| Natural   | рН                | Hardness<br>mg/L<br>CaCO <sub>3</sub> | DOC _<br>mg/L | Zi  | nc  | Copper  |   |  |
|-----------|-------------------|---------------------------------------|---------------|---|---|---|---|--|
| water     |                   |                                       |               | EC <sub>10</sub> ª<br>(95% CL) μg/L<br>Zn <sup>2+</sup> | EC <sub>50</sub> ª<br>(95% CL) μg/L<br>Zn <sup>2+</sup> | EC <sub>10</sub> ª<br>(95% CL) μg/L<br>Cu <sup>2+</sup> | EC <sub>50</sub> ª<br>(95% CL) μg/L<br>Cu <sup>2+</sup> |  |
| Mahurangi | 7.58              | 73.5                                  | 2.18          | 511 (459-551)   | 826 (782-873)   | 89 (65-150)°  | 211 (159-255)   |  |
| Hoteo     | 7.49              | 58.1                                  | 3.73          | 374 (279-442)   | 751 (661-853)   | 180 (178-183) <sup>c</sup>                              | 272 (261-284)   |  |
| Okutua    | 6.03 <sup>b</sup> | 2.74                                  | 11.9          | 162 (78-208)  | 343 (282-416)   | 74 (n/a-93)   | 103 (78-136)  |  |
| Clutha    | 7.17              | 33.7                                  | 0.38          | 243 (124-311)   | 526 (432-641)   | 22 (n/a-26)   | 32 (26-39)  |  |
| Waihou    | 7.26              | 15.7                                  | <0.3          | 204 (121-252)   | 404 (344-474)   | 23 (n/a-30)   | 41 (33-51)  |  |

| Table E-1:    | Summary of the natural water sample characteristics and toxicity statistics for D. thomsoni |
|---------------|---|
| exposed to zi | inc and copper in 48-hour survival tests.   |

<sup>a</sup> EC<sub>x</sub>: The statistically determined test concentration causing a X% effect on the endpoint after the specified exposure period. <sup>b</sup> As pH adjusted for testing. <sup>c</sup> EC<sub>15</sub> value as EC<sub>10</sub> not available with the linear interpolation analysis used.

# 1 Introduction

Water quality guidelines, such as those established for Australia and New Zealand (ANZECC (2000) and ANZG (2018)), are designed to protect aquatic organisms from chronic (long-term) exposure to harmful substances in water bodies.

Acute guidelines focus on the concentrations of pollutants that may cause short-term or immediate toxic effects on aquatic life. They are essential for managing water quality across both natural and industrial environments, as they provide regulatory bodies with crucial benchmarks to prevent lethal impacts on aquatic organisms and the disruption of ecosystems.

A key consideration in setting guideline values is the role of *toxicity modifiers*—environmental factors that affect the bioavailability and, consequently, the toxicity of contaminants. Significant toxicity modifiers include pH, water hardness, and dissolved organic carbon (DOC). These factors influence the chemical speciation, solubility, and interaction of pollutants with aquatic organisms, ultimately modifying their toxic effects.

- pH: Variations in pH alter the speciation of metals and chemicals, influencing their solubility and bioavailability. Lower pH levels, for instance, increase the solubility of metals, making them more bioavailable and potentially more toxic.
- Water hardness: Primarily influenced by calcium and magnesium concentrations, hardness can decrease the toxicity of certain metals. Calcium and magnesium ions compete with toxic metals at biological uptake sites, reducing their toxic effects.
- Dissolved Organic Carbon (DOC): DOC can bind to pollutants such as metals, lowering their bioavailability and mitigating their toxicity.

Natural waters (e.g., streams and rivers) exhibit significant chemical diversity, influenced by factors such as geology, land use, climate, and biological activity. These variations can affect the natural waters (toxicity modifying) characteristics like pH, hardness, and the concentrations of organic and inorganic substances.

Incorporating toxicity modifiers into acute water quality guidelines is critical for ensuring that protection levels for aquatic life are accurate and site-specific. This approach enables guidelines to account for local water chemistry, improving their reliability and promoting healthier aquatic ecosystems.

This report presents research that tested the acute toxicity of zinc and copper in five natural waters collected from various regions in New Zealand. The tests were conducted using the native freshwater species *Daphnia thomsoni* (Order Cladocera, commonly referred to as "water fleas"). This project was funded by the Ministry for the Environment (MfE) under a sub-contract with Hydrotoxy Research.

# 2 Methods

#### 2.1 Water collection and characterisation

Five natural waters from New Zealand were collected, covering a range of chemistries, but broadly representative of a range of rivers and stream conditions found in New Zealand – rather than extreme examples. The natural waters were also required to be largely pristine – not affected by point sources or diffuse pollution such as intensive agriculture or urban land use.

The five natural waters collected were the same as those used in a preceding chronic zinc toxicity testing study which included chronic testing with *D. thomsoni* (Gadd et al. 2022).

Mahurangi, Hoteo, Okutua and Clutha samples were collected by NIWA personnel in clean 5 L HDPE containers. Samples were also collected for chemical analysis to characterise the water. All samples were packaged with ice or ice packs and shipped overnight to the NIWA Hamilton Laboratory, where they were refrigerated (<4°C, in dark) until ecotoxicity testing commenced, or transferred to the chemical analysis laboratory (Hill Laboratories)<sup>1</sup>. The Waihou sample was collected by CST Group on behalf of NIWA into a drinking water supply truck and transported to NIWA where it was held in a 2000 L HDPE tank at ambient temperature in the dark until ecotoxicity testing commenced or transferred to the chemical analysis laboratory (Hill Laboratory Experiment). The natural water site locations, characteristics and collection dates are summarised in Table 2-1.

| Natural water   | Easting, Northing NZTM | Region         | <b>Characteristics</b> <sup>a</sup>  | Date collected |
|---|------------------------|----------------|--|----------------|
| Mahurangi Stream @<br>Forestry Headquarters<br>(Redwoods Park)  | 1747750, 5965035       | North Auckland | Small lowland stream,<br>catchment of exotic<br>forestry                         | 7/02/2024      |
| Hoteo River at Gubb<br>(NRWQN site AK1)                         | 1735254, 5972546       | North Auckland | Lowland stream,<br>catchment includes<br>exotic forestry and some<br>agriculture | 7/02/2024      |
| Okutua Creek  | 1377965, 5212859       | West Coast     | Pristine, tannin-stained<br>stream, typical for West<br>Coast indigenous forest  | 7/02/2024      |
| Clutha River / Mata-Au<br>at Luggate Bridge<br>(NRWQN site AX1) | 1305431, 5040387       | Otago          | Pristine river from<br>glacial-fed lake  | 13/02/2024     |
| Waihou River  | 1847019, 5788327       | Waikato        | Spring-fed pristine clear<br>water stream  | 4/12/2023      |

#### Table 2-1: Summary of natural water sample sites and collection dates.

<sup>a</sup> from Gadd et al. (2022).

All natural water samples were analysed by Hill Laboratories for hardness (as calcium and magnesium) and DOC (as dissolved non-purgeable organic carbon, DNPOC). The analytical methods used are outlined in Appendix A. The physico-chemical parameters (pH, dissolved oxygen and electrical conductivity) of each sample were measured by NIWA upon arrival at the laboratory (Table 3-1).

<sup>&</sup>lt;sup>1</sup> Testing was delayed due to the unavailability of the initially selected test species (*Echyridella menziesi* larvae), necessitating the establishment of *D. thomsoni* cultures, which further postponed testing.

### 2.2 Acute Daphnia thomsoni toxicity testing

#### 2.2.1 Daphnia collection and laboratory maintenance

*Daphnia thomsoni* are freshwater micro-crustaceans belonging to the order Cladocera (commonly known as "water fleas") and are native to New Zealand. *D. thomsoni* were collected on 1<sup>st</sup> April 2024 from a known population in a local pond on private land and maintained in the NIWA Hamilton Ecotoxicology Laboratory. Initial acclimation to laboratory culture water (Table 2-2) was carried out in four steps:

- Day 0: 25% laboratory culture water and 75% source water.
- Day 1: 50% laboratory culture water and 50% source water.
- Day 2: 75% laboratory culture water and 25% source water.
- Day 3: 100% laboratory culture water.

Organisms were then cultured individually in 55 mL containers with 40 mL of laboratory culture water in a static renewal system. Water in the culture containers was changed three times per week by aliquoting culture water and food into new containers and transferring *Daphnia* to the new water using a wide mouthed plastic transfer pipette. *D. thomsoni* were fed daily with 150  $\mu$ L YTC (yeast-trout chow mixture) and 150  $\mu$ L of green alga *Raphidocellis subcapitata* (concentration of 1 x 10<sup>8</sup> cells/mL). Culture room temperature, nominally 20°C, was monitored periodically.

| 22). |
|------|
|      |

| Ingredient or parameter                 | Amount or Value | Units      |
|---|-----------------|------------|
| Fernhollow Spring Water (GF/C filtered) | 18.5            | %          |
| Aged UV nanopure water                  | 81.5            | %          |
| NaHCO <sub>3</sub>                      | 48              | mg/L       |
| CaSO <sub>4</sub>                       | 30              | mg/L       |
| MgSO <sub>4</sub>                       | 30              | mg/L       |
| KCI                                     | 2               | mg/L       |
| Vitamin B <sub>12</sub>                 | 0.01            | mg/L       |
| Se                                      | 0.002           | mg/L       |
| рН                                      | 7.8 ± 0.2       | pH units   |
| Conductivity                            | 0.23            | mS/m       |
| Hardness                                | 40 to 50        | mg CaCO₃/L |

After approximately one month of acclimation in the laboratory culture water and the production of a sufficient number of juveniles from the culture, testing commenced. Neonates less than 24 hours old were collected and used as needed for toxicity testing.

#### 2.2.2 Survival in test waters

*Daphnia* were exposed to each natural water prior to the start of each test to ensure high survival rates of control organisms could be achieved. Two replicates of 10 organisms were used for each and *Daphnia* survival and mobility was assessed after a 48-hour exposure.

#### 2.2.3 Toxicity testing

Acute toxicity was tested with *Daphnia thomsoni* using 48-hour survival endpoints. Standard toxicity testing protocols developed at NIWA (NIWA 2022b), based on OECD Test 202 (2004) were used in this study (as summarised in Table 2-3). Tests were performed under static conditions in 55 mL polypropylene containers. On test initiation, ten <24-hour old neonates from the laboratory culture (Section 2.2.1) were added to each test container holding the various test solutions, with 3 replicates for each zinc and copper treatment concentration and 5 negative control replicates per natural water test. Active organisms were selected using a clean narrow mouth transfer pipette into the test containers.

Test solutions were prepared in volumetric flasks by spiking 100 ppm or 1000 ppm stock solutions of zinc (as  $ZnSO_4, 7H_2O$ ) or copper (as  $CuSO_4$ ) to 0.45  $\mu$ m filtered natural waters at least 24 hours before aliquoting into the test containers and adding organisms. A minimum of five treatment test concentrations up to a maximum of nine (zinc or copper) were used in each test, with different concentrations for each natural water aiming to bracket the likely  $EC_{50}$  in a geometric series with a factor less than 3 between test concentrations. Nominal test concentrations were supplied by the client.

| Test protocol:               | NIWA SOP 10.0 (NIWA 2022b)  |
|------------------------------|---|
| Reference method:            | OECD (2004)   |
| Test organisms:              | Daphnia thomsoni (previously known as D. carinata, Burns<br>et al. (2017))  |
| Organism source:             | Fernhollow pond, Waikato  |
| Test duration:               | 48-hour   |
| Test type:                   | Static  |
| Test chambers:               | 55 mL polypropylene beakers   |
| Replicates:                  | 5 for controls, 3 for treatments  |
| Organisms/container:         | 10  |
| Age of test organisms:       | <24-hour neonates from pre-test acclimation   |
| Test dilutions               | Minimum of 5, maximum of 9 concentrations, varying from 45-11,000 μg/L for zinc and 1-1391 μg/L for copper  |
| Lighting:                    | 16:8h light:dark  |
| Temperature:                 | 20 ± 2°C  |
| Aeration:                    | Nil   |
| Feeding during test:         | None  |
| Chemical data:               | Initial and final conductivity, temperature, pH, dissolved<br>oxygen, zinc or copper of selected test treatments.<br>Hardness (as dissolved calcium and magnesium) and<br>dissolved organic carbon of natural waters. |
| Observation:                 | 24- and 48-hour survival  |
| Effect measured:             | Survival  |
| Test acceptability criteria: | Mean control survival ≥90%  |

| Table 2-3: | Summary of D. thomsoni toxicity test conditions. |
|------------|--|
|------------|--|

Tests were performed at  $20 \pm 2^{\circ}$ C with a 16 h light/8 h dark photoperiod. *Daphnia* mortality was observed and recorded after 24 hours and at test termination, 48 hours.

Temperature, dissolved oxygen (DO), pH and electrical conductivity were measured on the test solutions at test initiation and five selected treatments at test termination (Appendix K and Appendix L).

Subsamples of five selected test concentrations were collected and filtered (0.45  $\mu$ m) for dissolved metals analysis at the start (Time zero, T<sub>0</sub>) and end (Time 48, T<sub>48</sub>) of each test. At test initiation (T<sub>0</sub>) the subsamples were taken from the prepared solutions prior to aliquoting into test chambers and at test termination (T<sub>48</sub>) the subsamples were partly collected from all treatment replicates (n=5 for controls and n=3 for positive treatments) and composited.

The selection of treatments for physico-chemical parameter measurement and chemical analysis (zinc or copper) was guided by the 48-hour *D. thomsoni* test survival results. Five concentrations that encompassed partial responses were identified to be included in the statistical analysis, thereby resolving the dose-response curve that encompasses the full survival-response range.

#### 2.3 Reference toxicant

Reference toxicant testing was undertaken concurrently to measure the sensitivity and condition of the test organisms using the standard test procedures (NIWA 2022b). Zinc sulfate is used as the reference toxicant and results from this test were compared to the long-term data set (NIWA, unpublished). This is part of the quality control procedures and enables comparability between laboratory test results in standard dilution water at different times. The zinc sulfate stock concentration was validated by chemical analysis (Hill Labs, data not shown).

#### 2.4 Chemical analysis

Hill Laboratories analysed subsamples of each of the natural waters for total hardness (measured as dissolved calcium and dissolved magnesium) and DOC (measured as non-purgeable organic carbon, DNPOC) (Appendix A) and selected test solution subsamples (T<sub>0</sub> and T<sub>48</sub>) for the applicable dissolved metal (zinc or copper) (Appendix B, Appendix C and Appendix D).

#### 2.5 Test acceptability criteria

The test was deemed acceptable if control organisms had greater than or equal to 90% mean survival (NIWA 2022b) (Appendix E).

#### 2.6 Statistical analysis

The zinc and copper concentrations used in the statistical analyses were a mean of the concentrations measured at the test start  $(T_0)$  and end  $(T_{48})$ .

The Daphnia test results were statistically analysed using CETIS<sup>™</sup> v2.1.4.5 (Comprehensive Environmental Toxicity Information System) software and corresponding user manual by Tidepool Scientific (2001-2022). CETIS<sup>™</sup> is a statistical application designed for analysing and reporting doseresponse results from aquatic, terrestrial and sediment toxicity tests. All statistical analyses follow US EPA standard guidelines for toxicity data analysis.

Initial analysis determined if there was a survival concentration relationship and if so, an ANOVA compared the survival at each concentration to determine the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). A regression model (3P log-logistic non-linear regression) was fitted where possible, otherwise a linear interpolation was conducted to calculate point estimates (EC<sub>50</sub> and EC<sub>10</sub>) with associated 95% confidence intervals ( $\alpha$ =0.05). When linear interpolation was applied, EC<sub>15</sub> values were calculated rather than EC<sub>10</sub> values (Appendix F, Appendix G, Appendix H, Appendix I and Appendix J).

# 3 Results and discussion

#### 3.1 Characterisation of natural waters

The water hardness, DOC and physico-chemical measurements of the five natural water samples are summarised in Table 3-1.

| Table 3-1:      | Characteristics of the natural water samples. Okutua Creek water (shaded grey) was adjusted |
|-----------------|---|
| prior to toxici | ty testing due to low survival in test water.   |

| Natural<br>water    | NIWA<br>laboratory<br>ID | Temp.ª<br>°C | рН   | Conductivity<br>μS cm <sup>-1</sup> | Dissolved<br>Oxygen<br>(DO)<br>mg/L 0 <sub>2</sub> | Dissolved<br>calcium/<br>magnesium<br>mg/L | Hardness<br>mg/L<br>CaCO <sub>3</sub> | DOC⁵<br>mg/L | Dissolved<br>zincº µg/L | Dissolved<br>copper <sup>c</sup><br>µg/L |
|---------------------|--------------------------|--------------|------|-------------------------------------|--|--|---------------------------------------|--------------|-------------------------|--|
| Mahurangi<br>Stream | 24.003.1                 | 18.4         | 7.58 | 242                                 | 10.7   | 16/8.1                                     | 73.5                                  | 2.18         | 3.2                     | <0.5 <sup>d</sup>                        |
| Hoteo<br>River      | 24.003.2                 | 18.3         | 7.49 | 201                                 | 10.7   | 14/ 5.7                                    | 58.1                                  | 3.73         | 4.4                     | 1.1                                      |
| Okutua<br>Creek     | 24.003.3                 | 18.0         | 4.96 | 27                                  | 10.9   | 0.48/0.37                                  | 2.74                                  | 11.9         | 4.4                     | 1.3                                      |
| Clutha<br>River     | 24.003.4                 | 18.1         | 7.17 | 72.4                                | 11.2   | 12/ 0.74                                   | 33.7                                  | 0.38         | 2.9                     | <0.5 <sup>d</sup>                        |
| Waihou<br>River     | 24.003.5                 | 19.1         | 7.26 | 89.9                                | 8.9  | 3.3/ 1.8                                   | 15.7                                  | <0.3         | 3.3                     | 0.8                                      |

<sup>a</sup> At time of measurements. <sup>b</sup> Measured as dissolved non-purgeable organic carbon (DNPOC). <sup>c</sup> Test initiation and test termination mean measured concentrations. <sup>d</sup> Less than detection limit.

The pH of Okutua Creek was 4.96 and the water had very low hardness (2.74 mg/L, Appendix A), outside of the range that would be suitable for the *Daphnia* to survive. The pH of the Okutua water was therefore adjusted using sodium hydroxide (NaOH) to a higher pH. The final adjusted pH of the Okutua Creek natural water used in testing was 6.03.

#### 3.2 Daphnia toxicity testing

The measured zinc and copper concentrations for selected Mahurangi, Hoteo, Clutha and Waihou treatments are provided in Appendix B and Appendix C respectively. The zinc and copper measured concentrations for the pH adjusted Okutua natural water are provided in Appendix D. Raw data (Appendix E) and detailed results from the statistical analyses are provided for all toxicity tests in Appendix F to Appendix J. A summary of physico-chemical analyses for the zinc and copper tests are included in Appendix K and Appendix L respectively.

The air temperature of the controlled temperature room which held the test vessels was  $20 \pm 2.0$  °C throughout the duration of the tests. The zinc test solution temperatures at test initiation were 19-20 °C and the dissolved oxygen concentration 8.0-9.3 mg/L O<sub>2</sub>. At the test termination the dissolved oxygen was 8.6-9.2 mg/L O<sub>2</sub>, and the solution temperatures ranged from 20-21 °C.

The pH of the zinc test solutions used in statistical analysis measured at the start and end of the exposure ranged from 7.7-8.1 pH units for Mahurangi, 7.6-8.0 pH units for Hoteo, 5.7-6.5 pH units for pH adjusted Okutua, 7.3-7.6 pH units for Clutha and 7.5-7.9 pH units for Waihou (Appendix K).

The copper test solution temperatures at test initiation were 19-20°C and the dissolved oxygen concentration ranged from 8.2-9.8 mg/L  $O_2$ . At the test termination the dissolved oxygen ranged from 8.6-9.2 mg/L  $O_2$ , and the solution temperatures were 20°C. The pH of the copper test solutions used in statistical analysis measured at the start and end of the exposure ranged from 7.2-8.0 pH units for Mahurangi, 7.5-7.8 pH units for Hoteo, 5.6-6.6 pH units for pH adjusted Okutua, 7.5-8.0 pH units for Clutha and 7.5-7.9 pH units for Waihou (Appendix L).

The zinc and copper toxicity results are summarised in Table 3-7 and Table 3-13 respectively.

#### 3.2.1 Test acceptability

After 48 hours the average survival in the Mahurangi, Hoteo, Okutua, Clutha and Waihou natural water control treatments was 96%-100% thereby meeting the criterion for test validity ( $\leq$ 10% mortality in control treatments) (Appendix E).

The *D. thomsoni* reference toxicant 48 h survival  $EC_{50}$  for zinc was 998 (812-1,226) µg/L Zn<sup>2+</sup> (± 95% CL). The reference toxicant testing criterion is that the  $EC_{50}$  falls within 2 standard deviations of the long-term average, however NIWA has limited data for this species (Appendix M).

#### 3.2.2 Zinc tests

Zinc concentrations in most of the test samples measured by Hill Labs (Appendix B and Appendix D) were within 17% of the nominal concentrations. However, there was evidence of possible zinc contamination in the Mahurangi Stream and Hoteo River 'Concentration 1' samples (both nominally  $100 \ \mu g/L \ Zn^{2+}$ ) subsampled at test termination (T<sub>48</sub>). These two measurements were not used in statistical analysis as are considered as outliers, only the test initiation (T<sub>0</sub>) measured concentrations were used. Mean measured concentrations (excluding outliers) were used in all data analyses. Zinc concentrations measured in the Mahurangi, Hoteo, Okutua (pH adjusted), Clutha and Waihou natural waters test solutions at the initiation of the exposure period were all within 8% of the concentrations of zinc in the test solutions at the end of the exposure period, indicating stability throughout the test period (Table 3-2 to Table 3-6).

Table 3-2:Nominal and measured zinc concentrations at the start (T<sub>0</sub>) and end (T<sub>48</sub>) of the MahurangiStream Daphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differencescalculated for nominal and mean measured concentrations, and initial and final measured test concentrations.Shaded cells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between $T_0$ and<br>$T_{48}$ measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 100                                | 3.3  | 3.1   | 3.2   | 200  | 6   |
| Concentration<br>1           | 100  | 100                                | 103  | 460ª  | 103 <sup>b</sup>  | 3 <sup>b</sup>   | -   |

| Treatment          | Nominal<br>concentration<br>(μg/L Zn <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between T <sub>0</sub> and<br>T <sub>48</sub> measured<br>concentrations<br>(%) |
|--------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>2 | 220  | 100                                | 230  | 250   | 240   | 9  | 8   |
| Concentration<br>3 | 484  | 90                                 | 490  | 490   | 490   | 4  | 0   |
| Concentration<br>4 | 1,065  | 27                                 | 1,080  | 1,030   | 1,055   | 0  | 5   |
| Concentration<br>5 | 2,343  | 0                                  | 2,400  | 2,300   | 2,350   | 0  | 4   |
| Concentration<br>6 | 5,154  | 0                                  | nm   | nm  | -   | -  | -   |
| Concentration<br>7 | 11,000   | 0                                  | nm   | nm  | -   | -  | -   |

<sup>a</sup> Considered an outlier so not used in statistical analysis. <sup>b</sup> Based on T<sub>0</sub> measured concentration only.

Table 3-3:Nominal and measured zinc concentrations at the start  $(T_0)$  and end  $(T_{48})$  of the Hoteo RiverDaphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differences calculatedfor nominal and mean measured concentrations, and initial and final measured test concentrations. Shadedcells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(μg/L Zn <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between T <sub>0</sub> and<br>T <sub>48</sub> measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 100                                | 4.2  | 4.5   | 4.35  | 200  | 7   |
| Concentration<br>1           | 100  | 100                                | 105  | 510ª  | 105 <sup>b</sup>  | 5 <sup>b</sup>   | -   |
| Concentration<br>2           | 220  | 100                                | 230  | 240   | 235   | 9  | 4   |
| Concentration<br>3           | 484  | 70                                 | 520  | 490   | 505   | 1  | 6   |
| Concentration<br>4           | 1,065  | 33                                 | 1,080  | 1,030   | 1,055   | 2  | 5   |
| Concentration<br>5           | 2,343  | 0                                  | 2,400  | 2,400   | 2,400   | 2  | 0   |
| Concentration<br>6           | 5,154  | 0                                  | nm   | nm  | -   | -  | -   |
| Concentration<br>7           | 11,000   | 0                                  | nm   | nm  | -   | -  | -   |

 $^{\rm a}$  Considered an outlier so not used in statistical analysis.  $^{\rm b}$  Based on  $T_0$  measured concentration only.

Table 3-4:Nominal and measured zinc concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Okutua CreekDaphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differences calculatedfor nominal and mean measured concentrations, and initial and final measured test concentrations. Shadedcells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between $T_0$ and<br>$T_{48}$ measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 98                                 | 3.9  | 4.4   | 4.4   | 200  | 12  |
| Concentration<br>1           | 100  | 93                                 | 110  | 108   | 109   | 9  | 2   |
| Concentration<br>2           | 220  | 70                                 | 250  | 220   | 235   | 7  | 13  |
| Concentration<br>3           | 484  | 33                                 | 500  | 470   | 485   | 0  | 6   |
| Concentration<br>4           | 1,065  | 0                                  | 1,070  | 1,090   | 1,080   | 1  | 2   |
| Concentration<br>5           | 2,343  | 0                                  | 2,300  | 2,300   | 2,300   | 2  | 0   |
| Concentration<br>6           | 5,154  | 0                                  | nm   | nm  | -   | -  | -   |
| Concentration<br>7           | 11,000   | 0                                  | nm   | nm  | -   | -  | -   |

Table 3-5:Nominal and measured zinc concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Clutha RiverDaphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differences calculatedfor nominal and mean measured concentrations, and initial and final measured test concentrations. Shadedcells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between T <sub>0</sub> and<br>T <sub>48</sub> measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 98                                 | 2.7  | 3.0   | 2.85  | 200  | 11  |
| Concentration<br>1           | 45   | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>2           | 100  | 100                                | 106  | 132   | 119   | 17   | 22  |
| Concentration<br>3           | 220  | 87                                 | 230  | 220   | 225   | 4  | 4   |
| Concentration<br>4           | 484  | 53                                 | 520  | 490   | 505   | 4  | 6   |
| Concentration<br>5           | 1,065  | 13                                 | 1,120  | 1,040   | 1,080   | 3  | 7   |

| Treatment          | Nominal<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between T <sub>0</sub> and<br>T <sub>48</sub> measured<br>concentrations<br>(%) |
|--------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>6 | 2,343  | 0                                  | 2,500  | 2,300   | 2,400   | 2  | 8   |
| Concentration<br>7 | 5,154  | 0                                  | nm   | nm  | -   | -  | -   |

Table 3-6:Nominal and measured zinc concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Waihou RiverDaphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differences calculatedfor nominal and mean measured concentrations, and initial and final measured test concentrations. Shadedcells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(μg/L Zn <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Zn <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(μg/L Zn <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between T <sub>0</sub> and<br>T <sub>48</sub> measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 96                                 | 3.1  | 3.4   | 3.25  | 200  | 9   |
| Concentration<br>1           | 100  | 93                                 | 109  | 123   | 116   | 12   | 12  |
| Concentration<br>2           | 220  | 77                                 | 240  | 220   | 230   | 2  | 9   |
| Concentration<br>3           | 484  | 40                                 | 520  | 480   | 500   | 0  | 8   |
| Concentration<br>4           | 1,065  | 0                                  | 1,130  | 1,090   | 1,110   | 3  | 4   |
| Concentration<br>5           | 2,343  | 0                                  | 2,500  | 2,400   | 2,450   | 2  | 4   |
| Concentration<br>6           | 5,154  | 0                                  | nm   | nm  | -   | -  | -   |

The 48 h *D. thomsoni* survival tests showed progressive concentration-response relationships, where *Daphnia* survival decreased with increasing zinc concentrations for all natural water samples.

For the Mahurangi Stream natural water sample spiked with zinc, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 240 mg/L Zn<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 490 mg/L Zn<sup>2+</sup> resulted in a statistically significant reduction in survival by 10% relative to the control. Complete mortality (100%) was observed at the highest concentration tested (2,350 mg/L Zn<sup>2+</sup>). The acute toxicity test for *Daphnia* resulted in an EC<sub>10</sub> of 511 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 459-551 µg/L Zn<sup>2+</sup> and an EC<sub>50</sub> of 826 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 782-873 µg/L Zn<sup>2+</sup> (Figure 3-1, Table 3-7 and Appendix F).



Figure 3-1: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Mahurangi Stream natural water sample. 3P Log-Logistic fitted.

For the Hoteo natural water sample spiked with zinc, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 235 mg/L Zn<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 505 mg/L Zn<sup>2+</sup> resulted in a statistically significant reduction in survival by 30% relative to the control. Complete mortality (100%) was observed at the highest concentration tested (2,400 mg/L Zn<sup>2+</sup>). The acute toxicity test for *Daphnia* resulted in an EC<sub>10</sub> of 374 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 279-442 µg/L Zn<sup>2+</sup> and an EC<sub>50</sub> of 751 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 661-853 µg/L Zn<sup>2+</sup> (Figure 3-2, Table 3-7 and Appendix G).



Figure 3-2: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Hoteo River natural water sample. 3P Log-Logistic fitted.

For the pH adjusted Okutua Creek natural water sample spiked with zinc, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 109 mg/L Zn<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 235 mg/L Zn<sup>2+</sup> resulted in a statistically significant reduction in survival by 29% relative to the control. Complete mortality (100%) was observed at the second highest concentration tested (1,080 mg/L Zn<sup>2+</sup>).

The acute toxicity test for *Daphnia* resulted in an EC<sub>10</sub> of 162  $\mu$ g/L Zn<sup>2+</sup>, with a 95% confidence interval of 78-208  $\mu$ g/L Zn<sup>2+</sup> and an EC<sub>50</sub> of 343  $\mu$ g/L Zn<sup>2+</sup>, with a 95% confidence interval of 282-416  $\mu$ g/L Zn<sup>2+</sup> (Figure 3-3, Table 3-7 and Appendix H).



**Figure 3-3:** Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for pH adjusted Okutua Creek natural water sample. 3P Log-Logistic fitted.

For the Clutha natural water sample spiked with zinc, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 225 mg/L Zn<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 505 mg/L Zn<sup>2+</sup> resulted in a statistically significant reduction in survival by 46% relative to the control. Complete mortality (100%) was observed at the highest concentration tested (2,400 mg/L Zn<sup>2+</sup>). The acute toxicity test for *Daphnia* resulted in an EC<sub>10</sub> of 243 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 124-311 µg/L Zn<sup>2+</sup> and an EC<sub>50</sub> of 526 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 432-641 µg/L Zn<sup>2+</sup> (Figure 3-4, Table 3-7 and Appendix I).



Figure 3-4: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Clutha River natural water sample. 3P Log-Logistic fitted.

For the Waihou natural water sample spiked with zinc, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 116 mg/L Zn<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 230 mg/L Zn<sup>2+</sup> resulted in a statistically significant reduction in survival by 20% relative to the control. Complete mortality (100%) was observed at the second highest concentration tested (1,110 mg/L Zn<sup>2+</sup>). The acute toxicity test for *Daphnia* resulted in an EC<sub>10</sub> of 204 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 121-252 µg/L Zn<sup>2+</sup> and an EC<sub>50</sub> of 404 µg/L Zn<sup>2+</sup>, with a 95% confidence interval of 344-474 µg/L Zn<sup>2+</sup> (Figure 3-5, Table 3-7 and Appendix J).



Figure 3-5: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Waihou River natural water sample. 3P Log-Logistic fitted.

| Notural water       | EC <sub>10</sub> <sup>a</sup>  | EC <sub>50</sub> <sup>a</sup>  | NOEC <sup>b</sup>     | LOEC <sup>c</sup>        | TEC <sup>d</sup>      |
|---------------------|--------------------------------|--------------------------------|-----------------------|--------------------------|-----------------------|
| Natural Water       | (95% CL) μg/L Zn <sup>2+</sup> | (95% CL) μg/L Zn <sup>2+</sup> | µg/L Zn <sup>2+</sup> | μ <mark>g/L Zn</mark> ²+ | µg/L Zn <sup>2+</sup> |
| Mahurangi           | 511 (459-551)                  | 826 (782-873)                  | 240                   | 490                      | 343                   |
| Hoteo               | 374 (279-442)                  | 751 (661-853)                  | 235                   | 505                      | 345                   |
| Okutua <sup>e</sup> | 162 (78-208)                   | 343 (282-416)                  | 109                   | 235                      | 160                   |
| Clutha              | 243 (124-311)                  | 526 (432-641)                  | 225                   | 505                      | 337                   |
| Waihou              | 204 (121-252)                  | 404 (344-474)                  | 116                   | 230                      | 163                   |

Table 3-7: Toxicity statistics as statistically derived by CETIS<sup>TM</sup> ( $\mu$ g/L zinc) for *D. thomsoni* 48-hour survival in each natural water tested. Values in parentheses are the EC<sub>50</sub> value 95% confidence intervals.

<sup>a</sup> EC<sub>x</sub>: The statistically determined test Concentration causing a X% Effect on the endpoint after the specified exposure period. <sup>b</sup> NOEC: The highest tested Concentration causing No Observed Effect relative to the controls. <sup>c</sup> LOEC: The Lowest tested Concentration causing an Observed Effect relative to the controls. <sup>d</sup> TEC: Threshold Effect Concentration, the geometric mean of NOEC and LOEC. <sup>e</sup> As pH adjusted for testing.

#### 3.2.3 Copper tests

Copper concentrations in most test samples analysed by Hill Labs (Appendix C and Appendix D) were within 18% of the target (nominal) concentrations. However, for 'Concentration 4' using Okutua Creek natural water, there was a 28% discrepancy between the nominal (10.6  $\mu$ g/L Cu<sup>2+</sup>) and the mean measured (14  $\mu$ g/L Cu<sup>2+</sup>) concentration.

Copper levels measured at the start of the exposure period ( $T_0$ ) and at the end ( $T_{48}$ ) generally showed a decrease. For the Mahurangi, Hoteo, Okutua, and Clutha test solutions, copper concentrations at the beginning ( $T_0$ ) were within 18% of the levels at the end ( $T_{48}$ ). However, the Waihou test solutions exhibited greater variability, with differences up to 32%, indicating a significant loss of copper during the test period (Table 3-8 to Table 3-12).

The mean measured concentrations were used for statistical analysis.

Table 3-8:Nominal and measured copper concentrations at the start (T<sub>0</sub>) and end (T<sub>48</sub>) of the MahurangiStream Daphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differencescalculated for nominal and mean measured concentrations, and initial and final measured test concentrations.Shaded cells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(μg/L Cu <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between T <sub>0</sub> and<br>T <sub>48</sub> measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 100                                | <0.5 ª   | <0.5 °  | <0.5 °  | 0  | 0   |
| Concentration<br>1           | 1  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>2           | 2.2  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>3           | 4.8  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>4           | 10.6   | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>5           | 23.4   | 100                                | 25   | 21  | 23  | 2  | 17  |

| Treatment          | Nominal<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between T <sub>0</sub> and<br>T <sub>48</sub> measured<br>concentrations<br>(%) |
|--------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>6 | 51.5   | 100                                | 52   | 50  | 51  | 1  | 4   |
| Concentration<br>7 | 154.5  | 70                                 | 156  | 155   | 155.5   | 1  | 1   |
| Concentration<br>8 | 463.5  | 0                                  | 470  | 430   | 450   | 3  | 9   |
| Concentration<br>9 | 1390.5   | 0                                  | 1440   | 1200  | 1320  | 5  | 18  |

<sup>a</sup> Less than detection limit.

Table 3-9:Nominal and measured copper concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Hoteo RiverDaphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differences calculatedfor nominal and mean measured concentrations, and initial and final measured test concentrations. Shadedcells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between $T_0$ and<br>$T_{48}$ measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 100                                | 1.1  | 1   | 1.05  | 200  | 10  |
| Concentration<br>1           | 1  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>2           | 2.2  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>3           | 4.8  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>4           | 10.6   | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>5           | 23.4   | 100                                | 24   | 25  | 24.5  | 5  | 4   |
| Concentration<br>6           | 51.5   | 100                                | 53   | 53  | 53  | 3  | 0   |
| Concentration<br>7           | 154.5  | 100                                | 149  | 153   | 151   | 2  | 3   |
| Concentration<br>8           | 463.5  | 33                                 | 470  | 470   | 470   | 1  | 0   |
| Concentration<br>9           | 1390.5   | 0                                  | 1420   | 1220  | 1320  | 5  | 15  |

Table 3-10:Nominal and measured copper concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the OkutuaCreek Daphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differencescalculated for nominal and mean measured concentrations, and initial and final measured test concentrations.Shaded cells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(μg/L Cu <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between $T_0$ and<br>$T_{48}$ measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 98                                 | 1.3  | 1.3   | 1.3   | 200  | 0   |
| Concentration<br>1           | 1  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>2           | 2.2  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>3           | 4.8  | 97                                 | nm   | nm  | -   | -  | -   |
| Concentration<br>4           | 10.6   | 100                                | 14.3   | 13.7  | 14  | 28   | 4   |
| Concentration<br>5           | 23.4   | 100                                | 28   | 28  | 28  | 18   | 0   |
| Concentration<br>6           | 51.5   | 97                                 | 60   | 58  | 59  | 14   | 3   |
| Concentration<br>7           | 113.4  | 13                                 | 136  | 136   | 136   | 18   | 0   |
| Concentration<br>8           | 249  | 0                                  | 280  | 280   | 280   | 12   | 0   |

Table 3-11:Nominal and measured copper concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the Clutha RiverDaphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differences calculatedfor nominal and mean measured concentrations, and initial and final measured test concentrations. Shadedcells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between $T_0$ and<br>$T_{48}$ measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 98                                 | <0.5 ª   | <0.5 °  | <0.5 ª  | 0  | 0   |
| Concentration<br>1           | 1  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>2           | 2.2  | 97                                 | 2.4  | 2.0   | 2.2   | 0  | 18  |
| Concentration<br>3           | 4.8  | 100                                | 4.7  | 4.1   | 4.4   | 9  | 14  |
| Concentration<br>4           | 10.6   | 100                                | 10.2   | 10.3  | 10.25   | 3  | 1   |

| Treatment          | Nominal<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(μg/L Cu <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between $T_0$ and<br>$T_{48}$ measured<br>concentrations<br>(%) |
|--------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>5 | 23.4   | 87                                 | 23   | 22  | 22.5  | 4  | 4   |
| Concentration<br>6 | 51.5   | 7                                  | 54   | 47  | 50.5  | 2  | 14  |

<sup>a</sup> Less than detection limit.

Table 3-12:Nominal and measured copper concentrations at the start ( $T_0$ ) and end ( $T_{48}$ ) of the WaihouRiver Daphnia survival toxicity test.Measured by Hill Labs, nm = not measured. Percentage differencescalculated for nominal and mean measured concentrations, and initial and final measured test concentrations.Shaded cells indicate concentrations used in statistical analyses.

| Treatment                    | Nominal<br>concentration<br>(μg/L Cu <sup>2+</sup> ) | Mean<br>48-hour<br>survival<br>(%) | Measured T <sub>0</sub><br>concentration<br>(μg/L Cu <sup>2+</sup> ) | Measured T <sub>48</sub><br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Mean<br>measured<br>concentration<br>(µg/L Cu <sup>2+</sup> ) | Difference<br>between nominal<br>and mean<br>measured<br>concentrations<br>(%) | Difference<br>between $T_0$ and<br>$T_{48}$ measured<br>concentrations<br>(%) |
|------------------------------|--|------------------------------------|--|---|---|--|---|
| Concentration<br>0 - Control | 0  | 96                                 | 0.9  | 0.7   | 0.8   | 200  | 25  |
| Concentration<br>1           | 1  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>2           | 2.2  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>3           | 4.8  | 100                                | nm   | nm  | -   | -  | -   |
| Concentration<br>4           | 10.6   | 97                                 | 11.3   | 8.2   | 9.75  | 8  | 32  |
| Concentration<br>5           | 23.4   | 83                                 | 23   | 19.1  | 21.05   | 11   | 19  |
| Concentration<br>6           | 51.5   | 40                                 | 53   | 43  | 48  | 7  | 21  |
| Concentration<br>7           | 113  | 0                                  | 102  | 92  | 97  | 15   | 10  |
| Concentration<br>8           | 249  | 0                                  | 230  | 191   | 210.5   | 17   | 19  |

The 48 h *Daphnia thomsoni* survival tests showed progressive concentration-response relationships, where *Daphnia* survival decreased with increasing copper concentrations for all natural water samples.

For the Mahurangi Stream natural water sample spiked with copper, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 51 mg/L Cu<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 156 mg/L Cu<sup>2+</sup> resulted in a statistically significant reduction in survival by 30% relative to the control. Complete mortality (100%) was observed at the second highest concentration tested (450 mg/L Cu<sup>2+</sup>). The acute toxicity test for

Daphnia resulted in an EC<sub>15</sub> of 89  $\mu$ g/L Cu<sup>2+</sup>, with a 95% confidence interval of 65-150  $\mu$ g/L Cu<sup>2+</sup> and an EC<sub>50</sub> of 211  $\mu$ g/L Cu<sup>2+</sup>, with a 95% confidence interval of 159-255  $\mu$ g/L Cu<sup>2+</sup> (Figure 3-6, Table 3-13 and Appendix F).



Figure 3-6: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Mahurangi Stream natural water sample. Linear interpolation.

For the Hoteo natural water sample spiked with copper, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 151 mg/L Cu<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 470 mg/L Cu<sup>2+</sup> resulted in a statistically significant reduction in survival by 97% relative to the control. Complete mortality (100%) was observed at the highest concentration tested (1,320 mg/L Cu<sup>2+</sup>). The acute toxicity test for *Daphnia* resulted in an EC<sub>15</sub> of 180 µg/L Cu<sup>2+</sup>, with a 95% confidence interval of 178-183 µg/L Cu<sup>2+</sup> and an EC<sub>50</sub> of 272 µg/L Cu<sup>2+</sup>, with a 95% confidence interval of 261-284 µg/L Cu<sup>2+</sup> (Figure 3-7, Table 3-13 and Appendix G).



Figure 3-7: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Hoteo River natural water sample. Linear interpolation.

For the pH adjusted Okutua Creek natural water sample spiked with copper, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 59 mg/L Cu<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 136 mg/L Cu<sup>2+</sup> resulted in a statistically significant reduction in survival by 86% relative to the control. Complete mortality (100%) was observed at the highest concentration tested (280 mg/L Cu<sup>2+</sup>). The acute toxicity test for *Daphnia* resulted in an EC<sub>10</sub> of 74 µg/L Cu<sup>2+</sup>, with a upper 95% confidence limit of 93 µg/L Cu<sup>2+</sup> and an EC<sub>50</sub> of 103 µg/L Cu<sup>2+</sup>, with a 95% confidence interval of 78-136 µg/L Cu<sup>2+</sup> (Figure 3-8, Table 3-13 and Appendix H).





For the Clutha natural water sample spiked with copper, no statistically significant negative effects on Daphnia survival were observed at concentrations up to 22.5 mg/L Cu<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure at the highest tested concentration, 50.5 mg/L Cu<sup>2+</sup> resulted in a statistically significant reduction in survival by 93% relative to the control. The acute toxicity test for Daphnia resulted in an EC10 of 22  $\mu$ g/L Cu2+, with a upper 95% confidence limit of 26  $\mu$ g/L Cu2+ and an EC50 of 32  $\mu$ g/L Cu2+, with a 95% confidence interval of 26-39  $\mu$ g/L Cu2+ (Figure 3-9, Table 3-13 and Appendix I).



Figure 3-9: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Clutha River natural water sample. 3P Log-Logistic fitted.

For the Waihou natural water sample spiked with copper, no statistically significant negative effects on *Daphnia* survival were observed at concentrations up to 21.1 mg/L Cu<sup>2+</sup> after 48 hours when compared to the negative control. However, exposure to 48 mg/L Cu<sup>2+</sup> resulted in a statistically significant reduction in survival by 58% relative to the control. Complete mortality (100%) was observed at the second highest concentration tested (97 mg/L Cu<sup>2+</sup>). The acute toxicity test for *Daphnia* resulted in an EC<sub>10</sub> of 23 µg/L Cu<sup>2+</sup>, with a upper 95% confidence limit of 30 µg/L Cu<sup>2+</sup> and an EC<sub>50</sub> of 41 µg/L Cu<sup>2+</sup>, with a 95% confidence interval of 33-51 µg/L Cu<sup>2+</sup> (Figure 3-10, Table 3-13 and Appendix J).



Figure 3-10: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Waihou River natural water sample. 3P Log-Logistic fitted.

# Table 3-13: Toxicity statistics as statistically derived by CETIS<sup>TM</sup> ( $\mu$ g/L copper) for *D. thomsoni* 48-hour survival in each natural water tested. Values in parentheses are the EC<sub>50</sub> value 95% confidence intervals.

| Natural water       | EC <sub>10</sub> ª<br>(95% CL) μg/L Cu <sup>2+</sup> | EC <sub>50</sub> ª<br>(95% CL) μg/L Cu <sup>2+</sup> | NOEC <sup>b</sup><br>μg/L Cu <sup>2+</sup> | LOEC <sup>c</sup><br>µg/L Cu <sup>2+</sup> | TEC <sup>d</sup><br>μg/L Cu <sup>2+</sup> |
|---------------------|--|--|--|--|---|
| Mahurangi           | 89 (65-150) <sup>f</sup>                             | 211 (159-255)  | 51.0                                       | 156  | 89.2                                      |
| Hoteo               | 180 (178-183) <sup>f</sup>                           | 272 (261-284)  | 151  | 470  | 266                                       |
| Okutua <sup>e</sup> | 74 (n/a-93)  | 103 (78-136)   | 59.0                                       | 136  | 89.6                                      |
| Clutha              | 22 (n/a-26)  | 32 (26-39)   | 22.5                                       | 50.5                                       | 33.7                                      |
| Waihou              | 23 (n/a-30)  | 41 (33-51)   | 21.1                                       | 48.0                                       | 31.8                                      |

<sup>a</sup> EC<sub>x</sub>: The statistically determined test Concentration causing a X% Effect on the endpoint after the specified exposure period. <sup>b</sup> NOEC: The highest tested Concentration causing No Observed Effect relative to the controls. <sup>c</sup> LOEC: The Lowest tested Concentration causing an Observed Effect relative to the controls. <sup>d</sup> TEC: Threshold Effect Concentration, the geometric mean of NOEC and LOEC. <sup>e</sup> As pH adjusted for testing. <sup>f</sup> EC<sub>15</sub> value as EC<sub>10</sub> not available with the linear interpolation analysis used.

## 4 Summary

The five natural waters collected covered a range of pH, hardness and DOC concentrations that are found in most waters around New Zealand. The pH of the waters tested covered only a narrow range -6.0 to 7.6 - as the sample with low pH (Okutua Creek) needed to be adjusted to minimise pH-derived toxicity.

The survival of *D. thomsoni* was affected by zinc at concentrations of 230  $\mu$ g/L and higher, with EC<sub>50</sub> values ranging from 343 to 826  $\mu$ g/L in acute 48-hour tests, depending on the water chemistry. Zinc exhibited the highest toxicity in water with low pH, low hardness, and higher DOC, while the lowest toxicity occurred in water with the highest pH and hardness. For copper, survival decreased at concentrations of 48  $\mu$ g/L and above, with EC<sub>50</sub> values ranging from 32 to 272  $\mu$ g/L. Copper toxicity was highest in water with low DOC, while the lowest toxicity was observed in waters with environmentally moderate pH and hardness (Table 4-1).

# Table 4-1:Summary of the natural water sample characteristics and toxicity statistics for *D. thomsoni*exposed to zinc and copper in 48-hour survival test.

|                  | рН                | Hardness<br>mg/L<br>CaCO <sub>3</sub> | DOC  | Zinc  |   | Copper  |   |
|------------------|-------------------|---------------------------------------|------|---|---|---|---|
| Natural<br>water |                   |                                       | mg/L | EC <sub>10</sub> ª<br>(95% CL) μg/L<br>Zn <sup>2+</sup> | EC <sub>50</sub> ª<br>(95% CL) μg/L<br>Zn <sup>2+</sup> | EC <sub>10</sub> ª<br>(95% CL) μg/L<br>Cu <sup>2+</sup> | EC <sub>50</sub> ª<br>(95% CL) μg/L<br>Cu <sup>2+</sup> |
| Mahurangi        | 7.58              | 73.5                                  | 2.18 | 511 (459-551)   | 826 (782-873)   | 89 (65-150) <sup>c</sup>                                | 211 (159-255)   |
| Hoteo            | 7.49              | 58.1                                  | 3.73 | 374 (279-442)   | 751 (661-853)   | 180 (178-183) <sup>c</sup>                              | 272 (261-284)   |
| Okutua           | 6.03 <sup>b</sup> | 2.74                                  | 11.9 | 162 (78-208)  | 343 (282-416)   | 74 (n/a-93)   | 103 (78-136)  |
| Clutha           | 7.17              | 33.7                                  | 0.38 | 243 (124-311)   | 526 (432-641)   | 22 (n/a-26)   | 32 (26-39)  |
| Waihou           | 7.26              | 15.7                                  | <0.3 | 204 (121-252)   | 404 (344-474)   | 23 (n/a-30)   | 41 (33-51)  |

<sup>a</sup> EC<sub>x</sub>: The statistically determined test Concentration causing a X% Effect on the endpoint after the specified exposure period. <sup>b</sup> As pH adjusted for testing. <sup>c</sup> EC<sub>15</sub> value as EC<sub>10</sub> not available with the linear interpolation analysis used.

These data, along with other available published and unpublished acute toxicity data (Appendix M), can be used to develop acute water quality guidelines for zinc and copper, incorporating the influence of toxicity-modifying factors.

# 5 Acknowledgements

The author would like to thank the NIWA field staff involved in the sample collection, as follows: Pete Pattinson and Christian Hyde (Auckland), John Porteous (Greymouth/ West Coast) and Duncan Macpherson (Alexandra). The author also thanks Amelia Shepherd and Vageesha Neththikumara for assistance in laboratory work for the *Daphnia* tests.

# 6 Glossary of abbreviations and terms

| Acute toxicity                      | A lethal or adverse sub-lethal effect that occurs after exposure to a chemical for<br>a short period relative to the organism's life span. Refer to Warne et al. (Warne<br>et al. 2018) for examples of acute exposures.   |
|-------------------------------------|--|
| ANZECC                              | Australian and New Zealand Environment and Conservation Council  |
| ANZG                                | Australia and New Zealand Government, publishers of water quality guidelines for fresh and marine waters used in New Zealand   |
| Cu                                  | Copper   |
| Default<br>guideline value<br>(DGV) | A guideline value recommended for generic application to all Australian and<br>New Zealand fresh or marine waterbodies in the absence of a more specific<br>guideline value (for example site-specific) in the Australian and New Zealand<br>Guidelines for Fresh and Marine Water Quality |
| DOC                                 | Dissolved organic carbon – operational measurement of DOM using a carbon analyser  |
| DOM                                 | Dissolved organic matter – generic terminology for all forms of organic material in a filtered water sample  |
| EC <sub>50</sub>                    | The toxicant concentration that is expected to cause one or more specified effects in 50% of a group of organisms or a 50% effect under specified conditions   |
| ECx                                 | The toxicant concentration that is expected to cause one or more specified effects in x% of a group of organisms or a x% effect under specified conditions   |
| Endpoint                            | Measured attainment response, typically applied to ecotoxicity or management goals   |
| LOEC                                | Lowest Observed Effect Concentration; the lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.  |
| NOEC                                | No Observed Effect Concentration, the highest concentration of a material used<br>in a toxicity test that has no statistically significant adverse effect on the<br>exposed population of test organisms as compared with the controls.  |
| NRWQN                               | National River Water Quality Network, a network of monitoring sites run by NIWA on major rivers in New Zealand sampled monthly for water quality analyses  |
| Species                             | A group of organisms that resemble each other to a greater degree than<br>members of other groups and that form a reproductively isolated group that<br>will not normally breed with members of another group. (Chemical species are<br>differing compounds of an element)                 |
| Toxicity                            | The inherent potential or capacity of a material to cause adverse effects in a living organism.  |
| Toxicity test                       | The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a concentration of chemical.   |
| US EPA                              | United States Environmental Protection Agency  |
| Zn                                  | Zinc   |
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## Appendix A Hill Labs results - Natural water characterisation



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#### Page 1 of 2

| Client:<br>Contact:   | NIWA Corporate<br>K Thompson<br>C/- NIWA Corporate<br>PO Box 11115<br>Hillcrest<br>Hamilton 3251 | Lab No:<br>Date Received:<br>Date Reported:<br>Quote No:<br>Order No:<br>Client Reference:<br>Submitted By: |                    |                        | 3569077<br>03-May-20<br>09-May-20<br>130803<br>U333268<br>Requisition<br>K Thomps | SUPv1<br>124<br>124<br>n 138984<br>on |
|---|--|---|--------------------|------------------------|---|---------------------------------------|
| Sample Ty   | /pe: Aqueous   |   |                    |                        |   |                                       |
|   | Sample Name:   | Clutha 13-Feb-2024  | Waihou 30-Apr-2024 | Okutua                 | 07-Feb-2024   | Redwoods<br>07-Feb-2024               |
| Lab Number:   |  | 3569077.1   | 3569077.2          | 3569077.3              |   | 3569077.4                             |
| Total Hardne  | ess g/m³ as CaCO <sub>3</sub>  | 33.7 ± 2.0  | 15.66 ± 0.73       | 2.74 ± 0.17 73.5 ± 3.4 |   | 73.5 ± 3.4                            |
| Dissolved Ca  | alcium g/m <sup>3</sup>  | 12.27 ± 0.77  | 3.25 ± 0.21        | 0.48                   | 3 ± 0.045   | 16.1 ± 1.1                            |
| Dissolved Ma  | agnesium g/m³  | 0.737 ± 0.052   | 1.83 ± 0.13        | 0.37                   | 2 ± 0.029   | 8.09 ± 0.55                           |
| Dissolved No<br>(DNPOC)   | on-Purgeable Organic Carbon g/m <sup>3</sup>   | 0.38 ± 0.14   | < 0.3 ± 0.14       | 11.9 ± 2.4 2.18 :      |   | 2.18 ± 0.46                           |
|   | Sample Name:   |   | Hoteo 07-          | Feb-2024               |   |                                       |
|   | Lab Number:  |   | 3569               | 077.5                  |   |                                       |
| Total Hardness g/m <sup>3</sup> as CaCO <sub>3</sub> 58.1 ± 2.7 |  |   |                    |                        |   |                                       |
| Dissolved Ca  | alcium g/m <sup>3</sup>  | 3 13.87 ± 0.87  |                    |                        |   |                                       |
| Dissolved Ma  | agnesium g/m <sup>3</sup>  | 3 5.70 ± 0.39   |                    |                        |   |                                       |
| Dissolved No<br>(DNPOC)   | on-Purgeable Organic Carbon g/m <sup>3</sup>   |   | 3.73 :             | ± 0.76                 |   |                                       |

The reported uncertainty is an expanded uncertainty with a level of confidence of approximately 95 percent (i.e. two standard deviations, calculated using a coverage factor of 2). Reported uncertainties are calculated from the performance of typical matrices, and do not include variation due to sampling.

For further information on uncertainty of measurement at Hill Laboratories, refer to the technical note on our website: www.hill-laboratories.com/files/Intro\_To\_UOM.pdf, or contact the laboratory.

#### **Summary of Methods**

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicated, analyses were performed at Hill Labs, 28 Duke Street, Frankton, Hamilton 3204.

| Sample Type: Aqueous                              |   |                               |           |
|---|---|-------------------------------|-----------|
| Test  | Method Description  | Default Detection Limit       | Sample No |
| Total Hardness                                    | Calculation from Calcium and Magnesium. APHA 2340 B :<br>Online Edition.  | 1.0 g/m³ as CaCO <sub>3</sub> | 1-5       |
| Filtration for dissolved metals analysis          | Sample filtration through 0.45µm membrane filter and<br>preservation with nitric acid. APHA 3030 B : Online Edition.                                      | -                             | 1-5       |
| Dissolved Calcium                                 | Filtered sample, ICP-MS, trace level. APHA 3125 B : Online<br>Edition.  | 0.05 g/m <sup>3</sup>         | 1-5       |
| Dissolved Magnesium                               | Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.   | 0.02 g/m <sup>3</sup>         | 1-5       |
| Dissolved Non-Purgeable Organic<br>Carbon (DNPOC) | Acidification, purging to remove inorganic C, super-critical<br>persulphate oxidation at 375°C, IR detection. APHA 5310 C<br>(modified) : Online Edition. | 0.3 g/m <sup>3</sup>          | 1-5       |



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Testing was completed between 06-May-2024 and 09-May-2024. For completion dates of individual analyses please contact the laboratory.

Samples are held at the laboratory after reporting for a length of time based on the stability of the samples and analytes being tested (considering any preservation used), and the storage space available. Once the storage period is completed, the samples are discarded unless otherwise agreed with the customer. Extended storage times may incur additional charges.

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Lab No: 3569077-SUPv1

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# Appendix B Hill Labs results – Zinc



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Page 1 of 2

| Client:<br>Contact:             | NIWA Corporate<br>K Thompson<br>C/- NIWA Corporate | Lab No: 3578828<br>Date Received: 13-May-20<br>Date Reported: 16-May-20 |   |                     | SUPv1<br>)24<br>)24          |  |
|---------------------------------|--|---|---|---------------------|------------------------------|--|
|                                 | PO Box 11115                                       |   | Quote No: 130803                                |                     |                              |  |
|                                 | Hillcrest  |   | Order No:                                       |                     | U333504                      |  |
|                                 | Hamilton 3251                                      |   | Client Refe                                     | erence:             |                              |  |
|                                 | Submitted Bv: K Thomp                              |   |   |                     | son                          |  |
| Sample T                        | vne. Vaneous                                       |   |   | <u> </u>            | · ·                          |  |
| oumpie 13                       | Sample Name:                                       | Clutha 100ug/L T0   | Clutha 220ug/L T0                               | Clutha              | 484ug/L T0                   | Clutha 1056ug/L T0                             |
|                                 | Sample Name.                                       | 09-May-2024 1:00 pm   | 09-May-2024 1:00 pm                             | 09-May-2            | 024 1:00 pm                  | 09-May-2024 1:00 pm                            |
|                                 | Lab Number:  | 3578828.1   | 3578828.2                                       | 357                 | 8828.3                       | 3578828.4                                      |
| Dissolved Zir                   | nc g/m <sup>3</sup>                                | 0.106 ± 0.010   | 0.233 ± 0.022                                   | 0.51                | 7 ± 0.049                    | 1.12 ± 0.11                                    |
|                                 | Sample Name:                                       | Clutha 2343ug/L T0  | Clutha 100ug/L T48                              | Clutha 2            | 20ug/L T48                   | Clutha 484ug/L T48                             |
|                                 | Lab Numbari  | 2579929 5   | 2570020 c                                       | 09-1vlay-2          | 024 1:00 pm                  | 09-May-2024 1:00 pm                            |
| Dissolved Zir                   | Lab Nulliber.                                      | 2 47 + 0 23   | 0 132 + 0 013                                   | 0.219               | 0020.7<br>0+0.021            | 0.487 + 0.046                                  |
| Dissolved Zil                   | g/m  | 2.47 1 0.20   | 0.102 1 0.010                                   | 0.21                | 5 1 0.021                    | 0.407 1 0.040                                  |
|                                 | Sample Name:                                       | Clutha 1056ug/L T48   | Clutha 2343ug/L T48                             | Waihou              | 100ug/L T0                   | Waihou 220ug/L T0                              |
|                                 | Lab Numbor   | 2579929 0   | 2578929 10                                      | 09-1viay-2          | 024 1.00 pm                  | 2579929 12                                     |
| Dissolved Zir                   | nc g/m <sup>3</sup>                                | 1.043 ± 0.097   | 2.31 ± 0.22                                     | 0.109               | 9 ± 0.011                    | 0.236 ± 0.022                                  |
|                                 | <b>.</b>   |   |   |                     |                              |  |
|                                 | Sample Name:                                       | 09-May-2024 1:00 pm   | Walhou 1056ug/L 10<br>09-May-2024 1:00 pm       | Walhou              | 2343ug/L 10                  | Walhou 100ug/L 148<br>09-May-2024 1:00 pm      |
|                                 | Lab Number:  | 3578828.13  | 3578828.14                                      | 357                 | 8828.15                      | 3578828.16                                     |
| Dissolved Zinc g/m <sup>3</sup> |  | 0.518 ± 0.049   | 1.13 ± 0.11                                     | 2.40                | 6 ± 0.23                     | 0.123 ± 0.012                                  |
|                                 | Sample Name  | Waihou 220ug/L T48  | Waihou 484ug/L T48                              | Waihou 1            | 056ua/L T48                  | Waihou 2343ug/L T48                            |
|                                 | Campie Hame.                                       | 09-May-2024 1:00 pm   | 09-May-2024 1:00 pm                             | 09-May-2            | 024 1:00 pm                  | 09-May-2024 1:00 pm                            |
|                                 | Lab Number:  | 3578828.17  | 3578828.18                                      | 357                 | 8828.19                      | 3578828.20                                     |
| Dissolved Zir                   | nc g/m <sup>3</sup>                                | 0.223 ± 0.021   | 0.482 ± 0.045                                   | 1.09                | 9 ± 0.11                     | 2.36 ± 0.22                                    |
|                                 | Sample Name:                                       | Redwoods 100ug/L T0<br>10-May-2024 1:00 pm                              | Redwoods 220ug/L T0<br>10-May-2024 1:00 pm      | Redwood<br>10-May-2 | s 484ug/L T0<br>:024 1:00 pm | Redwoods 1056ug/L<br>T0 10-May-2024<br>1:00 pm |
|                                 | Lab Number:  | 3578828.21  | 3578828.22                                      | 357                 | 8828.23                      | 3578828.24                                     |
| Dissolved Zir                   | nc g/m <sup>3</sup>                                | 0.1032 ± 0.0097   | 0.232 ± 0.022                                   | 0.49                | 1 ± 0.046                    | 1.08 ± 0.11                                    |
|                                 | Sample Name:                                       | Redwoods 2343ug/L   | Redwoods 100ug/L                                | Redwoo              | ds 220ug/L                   | Redwoods 484ug/L                               |
|                                 |  | T0 10-May-2024  | T48 10-May-2024                                 | T48 10              | -May-2024                    | T48 10-May-2024                                |
|                                 | Lab Number:  | 3578828.25  | 3578828.26                                      | 357                 | 8828 27                      | 3578828.28                                     |
| Dissolved Zir                   | nc g/m <sup>3</sup>                                | 2.38 ± 0.23   | 0.457 ± 0.043                                   | 0.254               | 1 ± 0.024                    | 0.487 ± 0.046                                  |
|                                 | <b>3</b>   |   |   |                     |                              |  |
|                                 | Sample Name:                                       | T48 10-May-2024<br>1:00 pm  | Redwoods 2343ug/L<br>T48 10-May-2024<br>1:00 pm | Hoteo<br>10-May-2   | 100ug/L T0<br>2024 1:00 pm   | Hoteo 220ug/L 10<br>10-May-2024 1:00 pm        |
|                                 | Lab Number:  | 3578828.29  | 3578828.30                                      | 357                 | 8828.31                      | 3578828.32                                     |
| Dissolved Zir                   | nc g/m <sup>3</sup>                                | 1.034 ± 0.097   | 2.30 ± 0.22                                     | 0.104               | 7 ± 0.0098                   | 0.229 ± 0.022                                  |
|                                 | Sample Name:                                       | Hoteo 484ug/L T0<br>10-May-2024 1:00 pm                                 | Hoteo 1056ug/L T0<br>10-May-2024 1:00 pm        | Hoteo 2<br>10-May-2 | 343ug/L T0<br>024 1:00 pm    | Hoteo 100ug/L T48<br>10-May-2024 1:00 pm       |
|                                 | Lab Number:  | 3578828.33  | 3578828.34                                      | 357                 | 8828.35                      | 3578828.36                                     |
| Dissolved Zir                   | nc g/m <sup>3</sup>                                | 0.519 ± 0.049   | 1.08 ± 0.11                                     | 2.44                | 1 ± 0.23                     | 0.513 ± 0.048                                  |



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| Sample Type: Aqueous            |  |  |   |   |  |  |
|---------------------------------|--|--|---|---|--|--|
| Sample Name:                    | Hoteo 220ug/L T48<br>10-May-2024 1:00 pm | Hoteo 484ug/L T48<br>10-May-2024 1:00 pm | Hoteo 1056ug/L T48<br>10-May-2024 1:00 pm | Hoteo 2343ug/L T48<br>10-May-2024 1:00 pm |  |  |
| Lab Number:                     | 3578828.37                               | 3578828.38                               | 3578828.39                                | 3578828.40                                |  |  |
| Dissolved Zinc g/m <sup>3</sup> | 0.244 ± 0.023                            | 0.486 ± 0.046                            | 1.026 ± 0.096                             | 2.36 ± 0.22                               |  |  |

The reported uncertainty is an expanded uncertainty with a level of confidence of approximately 95 percent (i.e. two standard deviations, calculated using a coverage factor of 2). Reported uncertainties are calculated from the performance of typical matrices, and do not include variation due to sampling.

For further information on uncertainty of measurement at Hill Laboratories, refer to the technical note on our website: www.hill-laboratories.com/files/Intro\_To\_UOM.pdf, or contact the laboratory.

#### **Summary of Methods**

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicates the dilutions are available from the laboratory upon request.

| Sample Type: Aqueous                     |  |                         |           |  |  |  |
|--|--|-------------------------|-----------|--|--|--|
| Test                                     | Method Description   | Default Detection Limit | Sample No |  |  |  |
| Filtration for dissolved metals analysis | Sample filtration through 0.45µm membrane filter and<br>preservation with nitric acid. APHA 3030 B : Online Edition. | -                       | 1-40      |  |  |  |
| Dissolved Zinc                           | Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.  | 0.0010 g/m <sup>3</sup> | 1-40      |  |  |  |

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Testing was completed between 14-May-2024 and 16-May-2024. For completion dates of individual analyses please contact the laboratory.

Samples are held at the laboratory after reporting for a length of time based on the stability of the samples and analytes being tested (considering any preservation used), and the storage space available. Once the storage period is completed, the samples are discarded unless otherwise agreed with the customer. Extended storage times may incur additional charges.

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Hill Labs

# Appendix C Hill Labs results - Copper



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#### **Certificate of Analysis** Page 1 of 2 NIWA Corporate 3575453 Client: Lab No: SUPv1 09-May-2024 Contact: K Thompson Date Received: C/- NIWA Corporate Date Reported: 14-May-2024 PO Box 11115 130803 Quote No: Hillcrest Order No: U333436 Hamilton 3251 **Client Reference:** HYR24201 Cu Submitted By: K Thompson Sample Type: Aqueous Sample Name: Waihou Control T0 Waihou Control T48 Waihou Cu 10.6 T0 Waihou Cu 10.6 T48 Lab Number: 3575453.1 3575453 2 3575453.3 3575453 4 Dissolved Copper g/m<sup>3</sup> $0.00092 \pm 0.00035$ 0.00068 ± 0.00034 0.0113 ± 0.0011 0.00818 ± 0.00082 Dissolved Zind 0.00311 ± 0.00073 0.00338 ± 0.00074 g/m<sup>3</sup> Waihou Cu 23.4 T0 Waihou Cu 23.4 T48 Waihou Cu 51.5 T0 Waihou Cu 51.5 T48 Sample Name: Lab Number: 3575453.5 3575453.6 3575453.7 3575453.8 Dissolved Copper g/m<sup>3</sup> 0.0234 ± 0.0022 0.0191 ± 0.0018 $0.0533 \pm 0.0049$ 0.0431 ± 0.0040 Waihou Cu 113 T0 Waihou Cu 113 T48 Waihou Cu 249 T0 Waihou Cu 249 T48 Sample Name: 3575453.12 Lab Number: 3575453.9 3575453.10 3575453.11 Dissolved Copper g/m<sup>3</sup> $0.1016 \pm 0.0093$ $0.0918 \pm 0.0084$ $0.229 \pm 0.021$ $0.191 \pm 0.018$ Sample Name: Redwoods Control T0 Redwoods Control T48 Redwoods Cu 23.4 T0 Redwoods Cu 23.4 T48 Lab Number: 3575453.13 3575453.14 3575453.15 3575453.16 < 0.0005 ± 0.00034 < 0.0005 ± 0.00034 0.0249 ± 0.0023 0.0211 ± 0.0020 Dissolved Copper g/m<sup>3</sup> Dissolved Zinc g/m<sup>3</sup> 0.00330 ± 0.00073 0.00314 ± 0.00073 Redwoods Cu 51.5 Redwoods Cu 154.5 Redwoods Cu 51.5 T0 Redwoods Cu 154.5 Sample Name: T48 т0 T48 3575453.17 3575453.18 3575453.19 3575453.20 Lab Number: Dissolved Copper $0.0525 \pm 0.0048$ $0.0501 \pm 0.0046$ 0.156 ± 0.015 $0.155 \pm 0.015$ g/m<sup>3</sup> Sample Name: Redwoods Cu 463.5 Redwoods Cu 463.5 Redwoods Cu 1390.5 Redwoods Cu 1390.5 TO T48 то T48 3575453.22 Lab Number: 3575453.21 3575453.23 3575453.24 Dissolved Copper g/m<sup>3</sup> $0.466 \pm 0.043$ $0.429 \pm 0.040$ $1.44 \pm 0.14$ $1.20 \pm 0.11$ Sample Name: Hoteo Control T0 Hoteo Control T48 Hoteo Cu 23 4 T0 Hoteo Cu 23 4 T48 3575453.28 3575453.25 3575453.27 Lab Number: 3575453.26 Dissolved Copper 0.00105 ± 0.00035 0.00098 ± 0.00035 $0.0240 \pm 0.0023$ $0.0251 \pm 0.0024$ g/m Dissolved Zinc g/m<sup>3</sup> 0.00418 ± 0.00077 $0.00453 \pm 0.00079$ -Sample Name: Hoteo Cu 51.5 T0 Hoteo Cu 51.5 T48 Hoteo Cu 154.5 T0 Hoteo Cu 154.5 T48 3575453.31 3575453.32 Lab Number: 3575453.29 3575453.30 Dissolved Copper 0.0529 ± 0.0049 0.0527 ± 0.0049 0.149 ± 0.014 0.153 ± 0.014 g/m<sup>3</sup> Hoteo Cu 463.5 T0 Hoteo Cu 463.5 T48 Hoteo Cu 1390.5 T0 Hoteo Cu 1390.5 T48 Sample Name: Lab Number: 3575453.33 3575453.34 3575453.35 3575453.36 0.471 ± 0.043 Dissolved Copper 0.465 ± 0.043 $1.42 \pm 0.13$ $1.22 \pm 0.12$ g/m<sup>3</sup> Clutha Control T0 Clutha Control T48 Clutha Cu 2.2 T0 Clutha Cu 2.2 T48 Sample Name: 3575453.37 3575453.38 3575453.39 3575453.40 Lab Number: g/m<sup>3</sup> Dissolved Copper $< 0.0005 \pm 0.00034$ $< 0.0005 \pm 0.00034$ 0.00235 ± 0.00040 $0.00203 \pm 0.00038$



Dissolved Zinc

This Laboratory is accredited by International Accreditation New Zealand (IANZ), which represents New Zealand in the International Laboratory Accreditation Cooperation (ILAC). Through the ILAC Mutual Recognition Arrangement (ILAC-MRA) this accreditation is internationally recognised. The tests reported herein have been performed in accordance with the terms of accreditation, with the exception of tests marked \* or any comments and interpretations, which are not accredited.

0.00299 ± 0.00072

g/m<sup>3</sup>

0.00271 ± 0.00071

| Sample Type: Aqueous |              |                   |                    |                   |                    |  |
|----------------------|--------------|-------------------|--------------------|-------------------|--------------------|--|
|                      | Sample Name: | Clutha Cu 4.8 T0  | Clutha Cu 4.8 T48  | Clutha Cu 10.6 T0 | Clutha Cu 10.6 T48 |  |
|                      | Lab Number:  | 3575453.41        | 3575453.42         | 3575453.43        | 3575453.44         |  |
| Dissolved Copper     | g/m³         | 0.00470 ± 0.00055 | 0.00413 ± 0.00051  | 0.01020 ± 0.00099 | 0.01029 ± 0.0010   |  |
|                      | Sample Name: | Clutha Cu 23.4 T0 | Clutha Cu 23.4 T48 | Clutha Cu 51.5 T0 | Clutha Cu 51.5 T48 |  |
|                      | Lab Number:  | 3575453.45        | 3575453.46         | 3575453.47        | 3575453.48         |  |
| Dissolved Copper     | g/m³         | 0.0228 ± 0.0021   | 0.0221 ± 0.0021    | 0.0541 ± 0.0050   | 0.0474 ± 0.0044    |  |

The reported uncertainty is an expanded uncertainty with a level of confidence of approximately 95 percent (i.e. two standard deviations, calculated using a coverage factor of 2). Reported uncertainties are calculated from the performance of typical matrices, and do not include variation due to sampling.

For further information on uncertainty of measurement at Hill Laboratories, refer to the technical note on our website: www.hill-laboratories.com/files/Intro\_To\_UOM.pdf, or contact the laboratory.

#### **Summary of Methods**

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicates the indicates the indicates the same analyses were performed at Hill Labs, 28 Duke Street, Frankton, Hamilton 3204.

| Sample Type: Aqueous |   |                         |                                |  |  |  |
|----------------------|---|-------------------------|--------------------------------|--|--|--|
| Test                 | Method Description  | Default Detection Limit | Sample No                      |  |  |  |
| Dissolved Copper     | Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition. | 0.0005 g/m <sup>3</sup> | 1-48                           |  |  |  |
| Dissolved Zinc       | Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition. | 0.0010 g/m <sup>3</sup> | 1-2, 13-14,<br>25-26,<br>37-38 |  |  |  |

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Testing was completed between 13-May-2024 and 14-May-2024. For completion dates of individual analyses please contact the laboratory.

Samples are held at the laboratory after reporting for a length of time based on the stability of the samples and analytes being tested (considering any preservation used), and the storage space available. Once the storage period is completed, the samples are discarded unless otherwise agreed with the customer. Extended storage times may incur additional charges.

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Ara Heron BSc (Tech) Client Services Manager - Environmental

## Appendix D Hill Labs results – Okutua, zinc and copper



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|-------------------------------|
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| Certi        | ficate of Analy                   | sis               |                     |           |              | Page 1 of 2         |
|--------------|-----------------------------------|-------------------|---------------------|-----------|--------------|---------------------|
| Client:      | NIWA Corporate                    |                   | Lab No:             |           | 3599299      | SUPv2               |
| Contact:     | K Thompson                        |                   | Date Received:      |           | 05-Jun-20    | 24                  |
|              | C/- NIWA Corporate Date Reported: |                   | rted:               | 21-Jun-20 | 24 (Amended) |                     |
|              | PO Box 11115                      |                   | Quote No:           |           | 130803       |                     |
|              | Hillcrest                         |                   | Order No:           |           | U334528      |                     |
|              | Hamilton 3251                     |                   | Client Refe         | erence:   | Okutua       |                     |
|              |                                   |                   | Submitted           | By:       | K Thomps     | son                 |
| Sample Ty    | ype: Aqueous                      |                   |                     |           |              |                     |
|              | Sample Name:                      | Okutua Control To | Okutua Control Tend | Okutua    | Cu 10.6 To   | Okutua Cu 10.6 Tend |
|              | Lab Number:                       | 3599299.1         | 3599299.2           | 359       | 99299.3      | 3599299.4           |
| Dissolved Co | opper g/m <sup>3</sup>            | -                 | 0.00126 ± 0.00035   |           | -            | -                   |
| Total Copper | r g/m³                            | 0.00130 ± 0.00038 | -                   | 0.014     | 3 ± 0.0015   | 0.0137 ± 0.0015     |
| Dissolved Zi | nc g/m³                           | -                 | 0.00441 ± 0.00078   |           | -            | -                   |
| Total Zinc   | g/m <sup>3</sup>                  | 0.00390 ± 0.00080 | -                   |           | -            | -                   |
|              | Sample Name:                      | Okutua Cu 23.4 To | Okutua Cu 23.4 Tend | Okutua    | Cu 51.5 To   | Okutua Cu 51.5 Tend |
|              | Lab Number:                       | 3599299.5         | 3599299.6           | 359       | 99299.7      | 3599299.8           |
| Dissolved Co | opper g/m <sup>3</sup>            | -                 | 0.0283 ± 0.0026     | 0.060     | 1 ± 0.0055   | 0.0578 ± 0.0053     |
| Total Copper | r g/m³                            | 0.0284 ± 0.0029   | -                   |           | -            | -                   |
|              | Sample Name:                      | Okutua Cu 113 To  | Okutua Cu 113 Tend  | Okutua    | a Cu 249 To  | Okutua Cu 249 Tend  |
|              | Lab Number:                       | 3599299.9         | 3599299.10          | 359       | 9299.11      | 3599299.12          |
| Dissolved Co | opper g/m <sup>3</sup>            | -                 | -                   |           | -            | 0.284 ± 0.026       |
| Total Copper | r g/m <sup>3</sup>                | 0.136 ± 0.014     | 0.136 ± 0.014       | 0.28      | 3 ± 0.029    | -                   |
|              | Sample Name:                      | Okutua Zn 100 To  | Okutua Zn 100 Tend  | Okutua    | a Zn 220 To  | Okutua Zn 220 Tend  |
|              | Lab Number:                       | 3599299.13        | 3599299.14          | 359       | 9299.15      | 3599299.16          |
| Dissolved Zi | nc g/m³                           | -                 | 0.108 ± 0.011       |           | -            | 0.220 ± 0.021       |
| Total Zinc   | g/m <sup>3</sup>                  | 0.1099 ± 0.0089   | -                   | 0.25      | 2 ± 0.021    | -                   |
|              | Sample Name:                      | Okutua Zn 484 To  | Okutua Zn 484 Tend  | Okutua    | Zn 1065 To   | Okutua Zn 1065 Tend |
|              | Lab Number:                       | 3599299.17        | 3599299.18          | 359       | 9299.19      | 3599299.20          |
| Dissolved Zi | nc g/m³                           | 0.495 ± 0.047     | 0.468 ± 0.044       | 1.0       | 7 ± 0.10     | 1.09 ± 0.11         |
|              | Sample Name:                      | Okutua Z          | n 2343 To           |           | Okutua Zn    | 2343 Tend           |
|              | Lab Number:                       | 35992             | 299.21              |           | 35992        | 299.22              |
| Total Copper | r g/m³                            | 0.00137 :         | ± 0.00038           |           | 0.00137 :    | ± 0.00038           |
| Total Zinc   | g/m³                              | 2.34 :            | ± 0.19              |           | 2.31 :       | ± 0.19              |

The reported uncertainty is an expanded uncertainty with a level of confidence of approximately 95 percent (i.e. two standard deviations, calculated using a coverage factor of 2). Reported uncertainties are calculated from the performance of typical matrices, and do not include variation due to sampling.

For further information on uncertainty of measurement at Hill Laboratories, refer to the technical note on our website: www.hill-laboratories.com/files/Intro\_To\_UOM.pdf, or contact the laboratory.

#### **Analyst's Comments**

Amended Report: This certificate of analysis replaces report '3599299-SUPv1' issued on 19-Jun-2024 at 3:42 pm. Reason for amendment: Zinc results are now reported for the last two samples [sample registration error].



This Laboratory is accredited by International Accreditation New Zealand (IANZ), which represents New Zealand in the International Laboratory Accreditation Cooperation (ILAC). Through the ILAC Mutual Recognision Arrangement (ILAC-MRA) this accreditation is internationally recognised. The tests reported herein have been performed in accrediations, which are not accredited, with the terms of accredited.

#### **Summary of Methods**

The following table(s) gives a brief description of the methods used to conduct the analyses for this job. The detection limits given below are those attainable in a relatively simple matrix. Detection limits may be higher for individual samples should insufficient sample be available, or if the matrix requires that dilutions be performed during analysis. A detection limit range indicates the lowest and highest detection limits in the associated suite of analytes. A full listing of compounds and detection limits are available from the laboratory upon request. Unless otherwise indicated, analyses were performed at Hill Labs, 28 Duke Street, Frankton, Hamilton 3204.

| Sample Type: Aqueous |  |                          |                        |
|----------------------|--|--------------------------|------------------------|
| Test                 | Method Description   | Default Detection Limit  | Sample No              |
| Total Digestion      | Nitric acid digestion. APHA 3030 E (modified) : Online Edition.                          | -                        | 1, 3-5, 9-11,          |
|                      |  |                          | 13, 15,                |
|                      |  |                          | 21-22                  |
| Dissolved Copper     | Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.                      | 0.0005 g/m <sup>3</sup>  | 2, 6-8, 12             |
| Total Copper         | Nitric acid digestion, ICP-MS, trace level. APHA 3125 B : Online Edition / US EPA 200.8. | 0.00053 g/m <sup>3</sup> | 1, 3-5, 9-11,<br>21-22 |
| Dissolved Zinc       | Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.                      | 0.0010 g/m <sup>3</sup>  | 2, 14, 16-20           |
| Total Zinc           | Nitric acid digestion, ICP-MS, trace level. APHA 3125 B : Online Edition / US EPA 200.8. | 0.0011 g/m <sup>3</sup>  | 1, 13, 15,<br>21-22    |

These samples were collected by yourselves (or your agent) and analysed as received at the laboratory.

Testing was completed between 10-Jun-2024 and 21-Jun-2024. For completion dates of individual analyses please contact the laboratory.

Samples are held at the laboratory after reporting for a length of time based on the stability of the samples and analytes being tested (considering any preservation used), and the storage space available. Once the storage period is completed, the samples are discarded unless otherwise agreed with the customer. Extended storage times may incur additional charges.

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Ara Heron BSc (Tech) Client Services Manager - Environmental

Lab No: 3599299-SUPv2

Hill Labs

# Appendix E Toxicity tests raw data

## Zinc

| Acute D. the          | omson     | <i>i</i> results  |               |               |   |
|-----------------------|-----------|-------------------|---------------|---------------|---|
| Natural water Nominal |           |                   |               |               |   |
| Zinc µg/L             | Replicate | # Daphnia exposed | 24 h Survival | 48 h Survival | Comment                                 |
| Mahurangi Control 0   | 1         | 10                | 10            | 10            | Sent to hill labs with coppers          |
| Mahurangi Control 0   | 2         | 10                | 10            | 10            |   |
| Mahurangi Control 0   | 3         | 10                | 10            | 10            |   |
| Mahurangi Control 0   | 4         | 10                | 10            | 10            |   |
| Mahurangi Control 0   | 5         | 10                | 10            | 10            |   |
| Mahurangi 100         | 1         | 10                | 10            | 10            | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 10            | 10            |   |
|                       | 3         | 10                | 10            | 10            |   |
| Mahurangi 220         | 1         | 10                | 10            | 10            | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 10            | 10            |   |
|                       | 3         | 10                | 10            | 10            |   |
| Mahurangi 484         | 1         | 10                | 9             | 9             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 10            | 9             |   |
|                       | 3         | 10                | 10            | 9             |   |
| Mahurangi 1065        | 1         | 10                | 7             | 2             | Sent to hill labs for analysis TO & T48 |
|                       | 2         | 10                | 7             | 4             |   |
|                       | 3         | 10                | 6             | 2             |   |
| Mahurangi 2343        | 1         | 10                | 5             | 0             | Sent to hill labs for analysis TO & T48 |
|                       | 2         | 10                | 4             | 0             |   |
|                       | 2         | 10                | 4             | 0             |   |
| Mahurangi 5154        | 1         | 10                | 4             | 0             | Not sent to Hill Jahs                   |
|                       | 2         | 10                | 0             | 0             |   |
|                       | 2         | 10                | 0             | 0             |   |
| Mahurangi 11000       | 5         | 10                | 0             | 0             | Natsoatta Lilliaha                      |
| wanurangi 11000       | 1         | 10                | 0             | 0             |   |
|                       | 2         | 10                | 0             | 0             |   |
| Natural water Nominal | 5         | 10                | 0             | 0             |   |
| Zinc ug/L             | Replicate | # Daphnia exposed | 24 h Survival | 48 h Survival | Comment                                 |
|                       | 1         | 10                | 10            | 10            | Sent to hills with conners              |
| Hoteo Control 0       | 2         | 10                | 10            | 10            | Sent to mis with coppers                |
| Hoteo Control 0       | 2         | 10                | 10            | 10            |   |
| Hoteo Control 0       | 3         | 10                | 10            | 10            |   |
| Hoteo Control 0       | 4         | 10                | 10            | 10            |   |
| Hoteo 100             | 1         | 10                | 10            | 10            | Sont to hill labs for analysis TO & T48 |
|                       | 2         | 10                | 10            | 10            |   |
|                       | 2         | 10                | 10            | 10            |   |
| Listen 220            | 3         | 10                | 10            | 10            | Cont to hill lobe for anolysis TO 9 T49 |
| Hoteo 220             | 1         | 10                | 10            | 10            | Sent to hill labs for analysis 10 & 148 |
|                       | 2         | 10                | 10            | 10            |   |
|                       | 3         | 10                | 10            | 10            |   |
| Hoteo 484             | 1         | 10                | 9             | 6             | Sent to hill labs for analysis 10 & 148 |
|                       | 2         | 10                | 10            | 7             |   |
|                       | 3         | 10                | 10            | 8             |   |
| Hoteo 1065            | 1         | 10                | 8             | 2             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 7             | 3             |   |
|                       | 3         | 10                | 9             | 5             |   |
| Hoteo 2343            | 1         | 10                | 6             | 0             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 8             | 0             |   |
|                       | 3         | 10                | 6             | 0             |   |
| Hoteo 5154            | 1         | 10                | 0             | 0             | Not sent to Hill labs                   |
|                       | 2         | 10                | 0             | 0             |   |
|                       | 3         | 10                | 0             | 0             |   |
| Hoteo 11000           | 1         | 10                | 0             | 0             | Not sent to Hill labs                   |
|                       | 2         | 10                | 0             | 0             |   |
|                       | 3         | 10                | 0             | 0             |   |

| Natural water Nominal  |   |  |   |   |  |
|--|---|--|---|---|--|
| Zinc µg/L  | Replicate   | # Daphnia exposed  | 24 h Survival   | 48 h Survival   | Comment  |
| Okutua Control 0   | 1   | 10   | 10  | 10  | Sent to hill labs for analysis T0 & T48  |
| Okutua Control 0   | 2   | 10   | 10  | 10  |  |
| Okutua Control 0   | 3   | 10   | 10  | 10  |  |
| Okutua Control 0   | 4   | 10   | 10  | 10  |  |
| Okutua Control 0   | 5   | 10   | 10  | 9   |  |
| Okutua 100   | 1   | 10   | 10  | 10  | Sent to hill labs for analysis T0 & T48  |
|  | 2   | 10   | 10  | 8   |  |
|  | 3   | 10   | 10  | 10  |  |
| Okutua 220   | 1   | 10   | 10  | 6   | Sent to hill labs for analysis T0 & T48  |
|  | 2   | 10   | 10  | 6   |  |
|  | 3   | 10   | 10  | 9   |  |
| Okutua 484   | 1   | 10   | 9   | 1   | Sent to hill labs for analysis TO & T/8  |
|  | 2   | 10   | 10  | 3   |  |
|  | 2   | 10   | 10  | 2   |  |
| Okutua 1065  | 1   | 10   | 9   | 3   | Sont to hill lobe for analysis TO & TAP  |
|  | 2   | 10   | 0   | 0   |  |
|  | 2   | 10   | 0   | 0   |  |
| 01 1 2242  | 3   | 10   | 0   | 0   |  |
| Okutua 2343  | 1   | 10   | 0   | 0   | Sent to hill labs for analysis 10 & 148  |
|  | 2   | 10   | 0   | 0   |  |
|  | 3   | 10   | 0   | 0   |  |
| Okutua 5154  | 1   | 10   | 0   | 0   | Not sent to Hill labs  |
|  | 2   | 10   | 0   | 0   |  |
|  | 3   | 10   | 0   | 0   |  |
| Okutua 11000   | 1   | 10   | 0   | 0   | Not sent to Hill labs  |
|  | 2   | 10   | 0   | 0   |  |
|  | 3   | 10   | 0   | 0   |  |
| Notural water Naminal  |   |  |   |   |  |
|  |   | " D  |   |   |  |
|  | Replicate   | # Daphnia exposed  | 24 h Survival   | 48 h Survival   | Comment  |
| Zinc µg/L<br>Clutha Control 0  | Replicate   | # Daphnia exposed  | <b>24 h Survival</b><br>10  | 48 h Survival   | Comment<br>Sent to hill labs with coppers  |
| Zinc μg/L           Clutha Control 0           Clutha Control 0  | Replicate   | # Daphnia exposed<br>10<br>10  | 24 h Survival<br>10<br>10   | <b>48 h Survival</b><br>10<br>10  | Comment<br>Sent to hill labs with coppers  |
| Zinc µg/L<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0  | Replicate123  | # Daphnia exposed<br>10<br>10<br>10<br>10  | <b>24 h Survival</b><br>10<br>10<br>10                                    | <b>48 h Survival</b><br>10<br>10<br>10  | Comment<br>Sent to hill labs with coppers  |
| Zinc µg/L<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0  | Replicate 1 2 3 4   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10                                     | <b>48 h Survival</b><br>10<br>10<br>10<br>9   | Comment<br>Sent to hill labs with coppers  |
| Zinc µg/L<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0  | Replicate           1           2           3           4           5   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10                         | <b>48 h Survival</b><br>10<br>10<br>10<br>9<br>10   | Comment<br>Sent to hill labs with coppers  |
| Zinc µg/L<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha 45   | Replicate           1           2           3           4           5           1   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10             | <b>48 h Survival</b><br>10<br>10<br>10<br>9<br>10<br>10   | Comment<br>Sent to hill labs with coppers<br>Not sent to Hill labs   |
| Zinc µg/L<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha 45   | Replicate           1           2           3           4           5           1           2   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | <b>48 h Survival</b><br>10<br>10<br>9<br>10<br>10<br>10   | Comment<br>Sent to hill labs with coppers<br>Not sent to Hill labs   |
| Zinc µg/L<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha Control 0<br>Clutha 45   | Replicate           1           2           3           4           5           1           2           3   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | <b>48 h Survival</b><br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10   | Comment<br>Sent to hill labs with coppers<br>Not sent to Hill labs   |
| Zinc µg/L       Clutha Control 0       Clutha 45       Clutha 100  | Replicate           1           2           3           4           5           1           2           3           4           5           1           2           3           1   | # Daphnia exposed           10   | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | Comment<br>Sent to hill labs with coppers<br>Not sent to Hill labs<br>Sent to hill labs for analysis T0 & T48  |
| Zinc µg/L       Clutha Control 0       Clutha 45       Clutha 100  | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment<br>Sent to hill labs with coppers<br>Not sent to Hill labs<br>Sent to hill labs for analysis T0 & T48  |
| Zinc µg/L       Clutha Control 0       Clutha 45       Clutha 100  | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           3   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment<br>Sent to hill labs with coppers<br>Not sent to Hill labs<br>Sent to hill labs for analysis T0 & T48  |
| Zinc µg/L       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9                              | Comment<br>Sent to hill labs with coppers<br>Not sent to Hill labs<br>Sent to hill labs for analysis T0 & T48<br>Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha 100       Clutha 220   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9<br>9<br>9<br>9<br>9                | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220  | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9<br>9<br>8                          | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 100       Clutha 220       Clutha 484   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1   | # Daphnia exposed           10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9<br>9<br>8<br>6                     | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220       Clutha 484   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9<br>8<br>6<br>6                     | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220       Clutha 484   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           3           1           2           3           1           2           3   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9<br>8<br>6<br>6<br>4                | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 100       Clutha 220       Clutha 484       Clutha 1056   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9<br>8<br>6<br>6<br>6<br>4<br>0      | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 100       Clutha 220       Clutha 484       Clutha 1056   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2   | # Daphnia exposed           10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>9<br>9<br>8<br>6<br>6<br>6<br>4<br>0<br>1 | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 100       Clutha 220       Clutha 484       Clutha 1056   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10   | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220       Clutha 484       Clutha 1056       Clutha 2343                   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48     |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220       Clutha 484       Clutha 1056       Clutha 2343                   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48     |
| Zinc µg/L       Clutha Control 0       Clutha 100       Clutha 220       Clutha 484       Clutha 1056       Clutha 2343            | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3                                     | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48   |
| Zinc µg/L       Clutha Control 0       Clutha 100       Clutha 220       Clutha 1056       Clutha 2343       Clutha 5154           | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Not sent to Hill labs for analysis T0 & T48 |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220       Clutha 484       Clutha 1056       Clutha 2343       Clutha 5154 | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3 | # Daphnia exposed           10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Not sent to Hill labs                       |
| Zinc µg/L       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha Control 0       Clutha 45       Clutha 100       Clutha 220       Clutha 484       Clutha 1056       Clutha 2343       Clutha 5154 | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3 | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10  | 24 h Survival 10 10 10 10 10 10 10 10 10 10 10 10 10                      | 48 h Survival<br>10<br>10<br>10<br>9<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10                                  | Comment Sent to hill labs with coppers Not sent to Hill labs Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Sent to hill labs for analysis T0 & T48 Not sent to Hill labs                       |

| Natural water Nominal |           |                   |               |               |   |
|-----------------------|-----------|-------------------|---------------|---------------|---|
| Zinc µg/L             | Replicate | # Daphnia exposed | 24 h Survival | 48 h Survival | Comment                                 |
| Waihou Control 0      | 1         | 10                | 10            | 10            | Sent to hill labs with coppers          |
| Waihou Control 0      | 2         | 10                | 10            | 9             |   |
| Waihou Control 0      | 3         | 10                | 10            | 10            |   |
| Waihou Control 0      | 4         | 10                | 10            | 9             |   |
| Waihou Control 0      | 5         | 10                | 10            | 10            |   |
| Waihou 100            | 1         | 10                | 10            | 9             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 10            | 10            |   |
|                       | 3         | 10                | 10            | 9             |   |
| Waihou 220            | 1         | 10                | 9             | 9             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 9             | 7             |   |
|                       | 3         | 10                | 9             | 7             |   |
| Waihou 484            | 1         | 10                | 9             | 5             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 10            | 4             |   |
|                       | 3         | 10                | 9             | 3             |   |
| Waihou 1065           | 1         | 10                | 8             | 0             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 5             | 0             |   |
|                       | 3         | 10                | 5             | 0             |   |
| Waihou 2343           | 1         | 10                | 2             | 0             | Sent to hill labs for analysis T0 & T48 |
|                       | 2         | 10                | 0             | 0             |   |
|                       | 3         | 10                | 0             | 0             |   |
| Waihou 5145           | 1         | 10                | 0             | 0             | Not sent to Hill labs                   |
|                       | 2         | 10                | 0             | 0             |   |
|                       | 3         | 10                | 0             | 0             |   |

# Copper

# Acute D. thomsoni results

| Natural water Nominal   |  |   |   |  |   |
|---|--|---|---|--|---|
| Copper µg/L   | Replicate  | # Daphnia exposed   | 24 h Survival   | 48 h Survival  | Comment   |
| Mahurangi Control 0   | 1  | 10  | 10  | 10   | Sent to Hill labs for analysis T0 and T48   |
| Mahurangi Control 0   | 2  | 10  | 10  | 10   |   |
| Mahurangi Control 0   | 3  | 10  | 10  | 10   |   |
| Mahurangi Control 0   | 4  | 10  | 10  | 10   |   |
| Mahurangi Control 0   | 5  | 10  | 10  | 10   |   |
| Mahurangi 1.0   | 1  | 10  | 10  | 10   | Not sent to Hill labs   |
| Mahurangi 1.0   | 2  | 10  | 10  | 10   |   |
| Mahurangi 1.0   | 3  | 10  | 10  | 10   |   |
| Mahurangi 2.2   | 1  | 10  | 10  | 10   | Not sent to Hill labs   |
| Mahurangi 2.2   | 2  | 10  | 10  | 10   |   |
| Mahurangi 2.2   | 3  | 10  | 10  | 10   |   |
| Mahurangi 4.8   | 1  | 10  | 10  | 10   | Not sent to Hill labs   |
| Mahurangi 4.8   | 2  | 10  | 10  | 10   |   |
| Mahurangi 4.8   | 3  | 10  | 10  | 10   |   |
| Mahurangi 10.6  | 1  | 10  | 10  | 10   | Not sent to Hill labs   |
| Mahurangi 10.6  | 2  | 10  | 10  | 10   |   |
| Mahurangi 10.6  | 3  | 10  | 10  | 10   |   |
| Mahurangi 23.4  | 1  | 10  | 10  | 10   | Sent to Hill labs for analysis T0 and T48   |
| Mahurangi 23.4  | 2  | 10  | 10  | 10   |   |
| Mahurangi 23.4  | 3  | 10  | 10  | 10   |   |
| Mahurangi 51.5  | 1  | 10  | 10  | 10   | Sent to Hill labs for analysis T0 and T48   |
| Mahurangi 51.5  | 2  | 10  | 10  | 10   |   |
| Mahurangi 51.5  | 3  | 10  | 10  | 10   |   |
| Mahurangi 154.5   | 1  | 10  | 10  | 8  | Sent to Hill labs for analysis T0 and T48   |
| Mahurangi 154.5   | 2  | 10  | 7   | 6  | ,   |
| Mahurangi 154.5   | 3  | 10  | 8   | 7  |   |
| Mahurangi 463.5   | 1  | 10  | 0   | 0  | Sent to Hill labs for analysis T0 and T48   |
| Mahurangi 463.5   | 2  | 10  | 0   | 0  |   |
| Mahurangi 463.5   | 3  | 10  | 0   | 0  |   |
| Mahurangi 1390.5  | 1  | 10  | 0   | 0  | Sent to Hill labs for analysis T0 and T48   |
| Mahurangi 1390.5  | 2  | 10  | 0   | 0  | ,,,,,,  |
| Mahurangi 1390.5  | 3  | 10  | 0   | 0  |   |
|   |  |   | -   | -  |   |
| Natural water Nominal   |  |   |   |  |   |
| Natural water Nominal<br>Copper µg/L  | Replicate  | # Daphnia exposed   | 24 h Survival   | 48 h Survival  | Comment   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0   | Replicate  | # Daphnia exposed   | 24 h Survival   | 48 h Survival  | Comment<br>Sent to Hill labs for analysis TO and T48  |
| Natural water Nominal<br>Copper μg/L<br>Hoteo Control 0<br>Hoteo Control 0  | Replicate  | # Daphnia exposed<br>10<br>10   | <b>24 h Survival</b><br>10<br>10  | <b>48 h Survival</b><br>10<br>10   | <b>Comment</b><br>Sent to Hill labs for analysis TO and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0   | Replicate 1 2 3  | # Daphnia exposed<br>10<br>10<br>10   | <b>24 h Survival</b><br>10<br>10<br>10                                    | <b>48 h Survival</b><br>10<br>10<br>10   | Comment<br>Sent to Hill labs for analysis T0 and T48  |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0  | Replicate 1 2 3 4  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10                               | 24 h Survival<br>10<br>10<br>10<br>10                                     | <b>48 h Survival</b><br>10<br>10<br>10<br>10                                     | Comment<br>Sent to Hill labs for analysis T0 and T48  |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0   | Replicate           1           2           3           4           5  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10                         | <b>24 h Survival</b><br>10<br>10<br>10<br>10<br>10                        | <b>48 h Survival</b><br>10<br>10<br>10<br>10<br>10                               | Comment<br>Sent to Hill labs for analysis T0 and T48  |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0  | Replicate           1           2           3           4           5           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10                   | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10                         | <b>48 h Survival</b><br>10<br>10<br>10<br>10<br>10<br>10                         | Comment<br>Sent to Hill labs for analysis T0 and T48<br>Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0   | Replicate           1           2           3           4           5           1           2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10       | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10       | <b>48 h Survival</b><br>10<br>10<br>10<br>10<br>10<br>10<br>10                   | Comment<br>Sent to Hill labs for analysis TO and T48<br>Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0  | Replicate           1           2           3           4           5           1           2           3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | <b>48 h Survival</b><br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | Comment<br>Sent to Hill labs for analysis TO and T48<br>Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2  | Replicate           1           2           3           4           5           1           2           3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | <b>48 h Survival</b><br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | Comment<br>Sent to Hill labs for analysis TO and T48<br>Not sent to Hill labs<br>Not sent to Hill labs  |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2  | Replicate           1           2           3           4           5           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment<br>Sent to Hill labs for analysis TO and T48<br>Not sent to Hill labs<br>Not sent to Hill labs  |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2   | Replicate 1 2 3 4 5 1 2 3 4 5 1 2 3 1 2 3 1 2 3 3 3 1 2 3 3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment<br>Sent to Hill labs for analysis TO and T48<br>Not sent to Hill labs<br>Not sent to Hill labs  |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment<br>Sent to Hill labs for analysis T0 and T48<br>Not sent to Hill labs<br>Not sent to Hill labs<br>Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | <b>48 h Survival</b><br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | Comment<br>Sent to Hill labs for analysis T0 and T48<br>Not sent to Hill labs<br>Not sent to Hill labs<br>Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | <b>48 h Survival</b><br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | Comment<br>Sent to Hill labs for analysis T0 and T48<br>Not sent to Hill labs<br>Not sent to Hill labs<br>Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 1.0   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4  | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs Sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4  | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs Sent to Hill labs   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4  | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival 10 10 10 10 10 10 10 10 10 10 10 10 10                             | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 2.2<br>Hoteo 5.5<br>Hoteo 51.5<br>Hoteo 51.5           | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5   | Replicate         1         2         3         4         5         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 154.5<br>Hoteo 154.5  | Replicate         1         2         3         4         5         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3          1         2         3          1          2         3          1          2          3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 463.5     | Replicate         1         2         3         4         5         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2          3          1          2   | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival 10 10 10 10 10 10 10 10 10 10 10 10 10                             | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 463.5<br>Hoteo 463.5<br>Hoteo 463.5                 | Replicate         1         2         3         4         5         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3          1          2 </td <td># Daphnia exposed<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10</td> <td>24 h Survival<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10</td> <td>48 h Survival<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10<br/>10</td> <td>Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48</td> | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48   |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 463.5<br>Hoteo 463.5<br>Hoteo 463.5<br>Hoteo 463.5<br>Hoteo 463.5 | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival 10 10 10 10 10 10 10 10 10 10 10 10 10                             | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48                       |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 463.5<br>Hoteo 1390.5<br>Hoteo 1390.5                   | Replicate           1           2           3           4           5           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3           1           2           3  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival 10 10 10 10 10 10 10 10 10 10 10 10 10                             | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Not sent to Hill labs Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48                       |
| Natural water Nominal<br>Copper µg/L<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo Control 0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 1.0<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 2.2<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 4.8<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 10.6<br>Hoteo 23.4<br>Hoteo 23.4<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 51.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 154.5<br>Hoteo 463.5<br>Hoteo 1390.5<br>Hoteo 1390.5     | Replicate         1         2         3         4         5         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2         3         1         2  | # Daphnia exposed<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 24 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10 | 48 h Survival<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10<br>10        | Comment Sent to Hill labs for analysis T0 and T48 Not sent to Hill labs Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 Sent to Hill labs for analysis T0 and T48 |

| Natural water Nominal |           |                    |                 |                 |  |
|-----------------------|-----------|--------------------|-----------------|-----------------|--|
| Copper µg/L           | Replicate | # Daphnia exposed  | 24 h Survival   | 48 h Survival   | Comment                                    |
| Okutua Control        | 1         | 10                 | 10              | 10              |  |
| Okutua Control        | 2         | 10                 | 10              | 10              |  |
| Okutua Control        | 3         | 10                 | 10              | 10              |  |
| Okutua Control        | 4         | 10                 | 10              | 10              |  |
| Okutua Control        | 5         | 10                 | 10              | 9               |  |
| Okutua 1.0            | 1         | 10                 | 10              | 10              |  |
| Okutua 1.0            | 2         | 10                 | 10              | 10              |  |
| Okutua 1.0            | 3         | 10                 | 10              | 10              |  |
| Okutua 2.2            | 1         | 10                 | 10              | 10              |  |
| Okutua 2.2            | 2         | 10                 | 10              | 10              |  |
| Okutua 2.2            | 3         | 10                 | 10              | 10              |  |
| Okutua 4.8            | 1         | 10                 | 10              | 10              |  |
| Okutua 4.8            | 2         | 10                 | 10              | 9               |  |
| Okutua 4.8            | 3         | 10                 | 10              | 10              |  |
| Okutua 10.6           | 1         | 10                 | 10              | 10              |  |
| Okutua 10.6           | 2         | 10                 | 10              | 10              |  |
| Okutua 10.6           | 3         | 10                 | 10              | 10              |  |
| Okutua 23.4           | 1         | 10                 | 10              | 10              |  |
| Okutua 23.4           | 2         | 10                 | 10              | 10              |  |
| Okutua 23.4           | 3         | 10                 | 10              | 10              |  |
| Okutua 51 5           | 1         | 10                 | 10              | 10              |  |
| Okutua 51.5           | 2         | 10                 | 10              | 10              |  |
| Okutua 51.5           | 3         | 10                 | 10              | 9               |  |
| Okutua 113            | 1         | 10                 | 0               | 0               |  |
| Okutua 113            | 2         | 10                 | 10              | 0               |  |
| Okutua 113            | 2         | 10                 | 10              | 4               |  |
| Okutua 113            | 1         | 10                 | 0               | 0               |  |
| Okutua 249            | 2         | 10                 | 0               | 0               |  |
| Okutua 249            | 2         | 10                 | 0               | 0               |  |
| Natural water Nominal | 5         | 10                 | 0               | 0               |  |
|                       | Replicate | # Danhnia exposed  | 24 h Survival   | 18 h Survival   | Comment                                    |
| Clutha Control 0      |           | # Dupinilu exposed | 24 II Sul Vival | 48 11 3ul vival | Contro Hill John for analysis TO and T49   |
| Clutha Control 0      | 1         | 10                 | 10              | 10              |  |
| Clutha Control 0      | 2         | 10                 | 10              | 10              |  |
| Clutha Control 0      | 3         | 10                 | 10              | 10              |  |
| Clutha Control 0      | 4<br>F    | 10                 | 10              | 9               |  |
|                       | 5         | 10                 | 10              | 10              |  |
| Clutha 1.0            | 1         | 10                 | 10              | 10              | Not sent to Hill labs                      |
| Clutha 1.0            | 2         | 10                 | 10              | 10              |  |
| Clutha 1.0            | 3         | 10                 | 10              | 10              | Cont to Uill labo for one lucis TO and T49 |
| Clutha 2.2            | 1         | 10                 | 10              | 10              | Sent to Hill labs for analysis 10 and 148  |
| Clutha 2.2            | 2         | 10                 | 10              | 9               |  |
| Clutha 2.2            | 3         | 10                 | 10              | 10              | Cont to Uill labo for one lucis TO and T49 |
| Clutha 4.8            | 1         | 10                 | 10              | 10              |  |
| Clutha 4.8            | 2         | 10                 | 10              | 10              |  |
|                       | 3         | 10                 | 10              | 10              |  |
|                       | 1         | 10                 | 10              | 10              |  |
| Clutha 10.6           | 2         | 10                 | 10              | 10              |  |
| Clutha 10.6           | 3         | 10                 | 10              | 10              |  |
| Clutha 23.4           | 1         | 10                 | 8               | /               | Sent to Hill labs for analysis 10 and 148  |
| Clutha 23.4           | 2         | 10                 | 8               | 10              |  |
| Clutha 23.4           | 3         | 10                 | 7               | 9               |  |
| Clutha 51.5           | 1         | 10                 | 1               | 1               | Sent to Hill labs for analysis TO and T48  |
| Clutha 51.5           | 2         | 10                 | 1               | 1               |  |
| Clutha 51.5           | 3         | 10                 | 0               | 0               |  |

|                       | 1         |                   |               |               |   |
|-----------------------|-----------|-------------------|---------------|---------------|---|
| Natural water Nominal |           |                   |               |               |   |
| Copper µg/L           | Replicate | # Daphnia exposed | 24 h Survival | 48 h Survival | Comment                                   |
| Waihou Control 0      | 1         | 10                | 10            | 10            | Sent to Hill labs for analysis TO and T48 |
| Waihou Control 0      | 2         | 10                | 10            | 9             |   |
| Waihou Control 0      | 3         | 10                | 10            | 10            |   |
| Waihou Control 0      | 4         | 10                | 10            | 9             |   |
| Waihou Control 0      | 5         | 10                | 10            | 10            |   |
| Waihou 1.0            | 1         | 10                | 10            | 10            | Not sent to Hill labs                     |
| Waihou 1.0            | 2         | 10                | 10            | 10            |   |
| Waihou 1.0            | 3         | 10                | 10            | 10            |   |
| Waihou 2.2            | 1         | 10                | 10            | 10            | Not sent to Hill labs                     |
| Waihou 2.2            | 2         | 10                | 10            | 10            |   |
| Waihou 2.2            | 3         | 10                | 10            | 10            |   |
| Waihou 4.8            | 1         | 10                | 10            | 10            | Not sent to Hill labs                     |
| Waihou 4.8            | 2         | 10                | 10            | 10            |   |
| Waihou 4.8            | 3         | 10                | 10            | 10            |   |
| Waihou 10.6           | 1         | 10                | 10            | 10            | Sent to Hill labs for analysis TO and T48 |
| Waihou 10.6           | 2         | 10                | 10            | 9             |   |
| Waihou 10.6           | 3         | 10                | 10            | 10            |   |
| Waihou 23.4           | 1         | 10                | 10            | 10            | Sent to Hill labs for analysis TO and T48 |
| Waihou 23.4           | 2         | 10                | 9             | 9             |   |
| Waihou 23.4           | 3         | 10                | 9             | 6             |   |
| Waihou 51.5           | 1         | 10                | 7             | 5             | Sent to Hill labs for analysis TO and T48 |
| Waihou 51.5           | 2         | 10                | 5             | 2             |   |
| Waihou 51.5           | 3         | 10                | 7             | 5             |   |
| Waihou 113            | 1         | 10                | 0             | 0             | Sent to Hill labs for analysis TO and T48 |
| Waihou 113            | 2         | 10                | 1             | 0             |   |
| Waihou 113            | 3         | 10                | 0             | 0             |   |
| Waihou 249            | 1         | 10                | 0             | 0             | Sent to Hill labs for analysis T0 and T48 |
| Waihou 249            | 2         | 10                | 0             | 0             |   |
| Waihou 249            | 3         | 10                | 0             | 0             |   |

# Appendix F CETIS statistical analyses – Mahurangi

#### Zinc

| CETIS Analytical Report  |  |  |  |  |                      |  |  |   |   | Report Date:         27 May-24 14:24 (p           Test Code/ID:         24.003.1 Zn / 15-88 |  |                                       |   | 24 (p 1 of 2)<br>5-8888-6853 |            |
|--|--|--|--|--|----------------------|--|--|---|---|---|--|---------------------------------------|---|------------------------------|------------|
| Daphnia thoms  | soni 48-h Ac   | ute Surviv   | al T   | est  |                      |  |  |   |   |   |  |                                       |   | NIWA Eco                     | toxicology |
| Analysis ID: Analyzed: 2<br>Edit Date:                             | 17-8062-837(<br>27 May-24 14                               | )<br>I:24  | End<br>Anal<br>MD5                                 | point:<br>lysis:<br>i Hash:                                | 48h<br>Para<br>224   | Survival Ra<br>ametric-Mul<br>9197577821 | ate<br>tiple Compa<br>DA5F34CA2                      | rison<br>39A904E49                                  | 97A   | CETI<br>Statu<br>Edito  | S Versi<br>Is Level<br>or ID:                      | on:<br>:                              | CETISv2<br>1  | .1.4                         |            |
| Batch ID: ()<br>Start Date: ()<br>Ending Date: 7<br>Test Length: 4 | 03-1254-1573<br>08 May-24<br>10 May-24<br>48h              | 3  | Test<br>Prot<br>Spe<br>Taxo                        | Type:<br>ocol:<br>cies:<br>on:                             | Sun<br>NIW<br>Dap    | vival (48h)<br>/A SOP 10<br>ohnia thoms  | (2022)<br>oni (Water fl                              | ea)   |   | Analy<br>Dilue<br>Brine<br>Sour   | yst:  <br>ent:  <br>e:  <br>ce:                    | Ecot<br>Redv<br>Not /<br>Field        | ox Team<br>voods<br>Applicable<br>I Collected   |                              | Age:       |
| Sample ID: (<br>Sample Date: (<br>Receipt Date: (<br>Sample Age: 2 | 09-2771-2013<br>07 May-24<br>07 May-24<br>24h              | 3  | Cod<br>Mate<br>CAS<br>Clie                         | e:<br>erial:<br>(PC):<br>nt:                               | 24.0<br>Zino<br>Hyd  | 003.1 Zn<br>c sulfate<br>Irotoxy Rese    | earch  |   |   | Proje<br>Sour<br>Statio   | ce: son: l   | Spec<br>Solu<br>Lab                   | ial Studies<br>tion made t<br>Solution  | DY NIWA                      |            |
| Data Transform   | n  | Alt H  | ур   |  |                      |  |  | NOEL  | LO  | EL  | TOEL   |                                       | Tox Units   | MSDu                         | PMSD       |
| Angular (Correc  | C > T  |  |  |  |                      |  | 240  | 490   |   | 342.9   |  |                                       | 0.06421   | 6.42%                        |            |
| Bonferroni Adj   | t Test   |  |  |  |                      |  |  |   |   |   |  |                                       |   |                              |            |
| Control  | vs Conc-µ  | lg/L   | df Test Stat Critic                                |  |                      | Critical                                 | MSD  | P-Type  | P-V   | alue  | Decisi   | on(                                   | a:5%)   |                              |            |
| Dilution Water   | 103  |  | 6 0  |  |                      | 2.56                                     | 0.09742  | CDF   | 1.0   | 000   | Non-S  | ignif                                 | icant Effect  |                              |            |
|  | 240  |  | 6  | 0  |                      | 2.56                                     | 0.09742  | CDF   | 1.0   | 000   | Non-S  | ignif                                 | icant Effect  |                              |            |
|  | 490°<br>1055*  |  | 6  | 4.283  |                      | 2.50                                     | 0.09742  | CDF   | <1.0  | DE-05   | Signifi  | cant<br>cant                          | Effect  |                              |            |
|  |  | -  |  |  |                      |  |  |   |   |   |  |                                       |   |                              |            |
| Source   | Sum S  | mares  |  | Mean   | Sau                  | are                                      | DE   | F Stat  | P.V   | alue  | Decision(a:5%)                                     |                                       |   |                              |            |
| Between  | 1 80484  | 1  |  | 0 451  | 209                  |  | 4  | 166.2   | <1 (  | 0F-05   | Signifi  | cant                                  | Effect  |                              |            |
| Error  | 0.03258  | 318  |  | 0.002  | 7152                 |  | 12   |   |   |   |  |                                       |   |                              |            |
| Total  | 1.83742  | 2  |  |  |                      |  | 16   | _   |   |   |  |                                       |   |                              |            |
| ANOVA Assum  | ptions Tests   | 6  |  |  |                      |  |  |   |   |   |  |                                       |   |                              |            |
| Attribute  | Test   |  |  |  |                      |  | Test Stat  | Critical  | P-V   | alue  | Decisi   | on(                                   | a:1%)   |                              |            |
| Variance   | Bartlett   | Equality of  | f Vai  | riance 1   | Fest                 |  |  |   |   |   | Indete   | rmin                                  | ate   |                              |            |
| Distribution   | Levene<br>Mod Le<br>Anders<br>D'Agosi<br>Kolmog<br>Shapiro | Equality o<br>vene Equa<br>on-Darling<br>tino Skewn<br>orov-Smin<br>o-Wilk W N | f Vai<br>ality o<br>A2 1<br>ness<br>nov 1<br>lorma | riance 1<br>of Varia<br>Test<br>Test<br>D Test<br>ality Te | Test<br>ince 1<br>st | Test                                     | 19.76<br>1.458<br>4.306<br>3.014<br>0.4412<br>0.5509 | 5.412<br>7.847<br>3.878<br>2.576<br>0.2405<br>0.848 | 3.3 <br>0.3<br><1.0<br>0.00<br><1.0<br><1.0 | E-05<br>105<br>DE-05<br>D26<br>DE-05<br>DE-05   | Unequ<br>Equal<br>Non-N<br>Non-N<br>Non-N<br>Non-N | al V<br>Varia<br>orma<br>orma<br>orma | ariances<br>ances<br>al Distributi<br>al Distributi<br>al Distributi<br>al Distributi | on<br>on<br>on               |            |
| 48h Survival R   | ate Summar   | у  |  |  |                      |  |  |   |   |   |  |                                       |   |                              |            |
| Conc-µg/L  | Code   | Count  | t  | Mean   |                      | 95% LCL                                  | 95% UCL  | Median  | Min   |   | Мах  |                                       | Std Err   | CV%                          | %Effect    |
| 3.2<br>103   | D  | 5  |  | 1.000  | 0                    | 1.0000                                   | 1.0000   | 1.0000  | 1.0   | 000   | 1.0000   |                                       | 0.0000  | 0.00%                        | 0.00%      |
| 240  |  | 3  |  | 1.000  | 0                    | 1.0000                                   | 1.0000   | 1.0000  | 1.0   | 000   | 1.0000   |                                       | 0.0000  | 0.00%                        | 0.00%      |
| 490  |  | 3  |  | 0.900  | 0                    | 0.8996                                   | 0.9004   | 0.9000  | 0.9   | 000   | 0.9000   | )                                     | 0.0000  | 0.00%                        | 10.00%     |
| 1055   |  | 3  |  | 0.266  | 7                    | 0.0000                                   | 0.5535   | 0.2000  | 0.2   | 000   | 0.4000   | )                                     | 0.0667  | 43.30%                       | 73.33%     |
| 2350   |  | 3  |  | 0.000  | 0                    | 0.0000                                   | 0.0000   | 0.0000  | 0.0   | 000   | 0.0000   | )                                     | 0.0000  |                              | 100.00%    |
| Angular (Corre   | cted) Transf   | ormed Su   | mm   | ary  |                      |  |  |   |   |   |  |                                       |   |                              |            |
| Conc-µg/L  | Code   | Count  | t  | Mean   |                      | 95% LCL                                  | 95% UCL  | Median  | Min   | 1   | Мах  |                                       | Std Err   | CV%                          | %Effect    |
| 3.2  | D  | 5  |  | 1.412  | 0                    | 1.4120                                   | 1.4120   | 1.4120  | 1.4   | 120   | 1.4120   |                                       | 0.0000  | 0.00%                        | 0.00%      |
| 103  |  | 3  |  | 1.412  | 0                    | 1.4110                                   | 1.4130   | 1.4120  | 1.4   | 120   | 1.4120   |                                       | 0.0000  | 0.00%                        | 0.00%      |
| 240<br>490   |  | 3  |  | 1.412  | 0                    | 1.2490                                   | 1.2490   | 1.4120  | 1.4   | 490   | 1.2490   |                                       | 0.0000  | 0.00%                        | 11.54%     |
| 1055   |  | 3  |  | 0.537  | 3                    | 0.2203                                   | 0.8544   | 0.4636  | 0.4   | 636   | 0.6847   | ,                                     | 0.0737  | 23.75%                       | 61.95%     |
| 2350   |  | 3  |  | 0.158  | 8                    | 0.1588                                   | 0.1588   | 0.1588  | 0.1   | 588   | 0.1588   |                                       | 0.0000  | 0.00%                        | 88.76%     |
|  |  |  |  |  |                      |  |  |   |   |   |  |                                       |   |                              |            |

Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS Analytical Report Daphnia thomsoni 48-h Acute Survival Test |                            |                                 |                  |   |  |                                  |                          | Report Date:<br>Test Code/ID:                 | 27 May-24 14:24 (p 2 of 2)<br>24.003.1 Zn / 15-8888-6853 |
|---|----------------------------|---------------------------------|------------------|---|--|----------------------------------|--------------------------|---|--|
| Dapl  | hnia tho                   | msoni 48-h Acute                | e Survival       | Test                                    |  |                                  |                          |   | NIWA Ecotoxicology                                       |
| Anal<br>Anal<br>Edit  | ysis ID:<br>yzed:<br>Date: | 17-8062-8370<br>27 May-24 14:24 | En<br>4 An<br>MC | dpoint: 48<br>alysis: Pa<br>)5 Hash: 22 | h Survival R<br>arametric-Mu<br>4919757782 | ate<br>Iltiple Comp<br>2DA5F34CA | oarison<br>\239A904E497A | CETIS Version:<br>Status Level:<br>Editor ID: | CETISv2.1.4<br>1   |
| 48h   | Survival                   | Rate Detail                     |                  |   |  |                                  |                          |   |  |
| Con   | c-µg/L                     | Code                            | Rep 1            | Rep 2                                   | Rep 3                                      | Rep 4                            | Rep 5                    |   |  |
| 3.2   |                            | D                               | 1.0000           | 1.0000                                  | 1.0000                                     | 1.0000                           | 1.0000                   |   |  |
| 103   |                            |                                 | 1.0000           | 1.0000                                  | 1.0000                                     |                                  |                          |   |  |
| 240   |                            |                                 | 1.0000           | 1.0000                                  | 1.0000                                     |                                  |                          |   |  |
| 490   |                            |                                 | 0.9000           | 0.9000                                  | 0.9000                                     |                                  |                          |   |  |
| 1055  | 5                          |                                 | 0.2000           | 0.4000                                  | 0.2000                                     |                                  |                          |   |  |
| 2350  | )                          |                                 | 0.0000           | 0.0000                                  | 0.0000                                     |                                  |                          |   |  |
| Ang   | ular (Co                   | rrected) Transfor               | med Detai        | I                                       |  |                                  |                          |   |  |
| Con   | c-µg/L                     | Code                            | Rep 1            | Rep 2                                   | Rep 3                                      | Rep 4                            | Rep 5                    |   |  |
| 3.2   |                            | D                               | 1.4120           | 1.4120                                  | 1.4120                                     | 1.4120                           | 1.4120                   |   |  |
| 103   |                            |                                 | 1.4120           | 1.4120                                  | 1.4120                                     |                                  |                          |   |  |
| 240   |                            |                                 | 1.4120           | 1.4120                                  | 1.4120                                     |                                  |                          |   |  |
| 490   |                            |                                 | 1.2490           | 1.2490                                  | 1.2490                                     |                                  |                          |   |  |
| 1055  | 5                          |                                 | 0.4636           | 0.6847                                  | 0.4636                                     |                                  |                          |   |  |
| 2350  | )                          |                                 | 0.1588           | 0.1588                                  | 0.1588                                     |                                  |                          |   |  |
| 48h   | Survival                   | Rate Binomials                  |                  |   |  |                                  |                          |   |  |
| Con   | c-µg/L                     | Code                            | Rep 1            | Rep 2                                   | Rep 3                                      | Rep 4                            | Rep 5                    |   |  |
| 3.2   |                            | D                               | 10/10            | 10/10                                   | 10/10                                      | 10/10                            | 10/10                    |   |  |
| 103   |                            |                                 | 10/10            | 10/10                                   | 10/10                                      |                                  |                          |   |  |
| 240   |                            |                                 | 10/10            | 10/10                                   | 10/10                                      |                                  |                          |   |  |
| 490   |                            |                                 | 9/10             | 9/10                                    | 9/10                                       |                                  |                          |   |  |
| 1055  |                            |                                 | 2/10             | 4/10                                    | 2/10                                       |                                  |                          |   |  |
| 2350  | )                          |                                 | 0/10             | 0/10                                    | 0/10                                       |                                  |                          |   |  |
| Grap  | ohics                      |                                 |                  |   |  |                                  |                          |   |  |
|   | 1.0 -                      |                                 | -                |   |  |                                  | 0.15 -                   |   | ۲  |
|   | 0.9 -                      |                                 |                  | -                                       |  |                                  |                          |   |  |
|   | 0.8 -                      |                                 |                  |   |  |                                  | 0.10 -                   |   |  |
| e   | 0.7 -                      |                                 |                  |   |  |                                  |                          |   |  |
| Rat   | 0.6                        |                                 |                  |   |  |                                  | <u>e</u>                 |   |  |
| val   | 0.0 -                      |                                 |                  |   |  |                                  | 0.05 -                   |   |  |
| urvi  | 0.5 -                      |                                 |                  |   |  |                                  |                          |   |  |
| sh S  | 0.4 -                      |                                 |                  |   |  | d                                | S 0.00 -                 |   |  |
| 48  | 0.3 -                      |                                 |                  |   | -  |                                  | 0.00                     |   |  |
|   | 0.2 -                      |                                 |                  |   |  |                                  |                          |   |  |
|   | 0.1 -                      |                                 |                  |   |  |                                  | -0.05 -                  |   |  |
|   | 0.0 -                      |                                 |                  |   |  | -                                | •                        | •   |  |
|   |                            | 3.2 D 103                       | 240              | 490 10                                  | 055 2350                                   |                                  | -1                       | .5 -1.0 -0.5                                  | 0.0 0.5 1.0 1.5  |
|   |                            |                                 | Conc-            | µg/L                                    |  |                                  |                          | R   | ankits   |
|   |                            |                                 |                  |   |  |                                  |                          |   |  |
|   |                            |                                 |                  |   |  |                                  |                          |   |  |
|   |                            |                                 |                  |   |  |                                  |                          |   |  |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS                                   | S Analyt   | ical Repo                           | ort                    |  |  |                                |                             | F<br>T                     | Report D<br>Test Cod                      | ate:<br>e/ID:                     | 27<br>24.0  | May-24 14:2<br>003.1 Zn / 1 | 25 (p 1 of 3)<br>5-8888-6853 |
|---|--|-------------------------------------|------------------------|--|--|--------------------------------|-----------------------------|----------------------------|---|-----------------------------------|---|-----------------------------|------------------------------|
| Daphni                                  | ia thomsor   | ni 48-h Acute                       | Surviva                | Test   |  |                                |                             |                            |   |                                   |   | NIWA Eco                    | toxicology                   |
| Analysi<br>Analyzo<br>Edit Da           | is ID: 15-<br>ed: 27<br>ite:                       | -8302-1928<br>May-24 14:24          | Er<br>Ar<br>Mi         | ndpoint: 48h<br>nalysis: Nor<br>D5 Hash: 224         | n Survival Ra<br>nlinear Regr<br>19197577821 | ate<br>ession (NLF<br>DA5F34CA | R)<br>239A904E49            | )7A                        | CETIS<br>Status<br>Editor                 | Version<br>Level:<br>ID:          | CETISV.<br>1  | 2.1.4                       |                              |
| Batch I<br>Start D<br>Ending<br>Test Le | D: 03-<br>ate: 08<br>Date: 10<br>ength: 48         | -1254-1573<br>May-24<br>May-24<br>h | Te<br>Pr<br>Sp<br>Ta   | est Type: Sur<br>otocol: NIV<br>becies: Dap<br>ixon: | vival (48h)<br>VA SOP 10<br>phnia thoms      | (2022)<br>oni (Water           | flea)                       |                            | Analys<br>Diluen<br>Brine:<br>Source      | t: Eco<br>t: Reo<br>Not<br>e: Fie | otox Team<br>dwoods<br>: Applicable<br>Id Collected | I                           | Age:                         |
| Sample<br>Sample<br>Receip<br>Sample    | e ID: 09-<br>e Date: 07<br>t Date: 07<br>e Age: 24 | -2771-2013<br>May-24<br>May-24<br>h | Co<br>Ma<br>C/<br>Cl   | ode: 24.<br>aterial: Zin<br>AS (PC):<br>ient: Hyd    | 003.1 Zn<br>c sulfate<br>drotoxy Rese        |                                | Projec<br>Source<br>Station | t: Spe<br>e: Sol<br>n: Lat | ecial Studie:<br>ution made<br>) Solution | s<br>by NIWA                      |   |                             |                              |
| Non-Li                                  | near Regre   | ession Optio                        | ns                     |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| Model                                   | Name and   | Function                            |                        |  |  | Weightin                       | g Function                  |                            |   | PTBS Fu                           | nction  | X Trans                     | Y Trans                      |
| 3P Log                                  | -Logistic: µ                                       | =α/[1+[x/δ]^γ]                      |                        |  |  | Binomial                       | [ω=n/[p·q]]                 |                            |   | Off [µ*=µ                         | ]   | None                        | None                         |
| Regres                                  | sion Sum   | mary                                |                        |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| Iters                                   | LL   | AICc                                | BIC                    | Adj R2   | PMSD   | Thresh                         | Optimize                    | FS                         | itat                                      | P-Value                           | Decisior  | n(α:5%)                     |                              |
| 4                                       | -7.72  | 22.94                               | 24.43                  | 0.9753   | 0.00%  | 1                              | Yes                         | 1.7                        | 02  | 0.2123                            | Non-Sigr  | hificant Lack-              | of-Fit                       |
| Point E                                 | stimates   |                                     |                        |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| Level                                   | µg/L   | 95% LCL                             | 95% UC                 | L  |  |                                |                             |                            |   |                                   |   |                             |                              |
| LC5                                     | 433.6  | 377.4                               | 473.3                  |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| LC10                                    | 510.6  | 459.2                               | 551                    |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| LC15                                    | 565  | 516.6                               | 605.7                  |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| LC20                                    | 640.4  | 503.4<br>604.5                      | 0.0C0                  |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| LC25                                    | 755.7  | 712.5                               | 799.2                  |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| LC50                                    | 825.9  | 781.6                               | 872.6                  |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| Regres                                  | sion Para  | meters                              |                        |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| Daramy                                  | otor   | Eetimato                            | Std Err                | or 05% I.Cl  | 05% [[[C]                                    | t Stat                         | D Value                     | Dec                        | cicion/a                                  | 5%)                               |   |                             |                              |
| Faranne                                 | eter   | 1                                   | 1 518EJ                | 05 1   | 1  | 65870                          | <1.0E-05                    | Sin                        | nificant F                                | Paramete                          | r   |                             |                              |
| v                                       |  | 4 57                                | 0.2874                 | 3 963  | 5 176  | 15.9                           | <1.0E-05                    | Sia                        | nificant F                                | Paramete                          | r   |                             |                              |
| ō                                       |  | 825.9                               | 21.46                  | 780.6  | 871.1  | 38.49                          | <1.0E-05                    | Sig                        | nificant F                                | Paramete                          | r   |                             |                              |
| ANOVA                                   | Table  |                                     |                        |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| Source                                  |  | Sum Squa                            | ares M                 | ean Square   | DF   | F Stat                         | P-Value                     | Dee                        | cision(a:                                 | :5%)                              |   |                             |                              |
| Model                                   |  | 50040000                            | ) 16                   | 6800000  | 3  | 1.446E+0                       | 9 <1.0E-05                  | Sig                        | nificant E                                | ffect                             |   |                             |                              |
| Lack of                                 | Fit  | 0.5239                              | 0.1                    | 1746   | 3  | 1.702                          | 0.2123                      | Nor                        | n-Signific                                | ant Lack                          | -of-Fit   |                             |                              |
| Pure Er                                 | ror  | 1.437                               | 0.1                    | 1026   | 14   |                                |                             |                            |   |                                   |   |                             |                              |
| Residua                                 | al   | 1.961                               | 0.1                    | 1153   | 17   |                                |                             |                            |   |                                   |   |                             |                              |
| Residu                                  | al Analysi   | s                                   |                        |  |  |                                |                             |                            |   |                                   |   |                             |                              |
| Attribu                                 | te   | Method                              |                        |  | Test Stat                                    | Critical                       | P-Value                     | Dee                        | cision(a                                  | :5%)                              |   |                             |                              |
| Model F                                 | Fit  | Likelihood                          | Ratio GO               | F Test   | 2.194  | 27.59                          | 1.0000                      | Nor                        | n-Signific                                | ant Hete                          | rogeneity   |                             |                              |
| Variand                                 | م  | Mod Lever                           | ni-oq GO<br>ne Equalit | r rest<br>v of Variance                              | 1 371  | 27.59                          | 0.3286                      | For                        | u-orgnino<br>ual Varia                    | ani Hete<br>nces                  | ogeneity  |                             |                              |
| Distribu                                | tion   | Anderson-                           | Darling A              | 2 Test   | 1.661  | 2 492                          | <1.0E-05                    | Nor                        | n-Normal                                  | Distribut                         | ion   |                             |                              |
|   |  | Shapiro-W                           | ilk W Nor              | mality Test  | 0.7373                                       | 0.9044                         | 0.0001                      | Nor                        | n-Normal                                  | Distribut                         | ion   |                             |                              |
| Overdis                                 | persion  | Tarone C(                           | a) Overdis             | spersion Test  | 1.291  | 1.645                          | 0.0984                      | Nor                        | n-Signific                                | ant Over                          | dispersion  |                             |                              |
|   |  |                                     |                        |  |  |                                |                             |                            |   |                                   |   |                             |                              |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS An                                | alytical Repo                   | ort              |   | Report<br>Test C                            | Date:<br>ode/ID:                | 27 May-24 14:25<br>24.003.1 Zn / 15-8 |                          | 5 (p 2 of 3)<br>-8888-6853         |              |           |           |
|---|---------------------------------|------------------|---|---|---------------------------------|---------------------------------------|--------------------------|------------------------------------|--------------|-----------|-----------|
| Daphnia tho                             | nsoni 48-h Acute                | Survival         | Test                                    |   |                                 |                                       |                          |                                    |              | NIWA Ecot | oxicology |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 15-8302-1928<br>27 May-24 14:24 | En<br>4 An<br>MC | dpoint: 48<br>alysis: No<br>)5 Hash: 22 | h Survival R<br>onlinear Regi<br>4919757782 | ate<br>ression (NL<br>2DA5F34CA | R)<br>\239A904E4                      | CET<br>Stat<br>197A Edit | IS Version:<br>us Level:<br>or ID: | CETISV.<br>1 | 2.1.4     |           |
| 48h Survival                            | Rate Summary                    |                  |   |   |                                 | Calculate                             | d Variate(A              | /B)                                |              |           |           |
| Conc-µg/L                               | Code                            | Count            | Mean                                    | Median                                      | Min                             | Мах                                   | Std Err                  | Std Dev                            | CV%          | %Effect   | ΣΑ/ΣΒ     |
| 3.2                                     | D                               | 5                | 1.0000                                  | 1.0000                                      | 1.0000                          | 1.0000                                | 0.0000                   | 0.0000                             | 0.00%        | 0.00%     | 50/50     |
| 103                                     |                                 | 3                | 1.0000                                  | 1.0000                                      | 1.0000                          | 1.0000                                | 0.0000                   | 0.0000                             | 0.00%        | 0.00%     | 30/30     |
| 240                                     |                                 | 3                | 1.0000                                  | 1.0000                                      | 1.0000                          | 1.0000                                | 0.0000                   | 0.0000                             | 0.00%        | 0.00%     | 30/30     |
| 490                                     |                                 | 3                | 0.9000                                  | 0.9000                                      | 0.9000                          | 0.9000                                | 0.0000                   | 0.0000                             | 0.00%        | 10.00%    | 27/30     |
| 1055                                    |                                 | 3                | 0.2667                                  | 0.2000                                      | 0.2000                          | 0.4000                                | 0.0667                   | 0.1155                             | 43.30%       | 73.33%    | 8/30      |
| 2350                                    |                                 | 3                | 0.0000                                  | 0.0000                                      | 0.0000                          | 0.0000                                | 0.0000                   | 0.0000                             |              | 100.00%   | 0/30      |
| 48h Survival                            | Rate Detail                     |                  |   |   |                                 |                                       |                          |                                    |              |           |           |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                   | Rep 3                                       | Rep 4                           | Rep 5                                 |                          |                                    |              |           |           |
| 3.2                                     | D                               | 1.0000           | 1.0000                                  | 1.0000                                      | 1.0000                          | 1.0000                                |                          |                                    |              |           |           |
| 103                                     |                                 | 1.0000           | 1.0000                                  | 1.0000                                      |                                 |                                       |                          |                                    |              |           |           |
| 240                                     |                                 | 1.0000           | 1.0000                                  | 1.0000                                      |                                 |                                       |                          |                                    |              |           |           |
| 490                                     |                                 | 0.9000           | 0.9000                                  | 0.9000                                      |                                 |                                       |                          |                                    |              |           |           |
| 1055                                    |                                 | 0.2000           | 0.4000                                  | 0.2000                                      |                                 |                                       |                          |                                    |              |           |           |
| 2350                                    |                                 | 0.0000           | 0.0000                                  | 0.0000                                      |                                 |                                       |                          |                                    |              |           |           |
| 48h Survival                            | Rate Binomials                  |                  |   |   |                                 |                                       |                          |                                    |              |           |           |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                   | Rep 3                                       | Rep 4                           | Rep 5                                 |                          |                                    |              |           |           |
| 3.2                                     | D                               | 10/10            | 10/10                                   | 10/10                                       | 10/10                           | 10/10                                 |                          |                                    |              |           |           |
| 103                                     |                                 | 10/10            | 10/10                                   | 10/10                                       |                                 |                                       |                          |                                    |              |           |           |
| 240                                     |                                 | 10/10            | 10/10                                   | 10/10                                       |                                 |                                       |                          |                                    |              |           |           |
| 490                                     |                                 | 9/10             | 9/10                                    | 9/10  |                                 |                                       |                          |                                    |              |           |           |
| 1055                                    |                                 | 2/10             | 4/10                                    | 2/10  |                                 |                                       |                          |                                    |              |           |           |

2350

0/10

0/10

0/10

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CETIS™ v2.1.4.5 (009-951-268-0)

# Copper

| CETIS Analytical Report                                    |                             |   |  |  |  |                    |   |   |  | Report Date:         27 May-24 14:09 (p 1           Test Code/ID:         24.003.1 Cu / 11-3123 |   |   | 9 (p 1 of 2)<br>I-3123-4564   |  |         |         |
|--|-----------------------------|---|--|--|--|--------------------|---|---|--|---|---|---|---|--|---------|---------|
| Daphnia thon   | nsoni                       | 48-h Acute  | e Survi  | ival T   | est  |                    |   |   |  |   | I   | NIWA Eco  | toxicology  |  |         |         |
| Analysis ID:<br>Analyzed:<br>Edit Date:                    | 06-4<br>27 N                | 463-1136<br>lay-24 14:08  | 3  | End<br>Anal<br>MD5                                       | point:<br>ysis:<br>Hash:                                   | 48h<br>Para<br>5D2 | Survival Ra<br>ametric-Mul<br>41F0A1645 | ate<br>tiple Compa<br>i8046CC042                      | rison<br>27C2488552                                | 2BC   | CETI<br>Statu<br>Edito                        | S Versio<br>Is Level:<br>or ID:                           | on:<br>:  | CETISv2.<br>1                            | 1.4     |         |
| Batch ID:<br>Start Date:<br>Ending Date:<br>Test Length:   | 15-9<br>04 N<br>06 N<br>48h | 420-8257<br>lay-24<br>lay-24  |  | Test<br>Prot<br>Spec<br>Taxo                             | Type:<br>ocol:<br>cies:<br>on:                             | Sun<br>NIW<br>Dap  | vival (48h)<br>/A SOP 10<br>hnia thoms  | (2022)<br>oni (Water fl                               | ea)  |   | Analy<br>Dilue<br>Brine<br>Sour               | yst: E<br>ent: R<br>e: N<br>ce: F                         | Ecoto<br>Redw<br>Not A<br>Field   | x Team<br>oods<br>pplicable<br>Collected |         | Age:    |
| Sample ID:<br>Sample Date:<br>Receipt Date:<br>Sample Age: | 04-1<br>03 N<br>03 N<br>24h | 166-2908<br>lay-24<br>lay-24  |  | Cod<br>Mate<br>CAS<br>Clier                              | e:<br>erial:<br>(PC):<br>nt:                               | 24.0<br>Cop<br>Hyd | 003.1 Cu<br>oper<br>irotoxy Rese        | earch   |  |   | Proje<br>Sour<br>Statie                       | ect: S<br>ce: S<br>on: L                                  | Speci<br>Soluti<br>.ab S  | al Studies<br>on made by<br>olution      | y NIWA  |         |
| Data Transfor  | m                           |   | Alt I  | Нур  |  |                    |   |   | NOEL   | LO  | EL  | TOEL  |   | Tox Units                                | MSDu    | PMSD    |
| Angular (Corre   | ected)                      |   | C > 1  | Г  |  |                    |   |   | 51   | 156   |   | 89.2  |   |  | 0.06018 | 6.02%   |
| Bonferroni A   | lj t Te                     | est   |  |  |  |                    |   |   |  |   |   |   |   |  |         |         |
| Control  | vs                          | Conc-µg/L   | L  | df   | Test 9   | Stat               | Critical                                | MSD   | P-Type   | P-V   | alue  | Decisi  | on(α  | :5%)                                     |         |         |
| Dilution Water   |                             | 23  |  | 6  | 0  |                    | 2.466                                   | 0.08906   | CDF  | 1.00  | 000   | Non-Si  | gnifio  | cant Effect                              |         |         |
|  |                             | 51<br>156*  |  | 6  | 0  |                    | 2.466                                   | 0.08906   | CDF  | 1.00  | 000   | Non-Signific  | gnific  | ant Effect                               |         |         |
|  |                             | 150   |  |  | 11.55  |                    | 2.400                                   | 0.00300   | CDI  | ~1.4  | 12-03   | Signino   | anti  | Lilect                                   |         |         |
| ANOVA Table  |                             |   |  |  |  | _                  |   | ~ ~   |  |   |   | <b>.</b>  |   | -  |         |         |
| Source   |                             | Sum Squa  | ares   |  | Mean   | Squ                | are                                     | DF  | F Stat   | P-V   | alue  | Decisi  | on(α  | :5%)                                     |         |         |
| Error  |                             | 0.0244562   |  |  | 0.130  | 773<br>4456        |   | 3<br>10   | 00.93  | \$1.0   | JE-05   | Signific  | anti  | Ellect                                   |         |         |
| Total  |                             | 0.434774  |  |  |  |                    |   | 13  | _  |   |   |   |   |  |         |         |
| ANOVA Assu   | nptic                       | ns Tests  |  |  |  |                    |   |   |  |   |   |   |   |  |         |         |
| Attribute  |                             | Test  |  |  |  |                    |   | Test Stat   | Critical   | P-V   | alue  | Decisi  | on(α  | :1%)                                     |         |         |
| Variance   |                             | Bartlett Eq   | uality   | of Vai   | iance 1  | fest               |   |   |  |   |   | Indeter   | mina  | ite                                      |         |         |
| Distribution   |                             | Levene Eq<br>Mod Lever<br>Anderson-<br>D'Agostino<br>Kolmogoro<br>Shapiro-W | quality<br>ne Equ<br>Darling<br>Skew<br>ov-Smi<br>/ilk W I | of Vai<br>iality o<br>g A2 1<br>iness<br>irnov 1<br>Norm | riance T<br>of Varia<br>Test<br>Test<br>D Test<br>ality Te | Fest<br>nce T      | Test                                    | 5.781<br>656.6<br>3.824<br>0.2587<br>0.4286<br>0.5624 | 6.552<br>9.78<br>3.878<br>2.576<br>0.262<br>0.8239 | 0.01<br><1.(<br><1.(<br>0.79<br><1.(<br>1.98  | 148<br>DE-05<br>DE-05<br>958<br>DE-05<br>E-05 | Equal V<br>Unequa<br>Non-No<br>Normal<br>Non-No<br>Non-No | Equal Variances<br>Unequal Variances<br>Non-Normal Distribution<br>Normal Distribution<br>Non-Normal Distribution |  |         |         |
| 48h Survival   | Rate                        | Summary   |  |  |  |                    |   |   |  |   |   |   |   |  |         |         |
| Conc-µg/L  |                             | Code  | Cou  | nt   | Mean   |                    | 95% LCL                                 | 95% UCL   | Median   | Min   |   | Мах   |   | Std Err                                  | CV%     | %Effect |
| 0.25   |                             | D   | 5  |  | 1.000  | 0                  | 1.0000                                  | 1.0000  | 1.0000   | 1.00  | 000   | 1.0000  |   | 0.0000                                   | 0.00%   | 0.00%   |
| 23   |                             |   | 3  |  | 1.000  | 0                  | 1.0000                                  | 1.0000  | 1.0000   | 1.00  | 000   | 1.0000  |   | 0.0000                                   | 0.00%   | 0.00%   |
| 51   |                             |   | 3  |  | 1.000  | 0                  | 1.0000                                  | 1.0000  | 1.0000   | 1.00  | 000   | 1.0000  |   | 0.0000                                   | 0.00%   | 20.00%  |
| 450  |                             |   | 3  |  | 0.000  | 0                  | 0.0000                                  | 0.0000  | 0.0000   | 0.00  | 000   | 0.0000  |   | 0.0000                                   | 14.2370 | 100.00% |
| 1320   |                             |   | 3  |  | 0.000  | 0                  | 0.0000                                  | 0.0000  | 0.0000   | 0.00  | 000   | 0.0000  |   | 0.0000                                   |         | 100.00% |
| Angular (Corr  | ected                       | l) Transfori  | med S  | umm  | ary  |                    |   |   |  |   |   |   |   |  |         |         |
| Conc-µg/L  |                             | Code  | Cour   | nt   | Mean   |                    | 95% LCL                                 | 95% UCL   | Median   | Min   |   | Мах   |   | Std Err                                  | CV%     | %Effect |
| 0.25   |                             | D   | 5  |  | 1.412  | 0                  | 1.4120                                  | 1.4120  | 1.4120   | 1.41  | 120   | 1.4120  |   | 0.0000                                   | 0.00%   | 0.00%   |
| 23   |                             |   | 3  |  | 1.412  | 0                  | 1.4110                                  | 1.4130  | 1.4120   | 1.41  | 120   | 1.4120  |   | 0.0000                                   | 0.00%   | 0.00%   |
| 51   |                             |   | 3  |  | 1.412  | 0                  | 1.4110                                  | 1.4130  | 1.4120   | 1.41  | 120   | 1.4120  |   | 0.0000                                   | 0.00%   | 0.00%   |
| 156  |                             |   | 3  |  | 0.994  | 8<br>0             | 0.7201                                  | 1.2690  | 0.9912   | 0.88  | 301<br>:00                                    | 1.1070  |   | 0.0638                                   | 11.12%  | 29.55%  |
| 450  |                             |   | 3  |  | 0.158  | 0<br>8             | 0.1588                                  | 0.1588  | 0.1588   | 0.13  | 588<br>588                                    | 0.1588  |   | 0.0000                                   | 0.00%   | 88 76%  |
| .020   |                             |   |  |  | 0.100  | -<br>-             | 3.1300                                  | 3.1000  | 3.1300   | 0.15  |   | 0.1000  |   | 0.0000                                   | 3.0070  | 50.1070 |

Convergent Rounding (4 sf)

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Analyst:\_\_\_\_\_ QA:\_\_\_\_

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| CET                      | IS An                      | nalytical Rep                    | ort              |   |  |                              |                         | Report Date:<br>Test Code/ID:                   | 27 May-24 14:09 (p 2 of 2)<br>24.003.1 Cu / 11-3123-4564 |
|--------------------------|----------------------------|----------------------------------|------------------|---|--|------------------------------|-------------------------|---|--|
| Daph                     | nia tho                    | omsoni 48-h Acut                 | e Survival       | Test                                      |  |                              |                         |   | NIWA Ecotoxicology                                       |
| Analy<br>Analy<br>Edit [ | /sis ID:<br>/zed:<br>Date: | : 06-4463-1136<br>27 May-24 14:0 | En<br>8 An<br>Mi | ndpoint: 48<br>nalysis: Pa<br>D5 Hash: 5D | h Survival Ra<br>arametric-Mult<br>0241F0A1645 | ite<br>tiple Comj<br>8046CC0 | parison<br>427C2488552B | CETIS Version:<br>Status Level:<br>C Editor ID: | CETISv2.1.4<br>1   |
| 48h S                    | urviva                     | I Rate Detail                    |                  |   |  |                              |                         |   |  |
| Conc                     | -µg/L                      | Code                             | Rep 1            | Rep 2                                     | Rep 3  | Rep 4                        | Rep 5                   |   |  |
| 0.25                     |                            | D                                | 1.0000           | 1.0000                                    | 1.0000   | 1.0000                       | 1.0000                  |   |  |
| 23                       |                            |                                  | 1.0000           | 1.0000                                    | 1.0000   |                              |                         |   |  |
| 51                       |                            |                                  | 1.0000           | 1.0000                                    | 1.0000   |                              |                         |   |  |
| 156                      |                            |                                  | 0.8000           | 0.6000                                    | 0.7000   |                              |                         |   |  |
| 450                      |                            |                                  | 0.0000           | 0.0000                                    | 0.0000   |                              |                         |   |  |
| 1320                     |                            |                                  | 0.0000           | 0.0000                                    | 0.0000   |                              |                         |   |  |
| Angu                     | lar (Co                    | orrected) Transfor               | med Deta         | il  |  |                              |                         |   |  |
| Conc                     | -μg/L                      | Code                             | Rep 1            | Rep 2                                     | Rep 3  | Rep 4                        | Rep 5                   |   |  |
| 0.25                     |                            | D                                | 1.4120           | 1.4120                                    | 1.4120   | 1.4120                       | 1.4120                  |   |  |
| 23                       |                            |                                  | 1.4120           | 1.4120                                    | 1.4120   |                              |                         |   |  |
| 51                       |                            |                                  | 1.4120           | 1.4120                                    | 1.4120   |                              |                         |   |  |
| 156                      |                            |                                  | 1.1070           | 0.8861                                    | 0.9912   |                              |                         |   |  |
| 450                      |                            |                                  | 0.1588           | 0.1588                                    | 0.1588   |                              |                         |   |  |
| 1320                     |                            |                                  | 0.1588           | 0.1588                                    | 0.1588   |                              |                         |   |  |
| 48h S                    | Surviva                    | I Rate Binomials                 |                  |   |  |                              |                         |   |  |
| Conc                     | -µg/L                      | Code                             | Rep 1            | Rep 2                                     | Rep 3  | Rep 4                        | Rep 5                   |   |  |
| 0.25                     |                            | D                                | 10/10            | 10/10                                     | 10/10  | 10/10                        | 10/10                   |   |  |
| 23                       |                            |                                  | 10/10            | 10/10                                     | 10/10  |                              |                         |   |  |
| 51                       |                            |                                  | 10/10            | 10/10                                     | 10/10  |                              |                         |   |  |
| 150                      |                            |                                  | 8/10             | 0/10                                      | 0/10   |                              |                         |   |  |
| 1320                     |                            |                                  | 0/10             | 0/10                                      | 0/10   |                              |                         |   |  |
| 0                        |                            |                                  |                  |   |  |                              |                         |   |  |
| Grap                     | nics                       |                                  |                  |   |  |                              |                         |   |  |
|                          | 1.0-                       |                                  |                  |   |  | •                            | 0.10 -                  |   | ٠  |
|                          | 0.9 -                      |                                  |                  |   |  |                              |                         |   |  |
|                          | 0.8 -                      |                                  |                  |   |  |                              | 0.05                    |   |  |
| Rate                     | 0.7-                       |                                  |                  |   |  |                              | 0.03 -                  |   |  |
| val                      | 0.6 -                      |                                  |                  |   |  |                              | Angl                    |   |  |
| urvi                     | 0.5-                       |                                  |                  |   |  |                              | 0.00 -                  |   | ****   |
| 8h S                     | 0.4 -                      |                                  |                  |   |  |                              | ŭ                       |   |  |
| 4                        | 0.3                        |                                  |                  |   |  |                              | -0.05 -                 |   |  |
|                          | 0.1 -                      |                                  |                  |   |  |                              |                         |   |  |
|                          | 0.0                        |                                  |                  |   | <u> </u>                                       |                              | -0.10 -                 |   |  |
|                          |                            | 0.25 D 23                        | 51               | 156 4                                     | 50 1320  |                              | -                       | 1.5 -1.0 -0.5                                   | 0.0 0.5 1.0 1.5  |
|                          |                            |                                  | Conc-            | ua/L                                      |  |                              |                         | R   | ankits   |
|                          |                            |                                  |                  |   |  |                              |                         |   |  |

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| CETIS Analytical Report   |   |                         |  |                                     |  |                          |                    |  |                             |             | R<br>T                         | eport<br>est Co            | Date:<br>de/ID:                |   | 27<br>24.    | May-24 14<br>003.1 Cu / | :09 (p<br>11-31: | 1 of 2)<br>23-4564 |        |
|---|---|-------------------------|--|-------------------------------------|--|--------------------------|--------------------|--|-----------------------------|-------------|--------------------------------|----------------------------|--------------------------------|---|--------------|-------------------------|------------------|--------------------|--------|
| Daphn   | ia thon   | nsoni 4                 | 48-h Acute                                 | e Surv                              | ival T   | est                      |                    |  |                             |             |                                |                            |                                |   |              |                         | NIWA Ec          | otoxi              | cology |
| Analys<br>Analyz<br>Edit Da   | is ID:<br>ed:<br>ate:   | 01-06<br>27 Ma          | 18-7910<br>iy-24 14:09                     | 9                                   | Endj<br>Anal<br>MD5  | point:<br>ysis:<br>Hash: | 48h<br>Line<br>5D2 | Survival Rai<br>ear Interpolat<br>241F0A1645 | te<br>tion (ICPI<br>8046CC0 | N)<br>427C2 | 488552                         | 2BC                        | CETI<br>Statu<br>Edito         | S Versi<br>Is Leve<br>or ID:                  | ion:<br>I:   | CETISv<br>1             | 2.1.4            |                    |        |
| Batch  <br>Start D<br>Ending<br>Test Le   | atch ID:     15-9420-8257     Test Type: Survival (48h)       tart Date:     04 May-24     Protocol:     NIWA SOP 10 (2022)       nding Date:     06 May-24     Species:     Daphnia thomsoni (Water flea)       est Length:     48h     Taxon: |                         |  |                                     |  |                          |                    |  |                             |             | Anal<br>Dilue<br>Brine<br>Sour | yst:<br>ent:<br>e:<br>rce: | Ecot<br>Redv<br>Not /<br>Field | ox Team<br>woods<br>Applicable<br>I Collected | 1            | Ag                      | je:              |                    |        |
| Sample ID: 04-1166-2908<br>Sample Date: 03 May-24<br>Receipt Date: 03 May-24<br>Sample Age: 24h<br>Linear Interpolation Options |   |                         |  |                                     | Code: 24.003.1 Cu<br>Material: Copper<br>CAS (PC):<br>Client: Hydrotoxy Research |                          |                    |  |                             |             | Proje<br>Sour<br>Stati         | ect:<br>ce:<br>on:         | Spec<br>Solut<br>Lab \$        | ial Studie<br>tion made<br>Solution           | s<br>by NIWA |                         |                  |                    |        |
| Linear  | Interp  | olation                 | Options                                    |                                     |  |                          |                    |  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| X Tran  | sform   | Y.                      | Transform                                  | 1                                   | Seed   | 1                        |                    | Resamples                                    | Exp 95                      | % CL        | Meth                           | od                         |                                |   |              |                         |                  |                    |        |
| Log(X+  | 1)  | Lir                     | near                                       |                                     | 8502   | 200                      | 1                  | 200  | Yes                         |             | Two-                           | Point                      | Interp                         | olation                                       |              |                         |                  |                    |        |
| Point E<br>Level<br>LC15<br>LC20<br>LC25<br>LC40  | Estimat<br>µg/L<br>89.3<br>107.0<br>129.0<br>181.5  | tes<br>5<br>6<br>6<br>5 | 95% LCL<br>64.88<br>69.26<br>73.2<br>127.9 | 95%<br>149.<br>209.<br>207.<br>227. | UCL<br>7<br>2<br>5<br>4  |                          |                    |  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| LC50  | 211.3   | 2                       | 158.6                                      | 255.                                | 3  |                          |                    |  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 48h Su  | irvival   | Rate S                  | ummarv                                     |                                     |  |                          |                    |  | Calculate                   | ed Vari     | iate(A/                        | B)                         |                                |   |              |                         | Isoto            | nic V              | ariate |
| Conc-   | ua/L  |                         | Code                                       | Cou                                 | nt   | Mean                     |                    | Median                                       | Min                         | Ma          | x                              | CV <sup>e</sup>            | %                              | %Effe   | ect          | ΣΑ/ΣΒ                   | Mean             | %                  | Effect |
| 0.25  | -g  |                         | D  | 5                                   |  | 1.000                    | 0                  | 1.0000                                       | 1.0000                      | 1.0         | 000                            | 0.00                       | 0%                             | 0.00%   | 6            | 50/50                   | 1.0000           | 0.                 | .00%   |
| 23  |   |                         |  | 3                                   |  | 1.000                    | D                  | 1.0000                                       | 1.0000                      | 1.0         | 000                            | 0.00                       | 0%                             | 0.00%   | 5            | 30/30                   | 1.0000           | 0.                 | .00%   |
| 51  |   |                         |  | 3                                   |  | 1.000                    | D                  | 1.0000                                       | 1.0000                      | 1.0         | 000                            | 0.00                       | 0%                             | 0.00%   | b            | 30/30                   | 1.0000           | 0.                 | .00%   |
| 156   |   |                         |  | 3                                   |  | 0.700                    | D                  | 0.7000                                       | 0.6000                      | 0.8         | 000                            | 14.3                       | 29%                            | 30.00   | %            | 21/30                   | 0.7000           | 30                 | 0.00%  |
| 450   |   |                         |  | 3                                   |  | 0.000                    | D<br>D             | 0.0000                                       | 0.0000                      | 0.0         | 000                            |                            |                                | 100.00  | 0%           | 0/30                    | 0.0000           | 10                 | 00.00% |
| 1320  |   |                         |  | 3                                   |  | 0.000                    |                    | 0.0000                                       | 0.0000                      | 0.0         | 000                            |                            |                                | 100.00  | 070          | 0/30                    | 0.0000           |                    | 00.00% |
| 48h Su  | irvival   | Rate D                  | etail                                      |                                     |  |                          |                    |  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| Conc-   | ıg/L  |                         | Code                                       | Rep                                 | 1  | Rep 2                    |                    | Rep 3  | Rep 4                       | Re          | p 5                            |                            |                                |   |              |                         |                  |                    |        |
| 0.25  |   |                         | U  | 1.00                                | 00   | 1.000                    | U<br>N             | 1.0000                                       | 1.0000                      | 1.0         | 000                            |                            |                                |   |              |                         |                  |                    |        |
| 23  |   |                         |  | 1.00                                | 00   | 1.000                    | n                  | 1.0000                                       |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 156   |   |                         |  | 0.80                                | 00   | 0.600                    | n                  | 0 7000                                       |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 450   |   |                         |  | 0.00                                | 00   | 0.000                    | 0                  | 0.0000                                       |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 1320  |   |                         |  | 0.00                                | 00   | 0.000                    | D                  | 0.0000                                       |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 48h Su  | irvival   | Rate B                  | inomials                                   |                                     |  |                          |                    |  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| Conc-   | ıg/L  |                         | Code                                       | Rep                                 | 1  | Rep 2                    |                    | Rep 3  | Rep 4                       | Re          | o 5                            |                            |                                |   |              |                         |                  |                    |        |
| 0.25  | -   |                         | D  | 10/1                                | 0  | 10/10                    |                    | 10/10  | 10/10                       | 10/         | 10                             |                            |                                |   |              |                         |                  |                    |        |
| 23  |   |                         |  | 10/1                                | 0  | 10/10                    |                    | 10/10  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 51  |   |                         |  | 10/1                                | 0  | 10/10                    |                    | 10/10  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 156   |   |                         |  | 8/10                                |  | 6/10                     |                    | 7/10   |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 450   |   |                         |  | 0/10                                |  | 0/10                     |                    | 0/10   |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
| 1320  |   |                         |  | 0/10                                |  | 0/10                     |                    | 0/10   |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |
|   |   |                         |  |                                     |  |                          |                    |  |                             |             |                                |                            |                                |   |              |                         |                  |                    |        |

CETIS™ v2.1.4.5 (009-951-268-0)

| <b>CETIS An</b>   | alvtical Report                 |  | Report Date:  | 27 May-24 14:09 (p 2 of 2) |
|---|---------------------------------|--|---|----------------------------|
|   |                                 |  | Test Code/ID:                                       | 24.003.1 Cu / 11-3123-4564 |
| Daphnia tho   | msoni 48-h Acute Su             | Irvival Test   |   | NIWA Ecotoxicology         |
| Analysis ID:<br>Analyzed:<br>Edit Date:   | 01-0618-7910<br>27 May-24 14:09 | Endpoint: 48h Survival Rate<br>Analysis: Linear Interpolation (ICPIN)<br>MD5 Hash: 5D241F0A16458046CC0427C2488 | CETIS Version:<br>Status Level:<br>552BC Editor ID: | CETISv2.1.4<br>1           |
| Graphics  |                                 |  |   |                            |
| 1.0-<br>0.9<br>0.8<br>0.7<br>0.6<br>0.5<br>0.6<br>0.5<br>0.4<br>0.3<br>0.4<br>0.3<br>0.2<br>0.1<br>0.1<br>0.0 |                                 |  |   |                            |

•

1200

1000

600

Conc-µg/L

800

400

0

200

Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

# Appendix G CETIS statistical analyses – Hoteo

#### Zinc

| CETIS Anal   |  |  | F<br>T   | leport<br>est Co   | Date:<br>de/ID:              |   | 27 I<br>24.0   | May-24 14:2<br>03.2 Zn / 1   | 26 (p 1 of 2)<br>8-3585-2031           |  |  |                                |   |              |                  |
|--|--|--|--|--|------------------------------|---|--|--|--|--|--|--------------------------------|---|--------------|------------------|
| Daphnia thoms  | soni 48-h  | Acute Surv   | ival T   | est  |                              |   |  |  |  |  |  |                                |   | NIWA Eco     | toxicology       |
| Analysis ID: 0<br>Analyzed: 2<br>Edit Date:  | )5-6314-4<br>27 May-24                               | 525<br>14:26   | End<br>Ana<br>MDS  | point:<br>lysis:<br>5 Hash:  | 48h<br>Par<br>8CE            | Survival Ra<br>ametric-Mul<br>3123CEE58 | ate<br>tiple Compa<br>01E9A4A60                      | irison<br>3D8DCEB3   | 372A                                   | CETI<br>Statu<br>Edito                     | S Versi<br>Is Leve<br>or ID:   | ion:<br>:                      | CETISv2<br>1                              | 2.1.4        |                  |
| Batch ID: 2<br>Start Date: 0<br>Ending Date: 1<br>Test Length: 4   | 20-4051-48<br>08 May-24<br>10 May-24<br>18h          | 304  | Test<br>Prot<br>Spe<br>Tax                                     | Type:<br>ocol:<br>cies:<br>on:   | Sur<br>NIW<br>Dap            | vival (48h)<br>/A SOP 10<br>ohnia thoms | (2022)<br>oni (Water fi                              | lea)   |  | Analy<br>Dilue<br>Brine<br>Sour            | yst:<br>ent:<br>e:<br>rce:   | Ecot<br>Hote<br>Not /<br>Field | ox Team<br>o<br>Applicable<br>I Collected |              | Age:             |
| Sample ID: 0<br>Sample Date: 0<br>Receipt Date: 0<br>Sample Age: 2   | 04-6256-93<br>07 May-24<br>07 May-24<br>24h          | 350  | Cod<br>Mate<br>CAS<br>Clie                                     | e:<br>erial:<br>(PC):<br>nt:   | 24.(<br>Zind                 | 003.2 Zn<br>c sulfate<br>irotoxy Rese   | earch  |  |  | Proje<br>Sour<br>Stati                     | ect:<br>rce:<br>on:  | Spec<br>Solut<br>Lab 3         | cial Studies<br>tion made l<br>Solution   | by NIWA      |                  |
| Data Transform   | ı  | Alt I  | Нур  |  |                              |   |  | NOEL   | LO                                     | EL   | TOEL   | -                              | Tox Units                                 | ≶ MSDu       | PMSD             |
| Angular (Correc  | ted)   | C > 1  | Г  |  |                              |   |  | 235  | 505                                    | j  | 344.5  |                                |   | 0.09253      | 9.25%            |
| Bonferroni Adj t Test  |  |  |  |  |                              |   |  |  |  |  |  |                                |   |              |                  |
| Control V  | s Con  | c-µg/L   | Stat   | Critical   | MSD                          | P-Type                                  | P-V  | alue   | Decis                                  | sion(e                                     | a:5%)  |                                |   |              |                  |
| Dilution Water   | 105<br>235<br>505*<br>1055                           | *  | 0<br>0<br>7.106<br>13.67                                       |  | 2.56<br>2.56<br>2.56<br>2.56 | 0.1503<br>0.1503<br>0.1503<br>0.1503    | CDF<br>CDF<br>CDF<br>CDF                             | 1.0000         Non-Significant Effect           1.0000         Non-Significant Effect           2.5E-05         Significant Effect           1.0000         Significant Effect |  |  |  |                                | t<br>t                                    |              |                  |
|  | 1055   |  |  | 15.01  |                              | 2.50                                    | 0.1505   | 001  | - 1.                                   | 02-05                                      | olgrin   | Ican                           | Lincor                                    |              |                  |
| ANOVA Table  |  |  |  |  |                              |   |  |  | _                                      |  |  |                                |   |              |                  |
| Source   | Sum  | Squares  |  | Mean   | Squ                          | are                                     | DF   | F Stat   | P-V                                    | alue                                       | Decis  | sion(                          | a:5%)                                     |              |                  |
| Between  | 1.66   | 046<br>75609   |  | 0.416  | 616<br>4634                  |   | 4<br>12  | 64.46  | <1.                                    | 0E-05                                      | Signit   | icant                          | Effect                                    |              |                  |
| Total  | 1.74   | 402  |  | 0.000  | 1001                         |   | 16   | _  |  |  |  |                                |   |              |                  |
| ANOVA Assum  | ptions Te  | sts  |  |  |                              |   |  |  |  |  |  |                                |   |              |                  |
| Attribute  | Test   |  |  |  |                              |   | Test Stat  | Critical   | P-V                                    | alue                                       | Decis  | sion(                          | a:1%)                                     |              |                  |
| Variance<br>Distribution   | Barti<br>Leve<br>Mod<br>Ande<br>D'Ag<br>Kolm<br>Shap | ett Equality<br>ne Equality<br>Levene Equ<br>erson-Darlin<br>ostino Skew<br>ogorov-Sm<br>piro-Wilk W | of Va<br>of Va<br>Jality (<br>g A2<br>vness<br>irnov l<br>Norm | riance 1<br>riance 1<br>of Varia<br>Test<br>Test<br>D Test<br>ality Te | Test<br>Test<br>nce<br>st    | Test                                    | 5.924<br>22.1<br>2.777<br>0.9691<br>0.3824<br>0.7463 | 5.412<br>7.847<br>3.878<br>2.576<br>0.2405<br>0.848  | 0.0<br>0.0<br><1.<br>0.3<br><1.<br>0.0 | 072<br>005<br>0E-05<br>325<br>0E-05<br>004 | Indeterminate<br>Unequal Variances<br>Unequal Variances<br>Non-Normal Distribution<br>Normal Distribution<br>Non-Normal Distribution |                                |   |              |                  |
| 48h Survival R   | ate Summ   | arv  |  |  |                              |   |  |  |  |  |  |                                |   |              |                  |
| Conc-µg/L  | Code   | e Cou  | nt   | Mean   |                              | 95% LCL                                 | 95% UCL  | Median   | Min                                    |  | Мах  |                                | Std Err                                   | CV%          | %Effect          |
| Conc-µg/L<br>4.35  | Code<br>D  | e Cou<br>5   | nt   | Mean<br>1.000  | 0                            | 95% LCL<br>1.0000                       | 95% UCL<br>1.0000                                    | Median<br>1.0000   | Mir<br>1.0                             | )<br>000                                   | Max<br>1.000   | 0                              | Std Err<br>0.0000                         | CV%<br>0.00% | %Effect<br>0.00% |
| 105  |  | 3  |  | 1.000  | 0                            | 1.0000                                  | 1.0000   | 1.0000   | 1.0                                    | 000  | 1.000  | 0                              | 0.0000                                    | 0.00%        | 0.00%            |
| 235  |  | 3  |  | 1.000  | 0                            | 1.0000                                  | 1.0000   | 1.0000   | 1.0                                    | 000  | 1.000  | 0                              | 0.0000                                    | 0.00%        | 0.00%            |
| 1055   |  | 3  |  | 0.333  | 3                            | 0.4510                                  | 0.9464   | 0.7000   | 0.0                                    | 000  | 0.600  | 0                              | 0.0377                                    | 45.83%       | 50.00%<br>66.67% |
| 2400   |  | 3  |  | 0.000  | 0                            | 0.0000                                  | 0.0000   | 0.0000   | 0.0                                    | 000  | 0.000  | 0                              | 0.0000                                    |              | 100.00%          |
| Angular (Corrected) Transformed Summary  |  |  |  |  |                              |   |  |  |  |  |  |                                |   |              |                  |
| Conc-µg/L Code Count Mean 95% L(   |  |  |  |  |                              |   | 95% UCL  | Median   | Mir                                    | 1  | Мах  |                                | Std Err                                   | CV%          | %Effect          |
| 4.35   | D  | 5  |  | 1.412  | 0                            | 1.4120                                  | 1.4120   | 1.4120   | 1.4                                    | 120  | 1.412  | 0                              | 0.0000                                    | 0.00%        | 0.00%            |
| 105  |  | 3  |  | 1.412  | 0                            | 1.4110                                  | 1.4130   | 1.4120   | 1.4                                    | 120  | 1.412  | 0                              | 0.0000                                    | 0.00%        | 0.00%            |
| 235  |  | 3  |  | 1.412  | 0                            | 1.4110                                  | 1.4130   | 1.4120   | 1.4                                    | 120  | 1.412  | 0                              | 0.0000                                    | 0.00%        | 0.00%            |
| 505<br>1055  | 0.7201   | 1.2090   | 0.9912   | 0.8  | 501<br>626                   | 1.10/                                   | 0  | 0.0638   | 11.12%<br>26.72%                       | 29.55%                                     |  |                                |   |              |                  |
| 305         3         0.000         0.12           1055         3         0.6096         0.20           2400         3         0.1588         0.15 |  |  |  |  |                              |   | 0.1588   | 0.1588   | 0.4                                    | 588  | 0.785  | 8                              | 0.0000                                    | 0.00%        | 88.76%           |

Convergent Rounding (4 sf)

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| CETIS And                               | alytical Repo                   | ort              |   |  |                                  |                        | Report Date:<br>Test Code/ID:                 | 27 May-24 14:26 (p 2 of 2)<br>24.003.2 Zn / 18-3585-2031 |
|---|---------------------------------|------------------|---|--|----------------------------------|------------------------|---|--|
| Daphnia thor                            | nsoni 48-h Acute                | e Survival       | Test                                    |  |                                  |                        |   | NIWA Ecotoxicology                                       |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 05-6314-4525<br>27 May-24 14:20 | En<br>6 An<br>MD | dpoint: 48<br>alysis: Pa<br>95 Hash: 8C | h Survival R<br>rametric-Mu<br>B123CEE58 | ate<br>Iltiple Comp<br>301E9A4A6 | arison<br>03D8DCEB3724 | CETIS Version:<br>Status Level:<br>Editor ID: | CETISv2.1.4<br>1   |
| 48h Survival                            | Rate Detail                     |                  |   |  |                                  |                        |   |  |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                   | Rep 3                                    | Rep 4                            | Rep 5                  |   |  |
| 4.35                                    | D                               | 1.0000           | 1.0000                                  | 1.0000                                   | 1.0000                           | 1.0000                 |   |  |
| 105                                     |                                 | 1.0000           | 1.0000                                  | 1.0000                                   |                                  |                        |   |  |
| 235                                     |                                 | 1.0000           | 1.0000                                  | 1.0000                                   |                                  |                        |   |  |
| 1055                                    |                                 | 0.6000           | 0.7000                                  | 0.8000                                   |                                  |                        |   |  |
| 2400                                    |                                 | 0.2000           | 0.0000                                  | 0.0000                                   |                                  |                        |   |  |
| Angular (Cor                            | rected) Transfor                | med Detai        | 1                                       |  |                                  |                        |   |  |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                   | Rep 3                                    | Rep 4                            | Rep 5                  |   |  |
| 4.35                                    | D                               | 1.4120           | 1.4120                                  | 1.4120                                   | 1.4120                           | 1.4120                 |   |  |
| 105                                     |                                 | 1.4120           | 1.4120                                  | 1.4120                                   |                                  |                        |   |  |
| 235                                     |                                 | 1.4120           | 1.4120                                  | 1.4120                                   |                                  |                        |   |  |
| 505                                     |                                 | 0.8861           | 0.9912                                  | 1.1070                                   |                                  |                        |   |  |
| 2400                                    |                                 | 0.4636           | 0.5796                                  | 0.7854                                   |                                  |                        |   |  |
| A0h Curuinal                            | Data Dinamiala                  |                  |   |  |                                  |                        |   |  |
| 4011 Survival                           | Code                            | Dop 1            | Dop 2                                   | Dop 2                                    | Bop 4                            | Dop 5                  |   |  |
| / 35                                    | Code                            | 10/10            | 10/10                                   | 10/10                                    | 10/10                            | 10/10                  |   |  |
| 105                                     | U                               | 10/10            | 10/10                                   | 10/10                                    | 10/10                            | 10/10                  |   |  |
| 235                                     |                                 | 10/10            | 10/10                                   | 10/10                                    |                                  |                        |   |  |
| 505                                     |                                 | 6/10             | 7/10                                    | 8/10                                     |                                  |                        |   |  |
| 1055                                    |                                 | 2/10             | 3/10                                    | 5/10                                     |                                  |                        |   |  |
| 2400                                    |                                 | 0/10             | 0/10                                    | 0/10                                     |                                  |                        |   |  |
| Graphics                                |                                 |                  |   |  |                                  |                        |   |  |
| 1.0 -                                   |                                 | -                |   |  |                                  |                        |   | ٠  |
| 0.9 -                                   |                                 |                  |   |  | _ ▼                              | 0.15 -                 |   |  |
| 0.8 -                                   |                                 |                  |   |  |                                  | 0.10 -                 |   | •  |
| <b>2</b> 0.7 –                          |                                 |                  |   |  |                                  |                        |   |  |
| <b>₩</b> 0.6 -                          |                                 | I                |   |  | -                                | 0.05 -                 |   |  |
| ≥ 0.5 –                                 |                                 |                  |   |  |                                  | <b>Z</b>               |   |  |
| 0.4 -                                   |                                 |                  |   |  |                                  | 5                      |   |  |
| <b>9</b> 0.3 -                          |                                 |                  |   |  |                                  | -0.05 -                |   |  |
| 0.2 -                                   |                                 |                  |   |  |                                  |                        |   |  |
| 0.1 -                                   |                                 |                  |   |  |                                  | -0.10 -                | •   |  |
| 0.0 -                                   |                                 |                  | _,,                                     |  | -                                | -0.15 -                |   |  |
|   | 4.35 D 105                      | 235              | 505 10                                  | 55 2400                                  |                                  | -1.                    | 5 -1.0 -0.5                                   | 0.0 0.5 1.0 1.5  |
|   |                                 | Conc-µ           | ig/L                                    |  |                                  |                        | R   | ankits   |
|   |                                 |                  |   |  |                                  |                        |   |  |
|   |                                 |                  |   |  |                                  |                        |   |  |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS                                     | Analy                                 | ytic                             | al Repo                   | ort     |                              |                                       |                        |   |                                 |                 | R    | leport l<br>est Co               | Date:<br>de/ID:          |                                  | 27 I<br>24.0                            | May-24 14:2<br>03.2 Zn / 18 | 7 (p 1 of 3)<br>-3585-2031 |
|---|---------------------------------------|----------------------------------|---------------------------|---------|------------------------------|---------------------------------------|------------------------|---|---------------------------------|-----------------|------|----------------------------------|--------------------------|----------------------------------|---|-----------------------------|----------------------------|
| Daphnia                                   | a thoms                               | oni 4                            | 48-h Acute                | Survi   | ival T                       | est                                   |                        |   |                                 |                 |      |                                  |                          |                                  |   | NIWA Ecot                   | oxicology                  |
| Analysi<br>Analyze<br>Edit Da             | s ID: 0<br>ed: 2<br>te:               | )5-74<br>27 Ma                   | 11-6460<br>ay-24 14:26    | i       | Endj<br>Anal<br>MD5          | point: 4<br>ysis: 1<br>Hash: 8        | 48h S<br>Nonli<br>BCB1 | Survival Ra<br>inear Regre<br>123CEE58( | ite<br>ession (NLF<br>01E9A4A60 | R)<br>D3D8DCEB3 | 72A  | CETIS<br>Statu<br>Edito          | SVers<br>sLeve<br>rID:   | ion:<br>el:                      | CETISv2<br>1                            | 2.1.4                       |                            |
| Batch II<br>Start Da<br>Ending<br>Test Le | D: 2<br>ate: 0<br>Date: 1<br>ngth: 4  | 20-40<br>)8 Ma<br>10 Ma<br>18h   | 51-4804<br>ay-24<br>ay-24 |         | Test<br>Prot<br>Spec<br>Taxo | Type: \$<br>ocol: 1<br>cies: [<br>on: | Survi<br>NIW/<br>Daph  | ival (48h)<br>A SOP 10 (<br>nnia thomso | (2022)<br>oni (Water f          | flea)           |      | Analy<br>Dilue<br>Brine<br>Sourc | vst:<br>nt:<br>::<br>ce: | Ecoto<br>Hoteo<br>Not A<br>Field | ox Team<br>o<br>Applicable<br>Collected |                             | Age:                       |
| Sample<br>Sample<br>Receipt<br>Sample     | ID: 0<br>Date: 0<br>Date: 0<br>Age: 2 | )4-62<br>)7 Ma<br>)7 Ma<br>)7 Ma | 56-9350<br>ay-24<br>ay-24 |         | Code<br>Mate<br>CAS<br>Clier | e: 2<br>erial: 2<br>(PC):<br>nt: H    | 24.00<br>Zinc<br>Hydro | 03.2 Zn<br>sulfate<br>otoxy Rese        | arch                            |                 |      | Proje<br>Soure<br>Static         | ct:<br>ce:<br>on:        | Spec<br>Solut<br>Lab S           | ial Studies<br>ion made I<br>Solution   | by NIWA                     |                            |
| Non-Lir                                   | iear Reg                              | gress                            | sion Option               | ns      |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Model N                                   | Name an                               | nd Fu                            | Inction                   |         |                              |                                       |                        |   | Weightin                        | g Function      |      |                                  | PTBS                     | 5 Fun                            | ction                                   | X Trans                     | Y Trans                    |
| 3P Log-                                   | Logistic:                             | : μ=α                            | /[1+[x/δ]^γ]              |         |                              |                                       |                        |   | Binomial [                      | [ω=n/[p·q]]     |      |                                  | Off [µ                   | ι*=μ]                            |   | None                        | None                       |
| Regres                                    | sion Su                               | mma                              | ry                        |         |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Iters                                     | LL                                    |                                  | AICc                      | BIC     |                              | Adj R2                                | 2                      | PMSD                                    | Thresh                          | Optimize        | FS   | tat                              | P-Va                     | lue                              | Decision                                | (α:5%)                      |                            |
| 8   | -12.01                                |                                  | 31.51                     | 33      |                              | 0.9001                                |                        | 0.63%                                   | 0.9987                          | Yes             | 4.81 | 16                               | 0.016                    | 66                               | Significan                              | t Lack-of-Fit               |                            |
| Point E                                   | stimates                              | s                                |                           |         |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Level                                     | µg/L                                  |                                  | 95% LCL                   | 95%     | UCL                          |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| LC5                                       | 295.4                                 |                                  | 188.7                     | 357.1   | 1                            |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| LC10                                      | 374.3                                 |                                  | 278.7                     | 441.9   | 9                            |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| LC15                                      | 433.4                                 |                                  | 343.4                     | 504.6   | 6                            |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| LC20                                      | 483.9                                 |                                  | 397.5                     | 558     |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| LC25                                      | 530.1                                 |                                  | 445.9                     | 606.9   | 9                            |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| LC40                                      | 660.3                                 |                                  | 576.1                     | 748.4   | 4                            |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| LC50                                      | 750.7                                 |                                  | 661.1                     | 852.5   | 5                            |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Regres                                    | sion Par                              | rame                             | ters                      |         |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Parame                                    | ter                                   |                                  | Estimate                  | Std E   | Error                        | 95% LO                                | CL                     | 95% UCL                                 | t Stat                          | P-Value         | Dec  | ision(                           | a:5%)                    |                                  |   |                             |                            |
| α   |                                       |                                  | 0.9987                    | 0.002   | 2998                         | 0.9924                                |                        | 1.005                                   | 333.1                           | <1.0E-05        | Sigr | nificant                         | Paran                    | neter                            |   |                             |                            |
| Y   |                                       |                                  | 3.157                     | 0.35    | 54                           | 2.407                                 |                        | 3.907                                   | 8.883                           | <1.0E-05        | Sigr | nificant                         | Paran                    | neter                            |   |                             |                            |
| ō   |                                       |                                  | 750.7                     | 44.71   | 7                            | 656.3                                 |                        | 845.2                                   | 16.77                           | <1.0E-05        | Sigr | nificant                         | Paran                    | neter                            |   |                             |                            |
| ANOVA                                     | Table                                 |                                  |                           |         |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Source                                    |                                       |                                  | Sum Squa                  | res     | Mea                          | n Square                              | е                      | DF                                      | F Stat                          | P-Value         | Dec  | ision(                           | a:5%)                    |                                  |   |                             |                            |
| Model                                     |                                       |                                  | 50060                     |         | 1669                         | 0                                     |                        | 3                                       | 38650                           | <1.0E-05        | Sig  | nificant                         | Effect                   |                                  |   |                             |                            |
| Lack of                                   | Fit                                   |                                  | 3.728                     |         | 1.24                         | 3                                     |                        | 3                                       | 4.816                           | 0.0166          | Sigr | nificant                         | Lack-                    | of-Fit                           |   |                             |                            |
| Pure En                                   | ror                                   |                                  | 3.612                     |         | 0.25                         | в                                     |                        | 14                                      |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Residua                                   | il                                    |                                  | 7.34                      |         | 0.43                         | 18                                    |                        | 17                                      |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Residua                                   | al Analy                              | sis                              |                           |         |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
| Attribut                                  | е                                     |                                  | Method                    |         |                              |                                       |                        | Test Stat                               | Critical                        | P-Value         | Dec  | ision(                           | a:5%)                    |                                  |   |                             |                            |
| Model F                                   | it                                    |                                  | Likelihood                | Ratio   | GOF                          | Test                                  |                        | 8.369                                   | 27.59                           | 0.9580          | Nor  | n-Signif                         | icant H                  | letero                           | geneity                                 |                             |                            |
|   |                                       |                                  | Pearson C                 | hi-Sq   | GOF '                        | Test                                  |                        | 7.34                                    | 27.59                           | 0.9787          | Nor  | n-Signif                         | icant H                  | letero                           | geneity                                 |                             |                            |
| Variance                                  | e                                     |                                  | Mod Leven                 | ie Equ  | ality o                      | of Varian                             | се                     | 15.46                                   | 3.687                           | 0.0006          | Une  | equal V                          | ariance                  | es                               |   |                             |                            |
| Distribut                                 | tion                                  |                                  | Anderson-I                | Darling | g A2 T                       | est                                   |                        | 1.153                                   | 2.492                           | 0.0052          | Nor  | n-Norma                          | al Dist                  | ributio                          | n                                       |                             |                            |
|   |                                       |                                  | Shapiro-W                 | ilk W I | Norma                        | ality Test                            |                        | 0.886                                   | 0.9044                          | 0.0227          | Nor  | n-Norma                          | al Dist                  | ributio                          | n                                       |                             |                            |
| Overdis                                   | persion                               |                                  | Tarone C(d                | a) Ove  | erdispe                      | ersion Te                             | est                    | 0.8812                                  | 1.645                           | 0.1891          | Nor  | n-Signif                         | icant C                  | verdi                            | spersion                                |                             |                            |
|   |                                       |                                  |                           |         |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |
|   |                                       |                                  |                           |         |                              |                                       |                        |   |                                 |                 |      |                                  |                          |                                  |   |                             |                            |

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| CETIS An                                  | alytical Repo                   | ort  |  |  | Repor<br>Test C  | t Date:<br>ode/ID:                                       | 27<br>24.0   | May-24 14:2<br>003.2 Zn / 18                             | 7 (p 2 of 3)<br>-3585-2031                  |  |   |
|---|---------------------------------|--|--|--|--|--|--|--|---|--|---|
| Daphnia tho                               | msoni 48-h Acute                | Survival   | Test   |  |  |  |  | NIWA Ecot  | oxicology                                   |  |   |
| Analysis ID:<br>Analyzed:<br>Edit Date:   | 05-7411-6460<br>27 May-24 14:26 | En<br>6 An<br>MC   | dpoint: 48<br>alysis: No<br>05 Hash: 80                  | h Survival R<br>onlinear Reg<br>B123CEE58                | ate<br>ression (NL<br>801E9A4A6                          | R)<br>03D8DCEB   | CE<br>Sta<br>372A Edi                                    | FIS Version:<br>tus Level:<br>tor ID:                    | CETISV2<br>1                                | 2.1.4  |   |
| 48h Survival                              | Rate Summary                    |  |  |  |  | Calculate  | d Variate(A  | /B)  |   |  |   |
| Conc-µg/L                                 | Code                            | Count  | Mean   | Median   | Min  | Мах  | Std Err  | Std Dev  | CV%   | %Effect  | ΣΑ/ΣΒ   |
| 4.35<br>105<br>235<br>505<br>1055<br>2400 | D                               | 5<br>3<br>3<br>3<br>3<br>3<br>3                          | 1.0000<br>1.0000<br>1.0000<br>0.7000<br>0.3333<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>0.7000<br>0.3000<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>0.6000<br>0.2000<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>0.8000<br>0.5000<br>0.0000 | 0.0000<br>0.0000<br>0.0000<br>0.0577<br>0.0882<br>0.0000 | 0.0000<br>0.0000<br>0.0000<br>0.1000<br>0.1528<br>0.0000 | 0.00%<br>0.00%<br>0.00%<br>14.29%<br>45.83% | 0.00%<br>0.00%<br>0.00%<br>30.00%<br>66.67%<br>100.00% | 50/50<br>30/30<br>30/30<br>21/30<br>10/30<br>0/30 |
| 48h Survival                              | Rate Detail                     |  |  |  |  |  |  |  |   |  |   |
| Conc-µg/L                                 | Code                            | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |  |  |   |  |   |
| 4.35<br>105<br>235<br>505<br>1055<br>2400 | D                               | 1.0000<br>1.0000<br>1.0000<br>0.6000<br>0.2000<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>0.7000<br>0.3000<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>0.8000<br>0.5000<br>0.0000 | 1.0000   | 1.0000   |  |  |   |  |   |
| 48h Survival                              | Rate Binomials                  |  |  |  |  |  |  |  |   |  |   |
| Conc-µg/L                                 | Code                            | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |  |  |   |  |   |
| 4.35<br>105<br>235<br>505<br>1055<br>2400 | D                               | 10/10<br>10/10<br>10/10<br>6/10<br>2/10<br>0/10          | 10/10<br>10/10<br>10/10<br>7/10<br>3/10<br>0/10          | 10/10<br>10/10<br>10/10<br>8/10<br>5/10<br>0/10          | 10/10  | 10/10  |  |  |   |  |   |

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# Copper

| CETIS Analytical Report Daphnia thomsoni 48-h Acute Survival Test |              |                          |         |                    |                          |                    |   |                                 | R                  | leport<br>est Co | Date:<br>de/ID:        |                                 | 27 M<br>24.00 | lay-24 14:1<br>3.2 Cu / 11 | 5 (p 1 of 2)<br>-1449-0779 |         |
|---|--------------|--------------------------|---------|--------------------|--------------------------|--------------------|---|---------------------------------|--------------------|------------------|------------------------|---------------------------------|---------------|----------------------------|----------------------------|---------|
| Daphnia thon  | nsoni        | 48-h Acute               | Surv    | ival T             | est                      |                    |   |                                 |                    |                  |                        | l                               | NIWA Eco      | toxicology                 |                            |         |
| Analysis ID:<br>Analyzed:<br>Edit Date:                           | 02-8<br>27 N | 623-2987<br>1ay-24 14:14 | ļ       | End<br>Anal<br>MD5 | point:<br>ysis:<br>Hash: | 48h<br>Para<br>754 | Survival Ra<br>ametric-Mul<br>0DE25745E | ite<br>tiple Compa<br>A65EB54EI | rison<br>BC125F9B5 | 5243             | CETI<br>Statu<br>Edito | S Versio<br>Is Level:<br>or ID: | on:<br>:      | CETISv2.<br>1              | 1.4                        |         |
| Batch ID:   | 19-5         | 192-3864                 |         | Test               | Type:                    | Sun                | vival (48h)                             |                                 |                    |                  | Analy                  | yst: E                          | Ecoto         | ox Team                    |                            |         |
| Start Date:   | 04 N         | lay-24                   |         | Prot               | ocol:                    | NIW                | A SOP 10                                | (2022)                          |                    |                  | Dilue                  | ent: H                          | lote          | 0                          |                            |         |
| Ending Date:  | 06 N         | lay-24                   |         | Spe                | cies:                    | Dap                | hnia thoms                              | oni (Water fl                   | lea)               |                  | Brine                  | e: N                            | Not A         | Applicable                 |                            | Ago:    |
| rest Lengui.  | 4011         |                          |         | Tax                | л.                       |                    |   |                                 |                    |                  | Sour                   | ce. r                           | leiu          | Collected                  |                            | Age.    |
| Sample ID:  | 03-6         | 699-5858                 |         | Cod                | e:<br>vriete             | 24.0               | 03.2 Cu                                 |                                 |                    |                  | Proje                  | ect: S                          | Spec          | ial Studies                |                            |         |
| Receipt Date:   | 03 N         | 1ay-24<br>1ay-24         |         | CAS                | (DC)                     | Zind               | sunate                                  |                                 |                    |                  | Stati                  | ce: a<br>on: l                  | ah 9          | Solution                   | y NIWA                     |         |
| Sample Age:   | 24h          | iay 24                   |         | Clier              | nt:                      | Hyd                | rotoxy Rese                             | earch                           |                    |                  | Jun                    |                                 |               | Johanon                    |                            |         |
| Data Transfor   | m            |                          | Alt I   | Нур                |                          |                    |   |                                 | NOEL               | LO               | EL                     | TOEL                            |               | Tox Units                  | MSDu                       | PMSD    |
| Angular (Corre  | ected)       |                          | C > 1   | T.                 |                          |                    |   |                                 | 151                | 470              |                        | 266.4                           |               |                            | 0.05224                    | 5.22%   |
| Bonferroni A  | ijtTe        | est                      |         |                    |                          |                    |   |                                 |                    |                  |                        |                                 |               |                            |                            |         |
| Control   | vs           | Conc-µg/L                | _       | df                 | Test S                   | stat               | Critical                                | MSD                             | P-Type             | P-V              | alue                   | Decisi                          | on(o          | 1:5%)                      |                            |         |
| Dilution Water  |              | 24.5                     |         | 6                  | 0                        |                    | 2.56                                    | 0.07182                         | CDF                | 1.00             | 000                    | Non-Si                          | gnifi         | cant Effect                |                            |         |
|   |              | 53                       |         | 6                  | 0                        |                    | 2.56                                    | 0.07182                         | CDF                | 1.00             | 000                    | Non-Si                          | gnifi         | cant Effect                |                            |         |
|   |              | 151                      |         | 6                  | 0                        |                    | 2.56                                    | 0.07182                         | CDF                | 1.00             | 000                    | Non-Si<br>Signific              | gnifi         | cant Effect                |                            |         |
|   |              | 470                      |         | 0                  | 42.14                    |                    | 2.30                                    | 0.07102                         | CDI                | 51.4             | JE-03                  | Signing                         | am            | Lileu                      |                            |         |
| ANOVA Table   |              | Sum Saua                 | ree     |                    | Mean                     | 6 au               |   | DE                              | E Stat             | D V              | alua                   | Desisi                          |               |                            |                            |         |
| Source  |              | 3 5512                   | ires    |                    | Mean                     | Squ                | are                                     |                                 | F Stat             | P-V              |                        | Decisi                          | on(c          | 1:5%)<br>Effect            |                            |         |
| Error   |              | 0.0177062                |         |                    | 0.0014                   | ,<br>1755          |   | 4<br>12                         | 001.7              | \$1.0            | JE-03                  | Signing                         | am            | Ellect                     |                            |         |
| Total   |              | 3.56891                  |         |                    |                          |                    |   | 16                              | -                  |                  |                        |                                 |               |                            |                            |         |
| ANOVA Assu  | mptic        | ons Tests                |         |                    |                          |                    |   |                                 |                    |                  |                        |                                 |               |                            |                            |         |
| Attribute   |              | Test                     |         |                    |                          |                    |   | Test Stat                       | Critical           | P-V              | alue                   | Decisi                          | on(o          | 1:1%)                      |                            |         |
| Variance  |              | Bartlett Eq              | uality  | of Vai             | riance T                 | est                |   |                                 |                    |                  |                        | Indeter                         | min           | ate                        |                            |         |
|   |              | Levene Eq                | uality  | of Vai             | riance T                 | est                | Tost                                    | 19.76                           | 5.412              | 3.3              | E-05                   | Unequa                          | al Va         | ariances                   |                            |         |
| Distribution  |              | Anderson-I               | Darlin  | n A2 1             | Fest                     | ice                | lest                                    | 4.306                           | 3.878              | <1.0             | DE-05                  | Non-No                          | orma          | ances<br>al Distributio    | n                          |         |
|   |              | D'Agostino               | Skew    | /ness              | Test                     |                    |   | 3.014                           | 2.576              | 0.00             | 026                    | Non-No                          | orma          | al Distributio             | n                          |         |
|   |              | Kolmogoro                | v-Smi   | irnov [            | D Test                   |                    |   | 0.4412                          | 0.2405             | <1.(             | DE-05                  | Non-No                          | orma          | al Distributio             | n                          |         |
|   |              | Shapiro-W                | ilk W I | Norma              | ality Tes                | st                 |   | 0.5509                          | 0.848              | <1.(             | DE-05                  | Non-No                          | orma          | al Distributio             | n                          |         |
| 48h Survival  | Rate         | Summary                  |         |                    |                          |                    |   |                                 |                    |                  |                        |                                 |               |                            |                            |         |
| Conc-µg/L   |              | Code                     | Cou     | nt                 | Mean                     |                    | 95% LCL                                 | 95% UCL                         | Median             | Min              | 1                      | Max                             |               | Std Err                    | CV%                        | %Effect |
| 1.1   |              | D                        | 5       |                    | 1.0000                   | )                  | 1.0000                                  | 1.0000                          | 1.0000             | 1.00             | 000                    | 1.0000                          |               | 0.0000                     | 0.00%                      | 0.00%   |
| 24.5  |              |                          | 3       |                    | 1.0000                   | )                  | 1.0000                                  | 1.0000                          | 1.0000             | 1.00             | 000                    | 1.0000                          |               | 0.0000                     | 0.00%                      | 0.00%   |
| 03<br>151   |              |                          | 3       |                    | 1.0000                   | ,<br>,             | 1.0000                                  | 1.0000                          | 1.0000             | 1.00             | 000                    | 1.0000                          |               | 0.0000                     | 0.00%                      | 0.00%   |
| 470   |              |                          | 3       |                    | 0.0333                   | 3                  | 0.0000                                  | 0.1768                          | 0.0000             | 0.00             | 000                    | 0.1000                          |               | 0.0333                     | 173.21%                    | 96.67%  |
| 1320  |              |                          | 3       |                    | 0.000                    | 0                  | 0.0000                                  | 0.0000                          | 0.0000             | 0.0              | 000                    | 0.0000                          |               | 0.0000                     |                            | 100.00% |
| Angular (Corr   | ected        | d) Transform             | ned S   | umm                | ary                      |                    |   |                                 |                    |                  |                        |                                 |               |                            |                            |         |
| Conc-µg/L   |              | Code                     | Cou     | nt                 | Mean                     |                    | 95% LCL                                 | 95% UCL                         | Median             | Min              |                        | Мах                             |               | Std Err                    | CV%                        | %Effect |
| 1.1   |              | D                        | 5       |                    | 1.4120                   | )                  | 1.4120                                  | 1.4120                          | 1.4120             | 1.41             | 120                    | 1.4120                          |               | 0.0000                     | 0.00%                      | 0.00%   |
| 24.5  |              |                          | 3       |                    | 1.4120                   | )                  | 1.4110                                  | 1.4130                          | 1.4120             | 1.4              | 120                    | 1.4120                          |               | 0.0000                     | 0.00%                      | 0.00%   |
| 53  |              |                          | 3       |                    | 1.4120                   | J                  | 1.4110                                  | 1.4130                          | 1.4120             | 1.4              | 120                    | 1.4120                          |               | 0.0000                     | 0.00%                      | 0.00%   |
| 101   |              |                          | 2       |                    | 1.4120                   | 1                  | -0.0206                                 | 0.4469                          | 0.1500             | 0.4              | 120                    | 1.4120                          |               | 0.0000                     | 1/1 15%                    | 0.00%   |
| 1320  |              |                          | 3       |                    | 0.1588                   | 3                  | 0.1588                                  | 0.1588                          | 0.1588             | 0.1              | 588                    | 0.3218                          |               | 0.0000                     | 0.00%                      | 88.76%  |
|   |              |                          | _       |                    |                          |                    |   |                                 |                    |                  |                        |                                 |               |                            |                            |         |
|   |              |                          |         |                    |                          |                    |   |                                 |                    |                  |                        |                                 |               |                            |                            |         |

Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS And                               | alytical Repo                   | ort            |   |   |                              |                            | Report Date:<br>Test Code/ID:                 | 27 May-24 14:15 (p 2 of 2)<br>24.003.2 Cu / 11-1449-0779 |
|---|---------------------------------|----------------|---|---|------------------------------|----------------------------|---|--|
| Daphnia thor                            | nsoni 48-h Acute                | Survival       | Test                                    |   |                              |                            |   | NIWA Ecotoxicology                                       |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 02-8623-2987<br>27 May-24 14:14 | En<br>An<br>MC | dpoint: 48<br>alysis: Pa<br>)5 Hash: 75 | h Survival Ra<br>arametric-Mu<br>40DE257458 | ate<br>Itiple Com<br>EA65EB5 | nparison<br>4EBC125F9B5243 | CETIS Version:<br>Status Level:<br>Editor ID: | CETISv2.1.4<br>1   |
| 48h Survival                            | Rate Detail                     |                |   |   |                              |                            |   |  |
| Conc-µg/L                               | Code                            | Rep 1          | Rep 2                                   | Rep 3                                       | Rep 4                        | Rep 5                      |   |  |
| 1.1                                     | D                               | 1.0000         | 1.0000                                  | 1.0000                                      | 1.0000                       | 1.0000                     |   |  |
| 24.5                                    |                                 | 1.0000         | 1.0000                                  | 1.0000                                      |                              |                            |   |  |
| 53                                      |                                 | 1.0000         | 1.0000                                  | 1.0000                                      |                              |                            |   |  |
| 151                                     |                                 | 1.0000         | 1.0000                                  | 1.0000                                      |                              |                            |   |  |
| 470                                     |                                 | 0.0000         | 0.0000                                  | 0.1000                                      |                              |                            |   |  |
| 1320                                    |                                 | 0.0000         | 0.0000                                  | 0.0000                                      |                              |                            |   |  |
| Angular (Cor                            | rected) Transforn               | ned Detai      | I                                       |   |                              |                            |   |  |
| Conc-µg/L                               | Code                            | Rep 1          | Rep 2                                   | Rep 3                                       | Rep 4                        | Rep 5                      |   |  |
| 1.1                                     | D                               | 1.4120         | 1.4120                                  | 1.4120                                      | 1.4120                       | 1.4120                     |   |  |
| 24.5                                    |                                 | 1.4120         | 1.4120                                  | 1.4120                                      |                              |                            |   |  |
| 53                                      |                                 | 1.4120         | 1.4120                                  | 1.4120                                      |                              |                            |   |  |
| 151                                     |                                 | 1.4120         | 1.4120                                  | 1.4120                                      |                              |                            |   |  |
| 1320                                    |                                 | 0.1588         | 0.1588                                  | 0.3218                                      |                              |                            |   |  |
| A8h Survival                            | Rate Binomials                  |                |   |   |                              |                            |   |  |
| Conc. ug/l                              | Code                            | Den 4          | Dep 2                                   | Dan 2                                       | Den 4                        | Den F                      |   |  |
| Conc-µg/L                               | Code                            | Kep 1          | Kep 2                                   | Rep 5                                       | Rep 4                        | Kep 5                      |   |  |
| 1.1                                     | D                               | 10/10          | 10/10                                   | 10/10                                       | 10/10                        | 10/10                      |   |  |
| 53                                      |                                 | 10/10          | 10/10                                   | 10/10                                       |                              |                            |   |  |
| 151                                     |                                 | 10/10          | 10/10                                   | 10/10                                       |                              |                            |   |  |
| 470                                     |                                 | 0/10           | 0/10                                    | 1/10  |                              |                            |   |  |
| 1320                                    |                                 | 0/10           | 0/10                                    | 0/10  |                              |                            |   |  |
| Graphics                                |                                 |                |   |   |                              |                            |   |  |
| 1.0 -                                   |                                 | -              | -                                       |   | <b>-</b>                     |                            |   | •  |
| 0.9 -                                   |                                 |                |   |   |                              | 0.10 -                     |   |  |
| 0.8 -                                   |                                 |                |   |   |                              | 0.08 -                     |   |  |
| <b>9</b> 0.7 -                          |                                 |                |   |   |                              | 0.06 -                     |   |  |
| <b>8</b> 06                             |                                 |                |   |   |                              | <u>e</u>                   |   |  |
|   |                                 |                |   |   |                              | 6 0.04 -                   |   |  |
| 2 0.5                                   |                                 |                |   |   |                              | <b>E</b> 0.02 -            |   |  |
| S 0.4 -                                 |                                 |                |   |   |                              | Ö                          |   |  |
| <b>¥</b> 0.3-                           |                                 |                |   |   |                              |                            |   |  |
| 0.2 -                                   |                                 |                |   |   |                              | -0.02 -                    |   |  |
| 0.1 -                                   |                                 |                |   |   |                              | -0.04 -                    |   |  |
| 0.0 -                                   |                                 |                |   |   |                              |                            | •   |  |
|   | 1.1 D 24.5                      | 53             | 151 4                                   | 70 1320                                     |                              | -1.                        | .5 -1.0 -0.5                                  | 0.0 0.5 1.0 1.5  |
|   |                                 | Conc-          | ug/L                                    |   |                              |                            | R   | ankits   |
|   |                                 |                |   |   |                              |                            |   |  |
|   |                                 |                |   |   |                              |                            |   |  |
|   |                                 |                |   |   |                              |                            |   |  |
|   |                                 |                |   |   |                              |                            |   |  |
|   |                                 |                |   |   |                              |                            |   |  |
|   |                                 |                |   |   |                              |                            |   |  |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS                         | S Ana                 | lytical Repo                    | ort                |                                    |   |                                 |                | F       | Report<br>Test Co      | Date:<br>de/ID:                  | 2<br>24       | 27 May-24 14<br>4.003.2 Cu / | :15 (p 1 of 2)<br>11-1449-0779 |
|-------------------------------|-----------------------|---------------------------------|--------------------|------------------------------------|---|---------------------------------|----------------|---------|------------------------|----------------------------------|---------------|------------------------------|--------------------------------|
| Daphni                        | a thom                | soni 48-h Acute                 | e Survival T       | est                                |   |                                 |                |         |                        |                                  |               | NIWA Ec                      | otoxicology                    |
| Analysi<br>Analyze<br>Edit Da | is ID:<br>ed:<br>ite: | 01-8576-5485<br>27 May-24 14:14 | End<br>4 Ana<br>MD | lpoint: 4<br>lysis: 1<br>5 Hash: 7 | 48h Survival Ra<br>Linear Interpola<br>7540DE25745E | ate<br>Ition (ICPII<br>EA65EB54 | N)<br>EBC125F9 | B5243   | CETI<br>Statu<br>Edito | S Version<br>Is Level:<br>Or ID: | n: CETIS<br>1 | Sv2.1.4                      |                                |
| Batch I                       | D:                    | 19-5192-3864                    | Tes                | t Type: 🖇                          | Survival (48h)                                      |                                 |                |         | Anal                   | yst: E                           | cotox Team    | ı                            |                                |
| Start D                       | ate:                  | 04 May-24                       | Pro                | tocol: 1                           | NIWA SOP 10   | (2022)                          |                |         | Dilue                  | ent: He                          | oteo          |                              |                                |
| Ending                        | Date:                 | 06 May-24                       | Spe                | cies: [                            | Daphnia thoms                                       | oni (Water                      | flea)          |         | Brine                  | e: No                            | ot Applicab   | le                           |                                |
| Test Le                       | ength:                | 48n                             | Tax                | on:                                |   |                                 |                |         | Sour                   | ce: FI                           | eld Collecte  | ed                           | Age:                           |
| Sample                        | D:                    | 03-6699-5858                    | Cod                | le: 2                              | 24.003.2 Cu   |                                 |                |         | Proje                  | ect: S                           | pecial Stud   | ies                          |                                |
| Sample                        | e Date:               | 03 May-24                       | Mat                | erial: 2                           | Zinc sulfate  |                                 |                |         | Sour                   | ce: So                           | olution mad   | le by NIWA                   |                                |
| Receip                        | t Date:               | 03 May-24                       | CAS                | 6 (PC):                            | kudastau Daas                                       |                                 |                |         | Stati                  | on: La                           | ab Solution   |                              |                                |
| Sample                        | e Age:                | 240                             | Cile               | nu: r                              | Hydroloxy Rese                                      | earch                           |                |         |                        |                                  |               |                              |                                |
| Linear                        | Interpo               | lation Options                  |                    |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| X Trans                       | sform                 | Y Transform                     | n See              | d                                  | Resamples   | Exp 95                          | %CL Me         | thod    |                        |                                  |               |                              |                                |
| Log(X+                        | 1)                    | Linear                          | 114                | 1069                               | 200   | Yes                             | Tw             | o-Point | Interp                 | olation                          |               |                              |                                |
| Point E                       | stimate               | s                               |                    |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| Level                         | µg/L                  | 95% LCL                         | 95% UCL            |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| LC15                          | 180.2                 | 177.9                           | 182.6              |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| LC20                          | 191.1                 | 187.9                           | 194.5              |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| LC25                          | 202.6                 | 198.5                           | 207.1              |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| LC40                          | 241.7                 | 233.8                           | 250.3              |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| LCSU                          | 2/1.0                 | 200.8                           | 263.9              |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| 48h Su                        | rvival H              | late Summary                    |                    |                                    |   | Calculate                       | ed Variate(    | A/B)    |                        |                                  | —             | Isoto                        | nic Variate                    |
| Conc-µ                        | ig/L                  | Code                            | Count              | Mean                               | Median  | Min                             | Max            | CV      | %                      | %Effect                          | ΣΑ/ΣΒ         | Mean                         | %Effect                        |
| 1.1                           |                       | D                               | 5                  | 1.0000                             | 1.0000  | 1.0000                          | 1.0000         | 0.0     | 0%                     | 0.00%                            | 50/50         | 1.0000                       | 0.00%                          |
| 24.5<br>53                    |                       |                                 | 3                  | 1.0000                             | 1.0000  | 1.0000                          | 1.0000         | 0.0     | 0%                     | 0.00%                            | 30/30         | 1.0000                       | 0.00%                          |
| 151                           |                       |                                 | 3                  | 1.0000                             | 1.0000  | 1.0000                          | 1.0000         | 0.0     | 0%                     | 0.00%                            | 30/30         | 1.0000                       | 0.00%                          |
| 470                           |                       |                                 | 3                  | 0.0333                             | 0.0000  | 0.0000                          | 0.1000         | 173     | 3.21%                  | 96.67%                           | 1/30          | 0.0333                       | 96.67%                         |
| 1320                          |                       |                                 | 3                  | 0.0000                             | 0.0000  | 0.0000                          | 0.0000         |         |                        | 100.009                          | 6 0/30        | 0.0000                       | 100.00%                        |
| 48h Su                        | rvival R              | ate Detail                      |                    |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| Conc-µ                        | ig/L                  | Code                            | Rep 1              | Rep 2                              | Rep 3   | Rep 4                           | Rep 5          |         |                        |                                  |               |                              |                                |
| 1.1                           |                       | D                               | 1.0000             | 1.0000                             | 1.0000  | 1.0000                          | 1.0000         |         |                        |                                  |               |                              |                                |
| 24.5                          |                       |                                 | 1.0000             | 1.0000                             | 1.0000  |                                 |                |         |                        |                                  |               |                              |                                |
| 53                            |                       |                                 | 1.0000             | 1.0000                             | 1.0000  |                                 |                |         |                        |                                  |               |                              |                                |
| 151                           |                       |                                 | 1.0000             | 1.0000                             | 1.0000  |                                 |                |         |                        |                                  |               |                              |                                |
| 470                           |                       |                                 | 0.0000             | 0.0000                             | 0.1000  |                                 |                |         |                        |                                  |               |                              |                                |
| 102.0                         | and the late          | te Dinemiele                    | 0.0000             | 0.0000                             | 0.0000  |                                 |                |         |                        |                                  |               |                              |                                |
| 48n Su                        | rvival H              | ate Binomials                   |                    |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
| Conc-µ                        | Ig/L                  | Code                            | Rep 1              | 10/10                              | 10/10   | Kep 4                           | 10/10          |         |                        |                                  |               |                              |                                |
| 24.5                          |                       | U                               | 10/10              | 10/10                              | 10/10   | 10/10                           | 10/10          |         |                        |                                  |               |                              |                                |
| 53                            |                       |                                 | 10/10              | 10/10                              | 10/10   |                                 |                |         |                        |                                  |               |                              |                                |
| 151                           |                       |                                 | 10/10              | 10/10                              | 10/10   |                                 |                |         |                        |                                  |               |                              |                                |
| 470                           |                       |                                 | 0/10               | 0/10                               | 1/10  |                                 |                |         |                        |                                  |               |                              |                                |
| 1320                          |                       |                                 | 0/10               | 0/10                               | 0/10  |                                 |                |         |                        |                                  |               |                              |                                |
|                               |                       |                                 |                    |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
|                               |                       |                                 |                    |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |
|                               |                       |                                 |                    |                                    |   |                                 |                |         |                        |                                  |               |                              |                                |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS An                                | alvtical Report                 |                                     |  |                           | Report Date:                                  | 27 May-24 14:15 (p 2  | of 2)  |
|---|---------------------------------|-------------------------------------|--|---------------------------|---|-----------------------|--------|
|   |                                 |                                     |  |                           | Test Code/ID:                                 | 24.003.2 Cu / 11-1449 | 9-0779 |
| Daphnia tho                             | msoni 48-h Acute Su             | vival Test                          |  |                           |   | NIWA Ecotoxic         | ology  |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 01-8576-5485<br>27 May-24 14:14 | Endpoint:<br>Analysis:<br>MD5 Hash: | 48h Survival Rate<br>Linear Interpolation (I<br>7540DE25745EA65E | CPIN)<br>B54EBC125F9B5243 | CETIS Version:<br>Status Level:<br>Editor ID: | CETISv2.1.4<br>1      |        |
| Graphics                                |                                 |                                     |  |                           |   |                       |        |
| 1.0                                     |                                 |                                     |  |                           |   |                       |        |

1200

Convergent Rounding (4 sf)

0

200

400

800

600

Conc-µg/L

1000

CETIS™ v2.1.4.5 (009-951-268-0)

# Appendix H CETIS statistical analyses – Okutua (pH adjusted)

#### Zinc

| CETIS Analytical Report Daphnia thomsoni 48-h Acute Survival Test |   |                                     |                  |                           |                                      |  |                                     |                     |      | eport<br>est Co               | Date:<br>ode/ID:              |                                 | 26 J<br>24.00                            | un-24 12:0<br>)3.3 Zn / 12 | 01 (p 1 of 3)<br>2-5207-5074 |
|---|---|-------------------------------------|------------------|---------------------------|--------------------------------------|--|-------------------------------------|---------------------|------|-------------------------------|-------------------------------|---------------------------------|--|----------------------------|------------------------------|
| Daphnia thom  | soni 4                                  | 48-h Acute                          | Surviva          | il Te                     | est                                  |  |                                     |                     |      |                               |                               |                                 |  | NIWA Eco                   | toxicology                   |
| Analysis ID:<br>Analyzed:<br>Edit Date:                           | 13-57<br>26 Ju<br>26 Ju                 | 20-1591<br>n-24 12:00<br>n-24 11:56 | E<br>A<br>M      | ndp<br>nal<br>ID5         | ooint: 4<br>ysis: P<br>Hash: 7       | 8h Survival F<br>arametric-M<br>64B00A97D1 | Rate<br>ultiple Compa<br>DBA7F034CE | arison<br>0818B89CF | 20D9 | CET<br>State<br>Edite         | IS Versi<br>us Leve<br>or ID: | ion:<br>I:                      | CETISv2.<br>1<br>008-408-4               | 1.4<br>107-6               |                              |
| Batch ID:<br>Start Date:<br>Ending Date:<br>Test Length:          | 21-12<br>29 Ma<br>31 Ma<br>48h          | 07-3831<br>ay-24<br>ay-24           | T<br>P<br>S<br>T | est<br>roto<br>pec<br>axo | Type: S<br>ocol: N<br>cies: D<br>on: | urvival (48h)<br>IWA SOP 1(<br>aphnia thom | ) (2022)<br>soni (Water f           | lea)                |      | Anal<br>Dilue<br>Brin<br>Sour | yst:<br>ent:<br>e:<br>rce:    | Ecoto<br>Okut<br>Not A<br>Field | ox Team<br>ua<br>Applicable<br>Collected |                            | Age:                         |
| Sample ID:<br>Sample Date:<br>Receipt Date:<br>Sample Age:        | 07-23<br>28 Ma<br>28 Ma<br>28 Ma<br>24h | 99-9627<br>ay-24<br>ay-24           | C<br>M<br>C<br>C | ode<br>late<br>AS<br>lier | e: 24<br>erial: Z<br>(PC):<br>nt: H  | 4.003.3 Zn<br>inc sulfate<br>ydrotoxy Re   | search                              |                     |      | Proj<br>Sou<br>Stati          | ect:<br>rce:<br>ion:          | Spec<br>Solut<br>Lab \$         | tial Studies<br>tion made b<br>Solution  | y NIWA                     |                              |
| Data Transform  | m                                       |                                     | Alt Hyp          | р                         |                                      |  |                                     | NOEL                | LO   | EL                            | TOEL                          |                                 | Tox Units                                | MSDu                       | PMSD                         |
| Angular (Corre  | cted)                                   |                                     | C > T            |                           |                                      |  |                                     | 109                 | 235  |                               | 160                           |                                 |  | 0.1552                     | 15.84%                       |
| Bonferroni Ad   | j t Tes                                 | st                                  |                  |                           |                                      |  |                                     |                     |      |                               |                               |                                 |  |                            |                              |
| Control   | vs                                      | Conc-µg/L                           |                  | df                        | Test Sta                             | t Critical                                 | MSD                                 | P-Type              | P-V  | alue                          | Decis                         | ion(d                           | a:5%)                                    |                            |                              |
| Dilution Water  |   | 109                                 |                  | 6                         | 0.7077                               | 2.466                                      | 0.2405                              | CDF                 | 0.74 | 430                           | Non-S                         | Signifi                         | icant Effect                             |                            |                              |
|   |   | 235*                                |                  | 6                         | 3.817                                | 2.466                                      | 0.2405                              | CDF                 | 0.0  | 051                           | Signif                        | icant                           | Effect                                   |                            |                              |
|   |   | 485*                                |                  | 6                         | 7.84                                 | 2.466                                      | 0.2405                              | CDF                 | 2.1  | E-05                          | Signif                        | icant                           | Effect                                   |                            |                              |
| Auxiliary Tests   | s                                       |                                     |                  |                           |                                      |  |                                     |                     |      |                               |                               |                                 |  |                            |                              |
| Attribute   |   | Test                                |                  |                           |                                      |  | Test Stat                           | Critical            | P-V  | alue                          | Decis                         | ion(c                           | a:5%)                                    |                            |                              |
| Control Trend   |   | Mann-Ken                            | dall Trer        | nd T                      | Test                                 |  | 0.395                               | 0.05                | 0.39 | 950                           | Non-S                         | Signifi                         | icant Contro                             | I Trend                    |                              |
| ANOVA Table   |   |                                     |                  |                           |                                      |  |                                     |                     |      |                               |                               |                                 |  |                            |                              |
| Source  |   | Sum Squa                            | res              |                           | Mean S                               | quare                                      | DF                                  | F Stat              | P-V  | P-Value Decision(α:5%)        |                               |                                 |  |                            |                              |
| Between   |   | 1.24958                             |                  |                           | 0.41652                              | 8  | 3 23.35 7.8E-05 Significant Effect  |                     |      |                               |                               | Effect                          |  |                            |                              |
| Error   |   | 0.178402                            |                  |                           | 0.01784                              | 02   | 10                                  | _                   |      |                               |                               |                                 |  |                            |                              |
| Total   |   | 1.42799                             |                  |                           |                                      |  | 13                                  |                     |      |                               |                               |                                 |  |                            |                              |
| ANOVA Assun   | nptior                                  | ns Tests                            |                  |                           |                                      |  |                                     |                     |      |                               |                               |                                 |  |                            |                              |
| Attribute   |   | Test                                |                  |                           |                                      |  | Test Stat                           | Critical            | P-V  | alue                          | Decis                         | ion(c                           | a:1%)                                    |                            |                              |
| Variance  |   | Bartlett Equ                        | ality of         | Var                       | iance Tes                            | st   | 4.308                               | 11.34               | 0.23 | 301                           | Equal                         | Varia                           | ances                                    |                            |                              |
|   |   | Levene Equ                          | ality of         | Var                       | iance Tes                            | st   | 4.538                               | 6.552               | 0.02 | 296                           | Equal                         | Varia                           | ances                                    |                            |                              |
|   |   | Mod Leven                           | e Equali         | ty o                      | of Varianc                           | e Test                                     | 0.5359                              | 9.78                | 0.67 | 746                           | Equal                         | Varia                           | ances                                    |                            |                              |
| Distribution  |   | Anderson-D                          | arling A         | \2 T                      | est                                  |  | 0.4094                              | 3.878               | 0.34 | 493                           | Norma                         | al Dis                          | stribution                               |                            |                              |
|   |   | D'Agostino                          | Skewne           | ss                        | Test                                 |  | 0.2473                              | 2.576               | 0.80 | 047                           | Norma                         | al Dis                          | stribution                               |                            |                              |
|   |   | Kolmogorov<br>Shaniro Wil           | /-Smirno         | OV L                      | ) lest                               |  | 0.181                               | 0.262               | 0.2  | 526<br>740                    | Norma                         | al Dis<br>al Dis                | stribution                               |                            |                              |
|   |   | Shapiro-wi                          |                  | IIIIa                     | anty rest                            |  | 0.9571                              | 0.62.59             | 0.01 | 49                            | Norma                         |                                 | stribution                               |                            |                              |
| 48h Survival R  | Rate S                                  | ummary                              |                  |                           |                                      |  |                                     |                     |      |                               |                               |                                 |  |                            |                              |
| Conc-µg/L   |   | Code                                | Count            |                           | Mean                                 | 95% LCI                                    | . 95% UCL                           | Median              | Min  |                               | Мах                           |                                 | Std Err                                  | CV%                        | %Effect                      |
| 4.15  |   | D                                   | 5                |                           | 0.9800                               | 0.9245                                     | 1.0000                              | 1.0000              | 0.90 | 000                           | 1.000                         | 0                               | 0.0200                                   | 4.56%                      | 0.00%                        |
| 109   |   |                                     | 3                |                           | 0.9333                               | 0.6465                                     | 1.0000                              | 1.0000              | 0.80 | 000                           | 1.000                         | 0                               | 0.0667                                   | 12.37%                     | 4.76%                        |
| 235   |   |                                     | 3                |                           | 0.7000                               | 0.2697                                     | 1.0000                              | 0.6000              | 0.60 | 000                           | 0.900                         | 0                               | 0.1000                                   | 24.74%                     | 28.57%                       |
| 485   |   |                                     | 3                |                           | 0.3333                               | 0.1899                                     | 0.4768                              | 0.3000              | 0.30 | 000                           | 0.400                         | 0                               | 0.0333                                   | 17.32%                     | 65.99%                       |
| 1080  |   |                                     | 3                |                           | 0.0000                               | 0.0000                                     | 0.0000                              | 0.0000              | 0.00 | 000                           | 0.000                         | 0                               | 0.0000                                   |                            | 100.00%                      |
| 2300  |   |                                     | 3                |                           | 0.0000                               | 0.0000                                     | 0.0000                              | 0.0000              | 0.00 | 000                           | 0.000                         | U                               | 0.0000                                   |                            | 100.00%                      |

Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (008-408-407-6)

| CETIS Ana                 | alytical Repo                   | ort        |                          |                               |                     |           | Re<br>Te | eport E<br>est Co | )ate:<br>de/ID:      | 26<br>24.0   | Jun-24 12:0<br>003.3 Zn / 12 | )1 (p 2 of 3)<br>2-5207-5074 |
|---------------------------|---------------------------------|------------|--------------------------|-------------------------------|---------------------|-----------|----------|-------------------|----------------------|--------------|------------------------------|------------------------------|
| Daphnia thor              | nsoni 48-h Acute                | Survival 1 | Test                     |                               |                     |           |          |                   |                      |              | NIWA Eco                     | toxicology                   |
| Analysis ID:<br>Analyzed: | 13-5720-1591<br>26 Jun-24 12:00 | Enc<br>Ana | lpoint: 48<br>alysis: Pa | h Survival Ra<br>rametric-Mul | ate<br>Itiple Compa | irison    |          | CETIS<br>Status   | Version:<br>s Level: | CETISV.<br>1 | 2.1.4                        |                              |
| Edit Date:                | 26 Jun-24 11:56                 | MD         | 5 Hash: 76               | 4B00A97DD                     | BA7F034CD           | 0818B89CF | 20D9     | Edito             | r ID:                | 008-408      | -407-6                       |                              |
| Angular (Cor              | rected) Transforr               | ned Sumn   | nary                     |                               |                     |           |          |                   |                      |              |                              |                              |
| Conc-µg/L                 | Code                            | Count      | Mean                     | 95% LCL                       | 95% UCL             | Median    | Min      |                   | Мах                  | Std Err      | CV%                          | %Effect                      |
| 4.15                      | D                               | 5          | 1.3790                   | 1.2890                        | 1.4700              | 1.4120    | 1.24     | 90                | 1.4120               | 0.0326       | 5.28%                        | 0.00%                        |
| 109                       |                                 | 3          | 1.3100                   | 0.8731                        | 1.7480              | 1.4120    | 1.10     | 70<br>61          | 1.4120               | 0.1016       | 13.43%                       | 5.00%                        |
| 235                       |                                 | 3          | 0.6147                   | 0.4600                        | 0.7654              | 0.0001    | 0.00     | 96                | 0.6847               | 0.1210       | 9.87%                        | 20.99%<br>55.44%             |
| 1080                      |                                 | 3          | 0.1588                   | 0.1588                        | 0.1588              | 0.1588    | 0.15     | 88                | 0.1588               | 0.0000       | 0.00%                        | 88.49%                       |
| 2300                      |                                 | 3          | 0.1588                   | 0.1588                        | 0.1588              | 0.1588    | 0.15     | 88                | 0.1588               | 0.0000       | 0.00%                        | 88.49%                       |
| 48h Survival              | Rate Detail                     |            |                          |                               |                     |           |          |                   |                      |              |                              |                              |
| Conc-µg/L                 | Code                            | Rep 1      | Rep 2                    | Rep 3                         | Rep 4               | Rep 5     |          |                   |                      |              |                              |                              |
| 4.15                      | D                               | 1.0000     | 1.0000                   | 1.0000                        | 1.0000              | 0.9000    |          |                   |                      |              |                              |                              |
| 109                       |                                 | 1.0000     | 0.8000                   | 1.0000                        |                     |           |          |                   |                      |              |                              |                              |
| 235                       |                                 | 0.6000     | 0.6000                   | 0.9000                        |                     |           |          |                   |                      |              |                              |                              |
| 485                       |                                 | 0.4000     | 0.3000                   | 0.3000                        |                     |           |          |                   |                      |              |                              |                              |
| 1080                      |                                 | 0.0000     | 0.0000                   | 0.0000                        |                     |           |          |                   |                      |              |                              |                              |
| 2300                      |                                 | 0.0000     | 0.0000                   | 0.0000                        |                     |           |          |                   |                      |              |                              |                              |
| Angular (Cor              | rected) Transforr               | ned Detail |                          |                               |                     |           |          |                   |                      |              |                              |                              |
| Conc-µg/L                 | Code                            | Rep 1      | Rep 2                    | Rep 3                         | Rep 4               | Rep 5     |          |                   |                      |              |                              |                              |
| 4.15                      | D                               | 1.4120     | 1.4120                   | 1.4120                        | 1.4120              | 1.2490    |          |                   |                      |              |                              |                              |
| 109                       |                                 | 1.4120     | 1.1070                   | 1.4120                        |                     |           |          |                   |                      |              |                              |                              |
| 235                       |                                 | 0.8861     | 0.8861                   | 1.2490                        |                     |           |          |                   |                      |              |                              |                              |
| 485                       |                                 | 0.6847     | 0.5796                   | 0.5796                        |                     |           |          |                   |                      |              |                              |                              |
| 1080                      |                                 | 0.1588     | 0.1588                   | 0.1588                        |                     |           |          |                   |                      |              |                              |                              |
| 2300                      |                                 | 0.1500     | 0.1500                   | 0.1500                        |                     |           |          |                   |                      |              |                              |                              |
| 48h Survival              | Rate Binomials                  | -          | -                        | -                             |                     |           |          |                   |                      |              |                              |                              |
| Conc-µg/L                 | Code                            | Rep 1      | Rep 2                    | Rep 3                         | Rep 4               | Rep 5     |          |                   |                      |              |                              |                              |
| 4.15                      | D                               | 10/10      | 9/10                     | 10/10                         | 10/10               | 9/10      |          |                   |                      |              |                              |                              |
| 225                       |                                 | 6/10       | 6/10                     | 0/10                          |                     |           |          |                   |                      |              |                              |                              |
| 485                       |                                 | 4/10       | 3/10                     | 3/10                          |                     |           |          |                   |                      |              |                              |                              |
| 1080                      |                                 | 0/10       | 0/10                     | 0/10                          |                     |           |          |                   |                      |              |                              |                              |
| 2300                      |                                 | 0/10       | 0/10                     | 0/10                          |                     |           |          |                   |                      |              |                              |                              |
| Graphics                  |                                 |            |                          |                               |                     |           |          |                   |                      |              |                              |                              |
| 10.                       |                                 |            |                          |                               |                     | 0.25      |          |                   |                      |              |                              |                              |
| 0.0                       |                                 |            |                          |                               |                     | 0.20      |          |                   |                      |              |                              | -                            |
| 0.9-                      |                                 |            |                          |                               |                     | 0.20 -    |          |                   |                      |              |                              | /                            |
| 0.8 -                     |                                 |            |                          |                               |                     | 0.15 -    |          |                   |                      |              |                              |                              |
| - 7.0 <b>ge</b>           |                                 |            |                          |                               |                     | 0.10 -    |          |                   |                      |              |                              | •                            |
| 0.6-                      |                                 |            |                          |                               | ale                 | 0.05 -    |          |                   |                      |              | <b>_</b>                     |                              |
| . <mark>≥</mark> 0.5 –    |                                 |            |                          |                               | . Ai                | 0.00      |          |                   |                      |              | • •                          |                              |
| <b>3</b> 0.4 -            |                                 | г          |                          |                               | Co.                 | 0.00 -    |          |                   |                      |              |                              |                              |
| <b>48</b><br>0.3 -        |                                 |            | -                        |                               |                     | -0.05 -   |          |                   | /                    |              |                              |                              |
| 0.2 -                     |                                 |            |                          |                               |                     | -0.10 -   |          |                   |                      |              |                              |                              |
| 0.1                       |                                 |            |                          |                               |                     | -0.15 -   | -        |                   |                      |              |                              |                              |
| 0.1                       |                                 |            |                          | •                             |                     | 0.20      |          |                   |                      |              |                              |                              |
| 0.0 -                     |                                 |            |                          |                               |                     | -0.20 -   |          | 1                 |                      |              |                              |                              |
|                           | 4.15 D 109                      | 235        | 485 10                   | 80 2300                       |                     |           | -1.5     | -1.0              | -0.5                 | 0.0 0.       | .5 1.0                       | 1.5                          |
|                           |                                 | Conc-µ     | ig/L                     |                               |                     |           |          |                   | R                    | ankits       |                              |                              |

CETIS™ v2.1.4.5 (008-408-407-6)
| CETIS                                    | Analyti  | cal Repo                               | ort      |                               |  |   |                                 |                  | R    | eport<br>est Co                | Date:<br>ode/ID:              |                                 | 26<br>24.0                               | Jun-24 12:0<br>03.3 Zn / 12 | )2 (p 1 of 3)<br>2-5207-5074 |
|--|--|--|----------|-------------------------------|--|---|---------------------------------|------------------|------|--------------------------------|-------------------------------|---------------------------------|--|-----------------------------|------------------------------|
| Daphni                                   | a thomsoni                                       | i 48-h Acute                           | e Survi  | val T                         | est  |   |                                 |                  |      |                                |                               |                                 |  | NIWA Eco                    | toxicology                   |
| Analysi<br>Analyze<br>Edit Da            | sID: 15-2<br>ed: 26 J<br>te: 26 J                | 921-5324<br>un-24 12:00<br>un-24 11:56 |          | Endı<br>Anal<br>MD5           | ooint: 48h<br>ysis: Nor<br>Hash: 764       | Survival Ra<br>Ilinear Regro<br>B00A97DDI | ite<br>ession (NLF<br>BA7F034CI | R)<br>D818B89CF2 | 20D9 | CET<br>Statu<br>Edito          | IS Versi<br>us Leve<br>or ID: | on:<br>I:                       | CETISv2<br>1<br>008-408-                 | 1.4<br>407-6                |                              |
| Batch I<br>Start Da<br>Ending<br>Test Le | D: 21-1<br>ate: 29 M<br>Date: 31 M<br>ngth: 48h  | 207-3831<br>/lay-24<br>/lay-24         |          | Test<br>Prote<br>Spec<br>Taxo | Type: Sur<br>ocol: NIV<br>cies: Dap<br>on: | vival (48h)<br>/A SOP 10<br>ohnia thoms   | (2022)<br>oni (Water t          | flea)            |      | Anal<br>Dilue<br>Brine<br>Sour | yst:<br>ent:<br>e:<br>rce:    | Ecoto<br>Okut<br>Not A<br>Field | ox Team<br>ua<br>Applicable<br>Collected |                             | Age:                         |
| Sample<br>Sample<br>Receipt<br>Sample    | ID: 07-2<br>Date: 28 M<br>Date: 28 M<br>Age: 24h | 2399-9627<br>Nay-24<br>Nay-24          |          | Code<br>Mate<br>CAS<br>Clier  | e: 24.0<br>rial: Zino<br>(PC):<br>nt: Hyd  | 003.3 Zn<br>c sulfate<br>Irotoxy Rese     | earch                           |                  |      | Proje<br>Sour<br>Stati         | ect:<br>rce:<br>on:           | Spec<br>Solut<br>Lab \$         | ial Studies<br>tion made l<br>Solution   | by NIWA                     |                              |
| Non-Lir<br>Model I                       | near Regres                                      | ssion Optio<br>Function                | ns       |                               |  |   | Weightin                        | a Function       |      |                                | PTBS                          | Fun                             | ction                                    | X Trans                     | Y Trans                      |
| 3P Log-                                  | P Log-Logistic: μ=α/[1+[x/δ]^γ]                  |  |          |                               |  |   | Binomial                        | [ω=n/[p·q]]      |      |                                | Off [µ'                       | *=µ]                            |  | None                        | None                         |
| Regres                                   | sion Summ  | ary                                    | -        |                               |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Iters                                    | Ц  | AICc                                   | BIC      |                               | Adi R2                                     | PMSD                                      | Thresh                          | Optimize         | F S  | tat                            | P-Val                         | ue                              | Decision                                 | (a:5%)                      |                              |
| 7  | -17.28   | 42.07                                  | 43.55    | 5                             | 0.8442                                     | 3.99%                                     | 0.9766                          | Yes              | 0.87 | 779                            | 0.476                         | 1                               | Non-Signi                                | ficant Lack-                | of-Fit                       |
| Point E                                  | stimates   |  |          |                               |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Level                                    | µg/L   | 95% LCL                                | 95%      | UCL                           |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| LC5                                      | 125.4  |  | 167.2    | )                             |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| LC10                                     | 161.8  | 77.84                                  | 207.5    | 5                             |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| LC15                                     | 189.5  | 118                                    | 238.8    | 3                             |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| LC20                                     | 213.4  | 147.4                                  | 265.7    | ,                             |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| LC25                                     | 235.5  | 172 7                                  | 290.5    | 5                             |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| LC40                                     | 298.3  | 239.2                                  | 362.4    | i                             |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| LC50                                     | 342.6  | 281.9                                  | 416.3    | 3                             |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Regres                                   | sion Param                                       | ieters                                 |          |                               |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Parame                                   | ter  | Estimate                               | Std E    | Frror                         | 95% LCL                                    | 95% UCL                                   | t Stat                          | P-Value          | Dec  | ision(                         | (α:5%)                        |                                 |  |                             |                              |
| α  |  | 0.9766                                 | 0.018    | 346                           | 0.9376                                     | 1.016                                     | 52.89                           | <1.0E-05         | Sig  | nifican                        | t Param                       | eter                            |  |                             |                              |
| v  |  | 2.93                                   | 0.487    | 7                             | 1.901                                      | 3.959                                     | 6.008                           | 1.4E-05          | Sig  | nifican                        | t Param                       | eter                            |  |                             |                              |
| ō  |  | 342.6                                  | 31.78    | 3                             | 275.5                                      | 409.6                                     | 10.78                           | <1.0E-05         | Sig  | nifican                        | t Param                       | eter                            |  |                             |                              |
| ANOVA                                    | Table  |  |          |                               |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Source                                   |  | Sum Squa                               | ares     | Mea                           | n Square                                   | DF  | F Stat                          | P-Value          | Dec  | ision(                         | (α:5%)                        |                                 |  |                             |                              |
| Model                                    |  | 2679                                   |          | 892.9                         | )  | 3   | 1072                            | <1.0E-05         | Sigr | nifican                        | t Effect                      |                                 |  |                             |                              |
| Lack of                                  | Fit  | 2.243                                  |          | 0.74                          | 76   | 3   | 0.8779                          | 0.4761           | Nor  | -Signi                         | ficant La                     | ack-o                           | f-Fit                                    |                             |                              |
| Pure Er                                  | ror  | 11.92                                  |          | 0.85                          | 16   | 14  |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Residua                                  | il   | 14.17                                  |          | 0.83                          | 33   | 17  |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Residu                                   | al Analysis                                      |  |          |                               |  |   |                                 |                  |      |                                |                               |                                 |  |                             |                              |
| Attribut                                 | e  | Method                                 |          |                               |  | Test Stat                                 | Critical                        | P-Value          | Dec  | ision(                         | (α:5%)                        |                                 |  |                             |                              |
| Model F                                  | it   | Likelihood                             | Ratio (  | GOF                           | Test                                       | 14.8                                      | 27.59                           | 0.6102           | Nor  | -Signi                         | ficant H                      | etero                           | geneity                                  |                             |                              |
|  |  | Pearson C                              | hi-Sq (  | GOF '                         | Fest                                       | 14.17                                     | 27.59                           | 0.6554           | Nor  | I-Signi                        | ficant H                      | etero                           | geneity                                  |                             |                              |
| Varianc                                  | е  | Mod Lever                              | ne Equ   | ality o                       | f Variance                                 | 0.5301                                    | 3.687                           | 0.7489           | Equ  | ial Var                        | iances                        |                                 |  |                             |                              |
| Distribu                                 | tion   | Anderson-                              | Darling  | ) A2 T                        | est  | 0.484                                     | 2.492                           | 0.2325           | Nor  | mal Di                         | istributio                    | n                               |  |                             |                              |
|  |  | Shapiro-W                              | ilk W N  | Norma                         | ality Test                                 | 0.9468                                    | 0.9044                          | 0.3214           | Nor  | mal Di                         | istributio                    | n                               |  |                             |                              |
| Control                                  | Trend  | Mann-Ken                               | dall Tre | end T                         | est  | 0.395                                     | 0.05                            | 0.3950           | Nor  | I-Signi                        | ficant C                      | ontro                           | l Trend                                  |                             |                              |
| Overdis                                  | persion  | Tarone C(                              | α) Ove   | rdispe                        | ersion Test                                | 1.162                                     | 1.645                           | 0.1226           | Nor  | I-Signi                        | ficant O                      | verdi                           | spersion                                 |                             |                              |

| CETIS Ana                                 | alytical Repo                                      | ort  |  |  |  |  | Report<br>Test Co  | Date:<br>ode/ID:   | 26<br>24.0                              | Jun-24 12:0<br>)03.3 Zn / 12                             | 2 (p 2 of 3)<br>-5207-5074                       |
|---|--|--|--|--|--|--|--|--|---|--|--|
| Daphnia thor                              | msoni 48-h Acute                                   | Survival T   | fest   |  |  |  |  |  |   | NIWA Ecot  | oxicology  |
| Analysis ID:<br>Analyzed:<br>Edit Date:   | 15-2921-5324<br>26 Jun-24 12:00<br>26 Jun-24 11:56 | End<br>Ana<br>MDS  | lpoint: 48<br>Iysis: No<br>5 Hash: 76                    | h Survival R<br>Ininear Regi<br>4B00A97DD                | ate<br>ression (NL<br>IBA7F034C                          | R)<br>D818B89CF  | CET<br>State<br>20D9 Edite                               | IS Version:<br>us Level:<br>or ID:                       | CETISv2<br>1<br>008-408-                | 2.1.4<br>-407-6  |  |
| 48h Survival                              | Rate Summary                                       |  |  |  |  | Calculate  | d Variate(A/   | В)   |   |  |  |
| Conc-µg/L                                 | Code   | Count  | Mean   | Median   | Min  | Мах  | Std Err  | Std Dev  | CV%                                     | %Effect  | ΣΑ/ΣΒ  |
| 4.15<br>109<br>235<br>485<br>1080<br>2300 | D<br>Rate Detail                                   | 5<br>3<br>3<br>3<br>3<br>3                               | 0.9800<br>0.9333<br>0.7000<br>0.3333<br>0.0000<br>0.0000 | 1.0000<br>1.0000<br>0.6000<br>0.3000<br>0.0000<br>0.0000 | 0.9000<br>0.8000<br>0.6000<br>0.3000<br>0.0000<br>0.0000 | 1.0000<br>1.0000<br>0.9000<br>0.4000<br>0.0000<br>0.0000 | 0.0200<br>0.0667<br>0.1000<br>0.0333<br>0.0000<br>0.0000 | 0.0447<br>0.1155<br>0.1732<br>0.0577<br>0.0000<br>0.0000 | 4.56%<br>12.37%<br>24.74%<br>17.32%<br> | 0.00%<br>4.76%<br>28.57%<br>65.99%<br>100.00%<br>100.00% | 49/50<br>28/30<br>21/30<br>10/30<br>0/30<br>0/30 |
|   | Code   | Ren 1  | Ren 2  | Ren 3  | Rep 4  | Ren 5  |  |  |   |  |  |
| 4.15<br>109<br>235<br>485<br>1080<br>2300 | D  | 1.0000<br>1.0000<br>0.6000<br>0.4000<br>0.0000<br>0.0000 | 1.0000<br>0.8000<br>0.6000<br>0.3000<br>0.0000<br>0.0000 | 1.0000<br>1.0000<br>0.9000<br>0.3000<br>0.0000<br>0.0000 | 1.0000   | 0.9000   |  |  |   |  |  |
| 48h Survival                              | Rate Binomials                                     |  |  |  |  |  |  |  |   |  |  |
| Conc-µg/L                                 | Code   | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |  |  |   |  |  |
| 4.15<br>109<br>235<br>485<br>1080<br>2300 | D  | 10/10<br>10/10<br>6/10<br>4/10<br>0/10<br>0/10           | 10/10<br>8/10<br>6/10<br>3/10<br>0/10<br>0/10            | 10/10<br>10/10<br>9/10<br>3/10<br>0/10<br>0/10           | 10/10  | 9/10   |  |  |   |  |  |



# Copper

| CETIS Anal   | ytical                                  | Report                         |                              |                                |                    |  |                                  |                    | R    | eport<br>est Co                | Date:<br>ode/ID:              |                                 | 26 J<br>24.00                            | lun-24 11:4<br>3.3 Cu / 16 | 8 (p 1 of 2)<br>-2293-8617 |
|--|---|--------------------------------|------------------------------|--------------------------------|--------------------|--|----------------------------------|--------------------|------|--------------------------------|-------------------------------|---------------------------------|--|----------------------------|----------------------------|
| Daphnia thoms  | soni 48-                                | h Acute Surv                   | vival T                      | est                            |                    |  |                                  |                    |      |                                |                               |                                 | I  | NIWA Ecot                  | oxicology                  |
| Analysis ID: 0<br>Analyzed: 2<br>Edit Date: 2                      | 08-9689<br>26 Jun-2<br>26 Jun-2         | -2974<br>24 11:47<br>24 11:46  | End<br>Anal<br>MD5           | point:<br>lysis:<br>Hash:      | 48h<br>Para<br>733 | Survival Ra<br>ametric-Mul<br>EADCD93E | ate<br>tiple Compa<br>:99B00DEDI | rison<br>E9048F1D8 | 118  | CETI<br>Statu<br>Edito         | IS Versi<br>Is Leve<br>or ID: | on:<br>I:                       | CETISv2.<br>1<br>008-408-4               | 1.4<br>107-6               |                            |
| Batch ID: 0<br>Start Date: 2<br>Ending Date: 3<br>Test Length: 4   | 01-3332<br>29 May-2<br>31 May-2<br>48h  | -2169<br>24<br>24              | Test<br>Prot<br>Spec<br>Taxo | Type:<br>ocol:<br>cies:<br>on: | Sun<br>NIW<br>Dap  | vival (48h)<br>/A SOP 10<br>hnia thoms | (2022)<br>oni (Water fl          | ea)                |      | Anal<br>Dilue<br>Brine<br>Sour | yst:<br>ent:<br>e:<br>rce:    | Ecoto<br>Okut<br>Not A<br>Field | ox Team<br>ua<br>Applicable<br>Collected |                            | Age:                       |
| Sample ID: 0<br>Sample Date: 2<br>Receipt Date: 2<br>Sample Age: 2 | 02-9373-<br>28 May-2<br>28 May-2<br>24h | -9603<br>24<br>24              | Cod<br>Mate<br>CAS<br>Clier  | e:<br>erial:<br>(PC):<br>nt:   | 24.0<br>Cop<br>Hyd | 03.3 Cu<br>per<br>rotoxy Rese          | earch                            |                    |      | Proje<br>Sour<br>Stati         | ect:<br>ce:<br>on:            | Spec<br>Solut<br>Lab (          | tial Studies<br>tion made by<br>Solution | y NIWA                     |                            |
| Data Transforn   | n                                       | Alt                            | Нур                          |                                |                    |  |                                  | NOEL               | LO   | EL                             | TOEL                          |                                 | Tox Units                                | MSDu                       | PMSD                       |
| Angular (Correc  | ted)                                    | C >                            | т                            |                                |                    |  |                                  | 59                 | 136  |                                | 89.58                         |                                 |  | 0.1873                     | 19.12%                     |
| Bonferroni Adj<br>Control  | t Test<br>vs Co                         | onc-µg/L                       | df                           | Test S                         | stat               | Critical                               | MSD                              | P-Type             | P-V  | alue                           | Decis                         | ion(c                           | a:5%)                                    |                            |                            |
| Dilution water   | 59                                      | 6*                             | 6                            | 0.1781                         | 1                  | 2.306                                  | 0.2814                           | CDF                | 0.8  | 531<br>=_05                    | Non-S                         | signiti<br>icant                | Effect                                   |                            |                            |
|  | 15                                      | 0                              | 0                            | 0.000                          |                    | 2.300                                  | 0.2014                           | CDF                | 2.11 | 2-05                           | Signii                        | luarii                          | Ellect                                   |                            |                            |
| Auxiliary Tests  |   |                                |                              |                                |                    |  |                                  |                    |      |                                |                               |                                 |  |                            |                            |
| Attribute  | Te                                      | est                            |                              |                                |                    |  | Test Stat                        | Critical           | P-V  | alue                           | Decis                         | ion(d                           | a:5%)                                    |                            |                            |
| Control Trend  | M                                       | ann-Kendall 1                  | Frend T                      | Test                           |                    |  | 0.395                            | 0.05               | 0.3  | 950                            | Non-S                         | Signifi                         | icant Contro                             | I Trend                    |                            |
| ANOVA Table  |   |                                |                              |                                |                    |  |                                  |                    |      |                                |                               |                                 |  |                            |                            |
| Source   | Su                                      | im Squares                     |                              | Mean                           | Squ                | are                                    | DF                               | F Stat             | P-V  | alue                           | Decis                         | ion(d                           | a:5%)                                    |                            |                            |
| Between  | 2.3                                     | 34796                          |                              | 1.1739                         | 98                 |  | 2                                | 42.05              | 5.7  | E-05                           | Signif                        | icant                           | Effect                                   |                            |                            |
| Error  | 0.2                                     | 223362                         |                              | 0.0279                         | 9202               |  | 8                                | _                  |      |                                |                               |                                 |  |                            |                            |
| Total  | 2.5                                     | 57132                          |                              |                                |                    |  | 10                               |                    |      |                                |                               |                                 |  |                            |                            |
| ANOVA Assum  | ptions                                  | Tests                          |                              |                                |                    |  |                                  |                    |      |                                |                               |                                 |  |                            |                            |
| Attribute  | Te                                      | st                             |                              |                                |                    |  | Test Stat                        | Critical           | P-V  | alue                           | Decis                         | ion(d                           | x:1%)                                    |                            |                            |
| Variance   | Ba                                      | rtlett Equality                | of Va                        | iance T                        | est                |  | 5.512                            | 9.21               | 0.0  | 635                            | Equal                         | Varia                           | ances                                    |                            |                            |
|  | Le                                      | vene Equality                  | of Va                        | riance T                       | est                | F                                      | 8.878                            | 8.649              | 0.0  | )93<br>)64                     | Unequ                         | ual Va                          | ariances                                 |                            |                            |
| Distribution   | MC                                      | od Levene Eq<br>Idoraon Darlin |                              | or variar<br>Foot              | nce                | lest                                   | 0.9841                           | 13.27              | 0.4  | 301                            | Equal                         | varia<br>ol Dia                 | ances                                    |                            |                            |
| Distribution   |   | Adostino Skev                  | NNess                        | Test                           |                    |  | 1.632                            | 2 576              | 0.0  | 126                            | Norm:                         | al Dis<br>al Dis                | stribution                               |                            |                            |
|  | Ko                                      | Imogorov-Sm                    | irnov l                      | D Test                         |                    |  | 0.2672                           | 0.2906             | 0.0  | 272                            | Norm                          | al Dis                          | stribution                               |                            |                            |
|  | Sh                                      | apiro-Wilk W                   | Norma                        | ality Tes                      | st                 |  | 0.838                            | 0.7725             | 0.0  | 298                            | Norma                         | al Dis                          | stribution                               |                            |                            |
| 48h Survival R   | ate Sun                                 | nmary                          |                              |                                |                    |  |                                  |                    |      |                                |                               |                                 |  |                            |                            |
| Conc-µg/L  | Co                                      | de Cou                         | nt                           | Mean                           |                    | 95% LCL                                | 95% UCL                          | Median             | Min  |                                | Мах                           |                                 | Std Err                                  | CV%                        | %Effect                    |
| 1.3  | D                                       | 5                              |                              | 0.9800                         | )                  | 0.9245                                 | 1.0000                           | 1.0000             | 0.9  | 000                            | 1.000                         | 0                               | 0.0200                                   | 4.56%                      | 0.00%                      |
| 59   |   | 3                              |                              | 0.9667                         | 7                  | 0.8232                                 | 1.0000                           | 1.0000             | 0.9  | 000                            | 1.000                         | 0                               | 0.0333                                   | 5.97%                      | 1.36%                      |
| 136  |   | 3                              |                              | 0.1333                         | 3                  | 0.0000                                 | 0.7070                           | 0.0000             | 0.0  | 000                            | 0.400                         | 0                               | 0.1333                                   | 173.21%                    | 86.39%                     |
| 280  |   | 3                              |                              | 0.0000                         | )                  | 0.0000                                 | 0.0000                           | 0.0000             | 0.0  | 000                            | 0.000                         | 0                               | 0.0000                                   |                            | 100.00%                    |
| Angular (Corre   | cted) Tr                                | ransformed §                   | Summ                         | ary                            |                    |  |                                  |                    |      |                                |                               |                                 |  |                            |                            |
| Conc-µg/L  | Co                                      | de Cou                         | nt                           | Mean                           |                    | 95% LCL                                | 95% UCL                          | Median             | Min  |                                | Мах                           |                                 | Std Err                                  | CV%                        | %Effect                    |
| 1.3  | D                                       | 5                              |                              | 1.3790                         | )                  | 1.2890                                 | 1.4700                           | 1.4120             | 1.24 | 490                            | 1.412                         | 0                               | 0.0326                                   | 5.28%                      | 0.00%                      |
| 59   |   | 3                              |                              | 1.3580                         |                    | 1.1240                                 | 1.5910                           | 1.4120             | 1.24 | 190                            | 1.412                         | 0                               | 0.0543                                   | 6.93%                      | 1.58%                      |
| 280  |   | 3                              |                              | 0.3341                         | 3                  | -0.4202                                | 0.1588                           | 0.1588             | 0.1  | 588<br>588                     | 0.684                         | /<br>8                          | 0.1753                                   | 90.89%                     | /5./8%<br>88.49%           |

Convergent Rounding (4 sf)

| CETIS An  | alytical Repo                                      | ort                                  |   |  |                                  |   | Report Date:<br>Test Code/ID:                 | 26 Jun-24 11:48 (p 2 of 2)<br>24.003.3 Cu / 16-2293-8617 |
|---|--|--------------------------------------|---|--|----------------------------------|---|---|--|
| Daphnia tho   | msoni 48-h Acute                                   | Survival                             | Test                                    |  |                                  |   |   | NIWA Ecotoxicology                                       |
| Analysis ID:<br>Analyzed:<br>Edit Date:                                       | 08-9689-2974<br>26 Jun-24 11:47<br>26 Jun-24 11:46 | En<br>An<br>MD                       | dpoint: 48<br>alysis: Pa<br>95 Hash: 73 | h Survival F<br>arametric-Mu<br>3EADCD93 | Rate<br>ultiple Comp<br>E99B00DE | parison<br>DE9048F1D8118                  | CETIS Version:<br>Status Level:<br>Editor ID: | CETISv2.1.4<br>1<br>008-408-407-6                        |
| 48h Survival  | Rate Detail  |                                      |   |  |                                  |   |   |  |
| Conc-µg/L   | Code   | Rep 1                                | Rep 2                                   | Rep 3                                    | Rep 4                            | Rep 5                                     |   |  |
| 1.3<br>59<br>136<br>280   | D  | 1.0000<br>1.0000<br>0.0000<br>0.0000 | 1.0000<br>1.0000<br>0.4000<br>0.0000    | 1.0000<br>0.9000<br>0.0000<br>0.0000     | 1.0000                           | 0.9000                                    |   |  |
| Angular (Cor  | rrected) Transforr                                 | ned Detai                            | I                                       |  |                                  |   |   |  |
| Conc-µg/L   | Code   | Rep 1                                | Rep 2                                   | Rep 3                                    | Rep 4                            | Rep 5                                     |   |  |
| 1.3<br>59<br>136<br>280   | D  | 1.4120<br>1.4120<br>0.1588<br>0.1588 | 1.4120<br>1.4120<br>0.6847<br>0.1588    | 1.4120<br>1.2490<br>0.1588<br>0.1588     | 1.4120                           | 1.2490                                    |   |  |
| 48h Survival  | Rate Binomials                                     |                                      |   |  |                                  |   |   |  |
| Conc-µg/L   | Code   | Rep 1                                | Rep 2                                   | Rep 3                                    | Rep 4                            | Rep 5                                     |   |  |
| 1.3<br>59<br>136<br>280   | D  | 10/10<br>10/10<br>0/10<br>0/10       | 10/10<br>10/10<br>4/10<br>0/10          | 10/10<br>9/10<br>0/10<br>0/10            | 10/10                            | 9/10                                      |   |  |
| Graphics  |  |                                      |   |  |                                  |   |   |  |
| 1.0 -<br>0.9 -<br>0.8 -<br>0.7 -<br>0.6 -<br>0.5 -<br>0.4 -<br>0.3 -<br>0.3 - |  |                                      |   |  |                                  | 0.3 -<br>0.2 -<br>0.1 -<br>0.1 -<br>0.0 - | •••   |  |

0.1 0.0 -

1.3 D

59

Conc-µg/L

136

280

CETIS™ v2.1.4.5 (008-408-407-6)

-0.1

-0.2

-1.5

-1.0

-0.5

0.0

Rankits

0.5

1.0

1.5

| CETIS                                    | S Analyt   | tical Repo                                 | ort     |                              |  |   |                               |                            | R    | eport Da<br>est Code                   | ite:<br>e/ID:           | 26<br>24.00  | Jun-24 11:4<br>03.3 Cu / 16 | 19 (p 1 of 3)<br>5-2293-8617 |
|--|--|--|---------|------------------------------|--|---|-------------------------------|----------------------------|------|--|-------------------------|--|-----------------------------|------------------------------|
| Daphni                                   | a thomso   | ni 48-h Acute                              | Surv    | ival T                       | est  |   |                               |                            |      |  |                         |  | NIWA Eco                    | toxicology                   |
| Analysi<br>Analyze<br>Edit Da            | is ID: 16<br>ed: 26<br>ite: 26                     | -0302-2229<br>Jun-24 11:48<br>Jun-24 11:46 |         | End<br>Anal<br>MD5           | point: 48ł<br>lysis: No<br>i Hash: 2F(     | h Survival Ra<br>nlinear Regr<br>6FF92B610D | ate<br>ession (NL<br>06BE0CFE | R)<br>8F75A74FEF           | -8CB | CETIS<br>Status<br>Editor I            | Versio<br>Level:<br>D:  | n: CETISv2<br>1<br>008-408-                            | .1.4<br>407-6               |                              |
| Batch I<br>Start Da<br>Ending<br>Test Le | D: 01-<br>ate: 29<br>Date: 31<br>ength: 48         | -3332-2169<br>May-24<br>May-24<br>h        |         | Test<br>Prot<br>Spec<br>Taxo | Type: Sur<br>ocol: NIV<br>cies: Dap<br>on: | rvival (48h)<br>NA SOP 10<br>phnia thoms    | (2022)<br>oni (Water          | flea)                      |      | Analyst<br>Diluent<br>Brine:<br>Source | : E<br>: O<br>N<br>: Fi | cotox Team<br>kutua<br>ot Applicable<br>ield Collected |                             | Age:                         |
| Sample<br>Sample<br>Receipt<br>Sample    | e ID: 02-<br>e Date: 28<br>t Date: 28<br>e Age: 24 | -9373-9603<br>May-24<br>May-24<br>h        |         | Code<br>Mate<br>CAS<br>Clier | e: 24.<br>erial: Co<br>(PC):<br>nt: Hyd    | 003.3 Cu<br>pper<br>drotoxy Rese            | earch                         |                            |      | Project<br>Source<br>Station           | : S <br>: S(<br>: La    | pecial Studies<br>olution made I:<br>ab Solution       | 99 NIWA                     |                              |
| Non-Li                                   | near Regro   | ession Optio                               | ns      |                              |  |   | Weightin                      | a Function                 |      |  | TDEF                    | unotion  | V Trana                     | V Trana                      |
| 3P Log-                                  | Logistic: u  | =α/[1+[x/δ]^v]                             |         |                              |  |   | Binomial                      | ig Function<br>[ω=n/[p·a]] |      | ۲<br>(                                 | )ff [u*=                | uncuon   | None                        | None                         |
| Pouroe                                   | sion Sum   | many                                       |         |                              |  |   | 2e                            | (                          |      |  | (p                      |  |                             |                              |
| Itore                                    |  |  | BIC     |                              | Adi R2                                     | DMSD  | Threeh                        | Ontimize                   | FS   | tat D                                  | Value                   | Decision/  | a:5%)                       |                              |
| 3  | -11.95   | 31.4                                       | 32.89   | 9                            | 0.8992                                     | 2.19%                                       | 0.9908                        | Yes                        | 0.28 | 343 0                                  | .8359                   | Non-Signi  | ficant Lack-                | of-Fit                       |
| Point F                                  | stimates   |  |         |                              |  |   |                               |                            |      |  |                         |  |                             |                              |
| Level                                    | µg/L   | 95% LCL                                    | 95%     | UCL                          |  |   |                               |                            |      |  |                         |  |                             |                              |
| LC5                                      | 66.66  |  | 83.00   | 6                            |  |   |                               |                            |      |  |                         |  |                             |                              |
| LC10                                     | 74.44  |  | 92.61   | 1                            |  |   |                               |                            |      |  |                         |  |                             |                              |
| LC15                                     | 79.71  |  | 99.40   | 6                            |  |   |                               |                            |      |  |                         |  |                             |                              |
| LC20                                     | 83.92  |  | 105.2   | 2                            |  |   |                               |                            |      |  |                         |  |                             |                              |
| LC25                                     | 87.56  | <br>E1 06                                  | 110.    | 5                            |  |   |                               |                            |      |  |                         |  |                             |                              |
| LC50                                     | 103  | 78.09                                      | 125.4   | 4<br>R                       |  |   |                               |                            |      |  |                         |  |                             |                              |
| Degree                                   | oion Doro  | motoro                                     | 100.    |                              |  |   |                               |                            |      |  |                         |  |                             |                              |
| Regres                                   | SION Para  | Estimate                                   | C 4 4   |                              | 05% 1.01                                   |   | 4.54-4                        | Divelue                    |      |  | 50/3                    |  |                             |                              |
| Parame                                   | eter   | Estimate                                   | Std     | Error                        | 95% LCL                                    | 95% UCL                                     | t Stat                        | P-Value                    | Dec  | ision(a:                               | 5%)                     |  |                             |                              |
| α  |  | 0.9908                                     | 0.010   | 03                           | 0.909                                      | 1.012                                       | 90.21                         | <1.0E-05                   | Sigi | nilicant Pa                            | aramet                  | ler  |                             |                              |
| δ  |  | 103  | 10.6    | 5                            | 2.556                                      | 125.5                                       | 9.667                         | <1.0E-05                   | Sig  | nificant P                             | aramet                  | er   |                             |                              |
|  | Table  |  |         |                              |  |   |                               |                            |      |  |                         |  |                             |                              |
| Source                                   | Tubic  | Sum Sau                                    | ares    | Mea                          | n Square                                   | DF  | E Stat                        | P-Value                    | Dec  | ision(a:                               | 5%)                     |  |                             |                              |
| Model                                    |  | 12680                                      |         | 4228                         | 3  | 3   | 3338                          | <1.0E-05                   | Sia  | nificant Et                            | ffect                   |  |                             |                              |
| Lack of                                  | Fit  | 1.236                                      |         | 0.41                         | 22   | 3   | 0.2843                        | 0.8359                     | Nor  | -Significa                             | ant Lac                 | k-of-Fit   |                             |                              |
| Pure Er                                  | ror  | 20.3                                       |         | 1.45                         |  | 14  |                               |                            |      |  |                         |  |                             |                              |
| Residua                                  | al   | 21.53                                      |         | 1.26                         | 7  | 17  |                               |                            |      |  |                         |  |                             |                              |
| Residu                                   | al Analysi   | s  |         |                              |  |   |                               |                            |      |  |                         |  |                             |                              |
| Attribut                                 | te   | Method                                     |         |                              |  | Test Stat                                   | Critical                      | P-Value                    | Dec  | ision(α:                               | 5%)                     |  |                             |                              |
| Model F                                  | it   | Likelihood                                 | Ratio   | GOF                          | Test                                       | 17.34                                       | 27.59                         | 0.4316                     | Nor  | -Significa                             | ant Het                 | erogeneity   |                             |                              |
|  |  | Pearson C                                  | hi-Sq   | GOF                          | Test                                       | 21.53                                       | 27.59                         | 0.2034                     | Nor  | -Significa                             | ant Het                 | erogeneity   |                             |                              |
| Varianc                                  | e  | Mod Lever                                  | ne Equ  | ality o                      | of Variance                                | 0.5486                                      | 3.687                         | 0.7365                     | Equ  | al Varian                              | ces                     |  |                             |                              |
| Distribu                                 | tion   | Anderson-                                  | Darling | g A2 1                       | est<br>I est                               | 2.284                                       | 2.492                         | <1.0E-05                   | Nor  | I-Normal                               | Distrib                 | ution  |                             |                              |
| Control                                  | Trand  | Snapiro-W                                  | dolUT-  | Norma                        | anty rest                                  | 0.7972                                      | 0.9044                        | 0.0008                     | Nor  | I-Normal                               | Distribi                | ution<br>strol Trend                                   |                             |                              |
| Overdia                                  | noreion  | Tarono C/                                  |         | ena l                        | est<br>areion Tect                         | 0.395                                       | 1.645                         | 0.3950                     | NOP  | i-Signinca                             | ant COP                 | nuor menu  |                             |                              |
| overuls                                  | persion  | raione C(                                  | u) Ove  | auspe                        | eraion rest                                | 2.001                                       | 1.040                         | 0.0037                     | Jugi | inicant O                              | veruist                 | Jer SIUT   |                             |                              |
|  |  |  |         |                              |  |   |                               |                            |      |  |                         |  |                             |                              |

| CETIS Ana                               | alytical Repo                                      | rt   |  |  |  |  | Report<br>Test C   | Date:<br>ode/ID:   | 26 -<br>24.00                               | Jun-24 11:4<br>)3.3 Cu / 16                             | 9 (p 2 of 3)<br>-2293-8617                       |
|---|--|--|--|--|--|--|--|--|---|---|--|
| Daphnia thor                            | nsoni 48-h Acute                                   | Survival T   | est  |  |  |  |  |  |   | NIWA Ecot   | oxicology  |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 16-0302-2229<br>26 Jun-24 11:48<br>26 Jun-24 11:46 | End<br>Ana<br>MD   | point: 48<br>lysis: No<br>5 Hash: 2F                     | h Survival R<br>nlinear Reg<br>6FF92B610                 | ate<br>ression (NL<br>D6BE0CFE                           | R)<br>8F75A74FE  | CET<br>Stat<br>F8CB Edit                                 | IS Version:<br>us Level:<br>or ID:                       | CETISv2<br>1<br>008-408-4                   | .1.4<br>407-6   |  |
| 48h Survival                            | Rate Summary                                       |  |  |  |  | Calculate  | d Variate(A  | В)   |   |   |  |
| Conc-µg/L                               | Code   | Count  | Mean   | Median   | Min  | Мах  | Std Err  | Std Dev  | CV%   | %Effect   | ΣΑ/ΣΒ  |
| 1.3<br>14<br>28<br>59<br>136<br>280     | D  | 5<br>3<br>3<br>3<br>3<br>3<br>3                          | 0.9800<br>1.0000<br>1.0000<br>0.9667<br>0.1333<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>1.0000<br>0.0000<br>0.0000 | 0.9000<br>1.0000<br>1.0000<br>0.9000<br>0.0000<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>1.0000<br>0.4000<br>0.0000 | 0.0200<br>0.0000<br>0.0000<br>0.0333<br>0.1333<br>0.0000 | 0.0447<br>0.0000<br>0.0000<br>0.0577<br>0.2309<br>0.0000 | 4.56%<br>0.00%<br>0.00%<br>5.97%<br>173.21% | 0.00%<br>-2.04%<br>-2.04%<br>1.36%<br>86.39%<br>100.00% | 49/50<br>30/30<br>30/30<br>29/30<br>4/30<br>0/30 |
| 48h Survival                            | Rate Detail  |  |  |  |  |  |  |  |   |   |  |
| Conc-µg/L                               | Code   | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |  |  |   |   |  |
| 1.3<br>14<br>28<br>59<br>136<br>280     | D  | 1.0000<br>1.0000<br>1.0000<br>0.0000<br>0.0000<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>1.0000<br>0.4000<br>0.0000 | 1.0000<br>1.0000<br>1.0000<br>0.9000<br>0.0000<br>0.0000 | 1.0000   | 0.9000   |  |  |   |   |  |
| 48h Survival                            | Rate Binomials                                     |  |  |  |  |  |  |  |   |   |  |
| Conc-µg/L                               | Code   | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |  |  |   |   |  |
| 1.3<br>14<br>28<br>59<br>136<br>280     | D  | 10/10<br>10/10<br>10/10<br>10/10<br>0/10<br>0/10         | 10/10<br>10/10<br>10/10<br>10/10<br>4/10<br>0/10         | 10/10<br>10/10<br>10/10<br>9/10<br>0/10<br>0/10          | 10/10  | 9/10   |  |  |   |   |  |



# Appendix I CETIS statistical analyses – Clutha

## Zinc

| CETIS Analy                                    | ytical Rep                          | oort        |                      |                          |                    |  |                                  |                    | R<br>T | eport<br>est Co         | Date:<br>de/ID:              |                        | 24 M<br>24.00                     | lay-24 13:4<br>)3.4 Zn / 02 | 3 (p 1 of 2)<br>2-6719-5256 |
|--|-------------------------------------|-------------|----------------------|--------------------------|--------------------|--|----------------------------------|--------------------|--------|-------------------------|------------------------------|------------------------|-----------------------------------|-----------------------------|-----------------------------|
| Daphnia thoms                                  | oni 48-h Acu                        | ite Surviv  | al T                 | est                      |                    |  |                                  |                    |        |                         |                              |                        | l                                 | NIWA Eco                    | toxicology                  |
| Analysis ID: 0<br>Analyzed: 2<br>Edit Date:    | 95-4485-8179<br>24 May-24 13:       | 41 /        | End<br>Anal<br>MD5   | point:<br>ysis:<br>Hash: | 48h<br>Para<br>A16 | Survival Ra<br>ametric-Mul<br>iB6493E9BF | ite<br>tiple Compa<br>F68A2A8531 | rison<br>143B3283F | DFA    | CETI<br>Statu<br>Edito  | S Versi<br>Is Leve<br>or ID: | ion:<br>I:             | CETISv2.<br>1                     | 1.4                         |                             |
| Batch ID: 1<br>Start Date: 0<br>Ending Date: 0 | 3-2989-6792<br>7 May-24<br>9 May-24 | 1           | Test<br>Prot<br>Spec | Type:<br>ocol:<br>cies:  | Sur<br>NIW<br>Dap  | vival (48h)<br>/A SOP 10<br>ohnia thoms  | (2022)<br>oni (Water f           | lea)               |        | Analy<br>Dilue<br>Brine | yst:<br>ent:<br>e:           | Ecot<br>Cluth<br>Not / | ox Team<br>ha river<br>Applicable |                             | A                           |
| Test Length: 4                                 |                                     |             | Taxo                 | on:                      |                    |  |                                  |                    |        | Sour                    | ce:                          | Field                  | Collected                         |                             | Age:                        |
| Sample ID: 1                                   | 6-8019-3246<br>6 May 24             |             | Cod                  | e:<br>vrial:             | 24.0<br>Zin/       | JU3.4 Zn                                 |                                  |                    |        | Proje                   | ect:                         | Spec                   | tion made by                      |                             |                             |
| Receipt Date: 0                                | 6 May-24                            |             | CAS                  | (PC):                    | 200                | sunate                                   |                                  |                    |        | Stati                   | on:                          | Lab                    | Solution                          |                             |                             |
| Sample Age: 2                                  | 4h                                  | (           | Clier                | nt:                      | Hyd                | Irotoxy Rese                             | earch                            |                    |        |                         |                              |                        |                                   |                             |                             |
| Data Transform                                 | 1                                   | Alt Hy      | /p                   |                          |                    |  |                                  | NOEL               | LO     | EL                      | TOEL                         |                        | Tox Units                         | MSDu                        | PMSD                        |
| Angular (Correct                               | ted)                                | C > T       |                      |                          |                    |  |                                  | 225                | 505    |                         | 337.1                        |                        |                                   | 0.1328                      | 13.55%                      |
| Bonferroni Adj                                 | t Test                              |             |                      |                          |                    |  |                                  |                    |        |                         |                              |                        |                                   |                             |                             |
| Control v                                      | s Conc-µg                           | J/L         | df                   | Test \$                  | Stat               | Critical                                 | MSD                              | P-Type             | P-V    | alue                    | Decis                        | ion(                   | a:5%)                             |                             |                             |
| Dilution Water                                 | 119                                 |             | 6                    | -0.396                   | 69                 | 2.56                                     | 0.2103                           | CDF                | 1.0    | 000                     | Non-S                        | Signif                 | icant Effect                      |                             |                             |
|  | 225                                 |             | 6                    | 2.163                    |                    | 2.56                                     | 0.2103                           | CDF                | 0.1    | 028                     | Non-S                        | Signif                 | icant Effect                      |                             |                             |
|  | 505°<br>1080*                       |             | 6<br>6               | 6.824<br>12.49           |                    | 2.56                                     | 0.2103                           | CDF                | 3.7    | E-05<br>DE-05           | Signif                       | icant<br>icant         | Effect                            |                             |                             |
| ANOVA Table                                    |                                     |             | -                    |                          |                    |  |                                  |                    |        |                         |                              |                        |                                   |                             |                             |
| Source   | Sum Sq                              | uares       |                      | Mean                     | Squ                | are                                      | DF                               | F Stat             | P-V    | alue                    | Decis                        | ion(                   | a:5%)                             |                             |                             |
| Between  | 2.61111                             |             |                      | 0.652                    | 778                |  | 4                                | 51.62              | <1.    | DE-05                   | Signif                       | icant                  | Effect                            |                             |                             |
| Error  | 0.15176                             | 4           |                      | 0.012                    | 647                |  | 12                               | _                  |        |                         | -                            |                        |                                   |                             |                             |
| Total  | 2.76287                             |             |                      |                          |                    |  | 16                               |                    |        |                         |                              |                        |                                   |                             |                             |
| ANOVA Assum                                    | ptions Tests                        |             |                      |                          |                    |  |                                  |                    |        |                         |                              |                        |                                   |                             |                             |
| Attribute                                      | Test                                |             |                      |                          |                    |  | Test Stat                        | Critical           | P-V    | alue                    | Decis                        | ion(                   | a:1%)                             |                             |                             |
| Variance                                       | Bartlett I                          | Equality of | f Var                | iance T                  | est                |  |                                  |                    |        |                         | Indete                       | ermin                  | ate                               |                             |                             |
|  | Levene I<br>Mod Lov                 | Equality of | f Vai                | nance 1                  | rest               | Tost                                     | 3.283                            | 5.412              | 0.0    | 190<br>195              | Equal                        | Varia                  | ances                             |                             |                             |
| Distribution                                   | Anderso                             | n-Darling   | A2 1                 | lest                     | nce                | Test                                     | 0.8802                           | 3.878              | 0.0    | 242                     | Norm                         | al Dis                 | stribution                        |                             |                             |
|  | D'Agosti                            | no Skewn    | ess                  | Test                     |                    |  | 0.05141                          | 2.576              | 0.9    | 590                     | Norm                         | al Dis                 | stribution                        |                             |                             |
|  | Kolmogo                             | prov-Smirr  | nov [                | D Test                   |                    |  | 0.2059                           | 0.2405             | 0.0    | 538                     | Norm                         | al Dis                 | stribution                        |                             |                             |
|  | Shapiro-                            | Wilk W N    | orma                 | ality Te                 | st                 |  | 0.9142                           | 0.848              | 0.1    | 178                     | Norm                         | al Dis                 | stribution                        |                             |                             |
| 48h Survival Ra                                | ate Summary                         | 1           |                      |                          |                    |  |                                  |                    |        |                         |                              |                        |                                   |                             |                             |
| Conc-µg/L                                      | Code                                | Count       |                      | Mean                     | _                  | 95% LCL                                  | 95% UCL                          | Median             | Min    |                         | Max                          |                        | Std Err                           | CV%                         | %Effect                     |
| 2.9  | D                                   | 5           |                      | 0.980                    | 0                  | 0.9245                                   | 1.0000                           | 1.0000             | 0.9    | 000                     | 1.000                        | 0                      | 0.0200                            | 4.56%                       | 0.00%                       |
| 119  |                                     | 3           |                      | 1.000                    | U<br>7             | 1.0000                                   | 1.0000                           | 1.0000             | 1.0    | 000                     | 1.000                        | 0                      | 0.0000                            | 0.00%                       | -2.04%                      |
| 505  |                                     | 3           |                      | 0.533                    | 3                  | 0.7252                                   | 0.8202                           | 0.6000             | 0.0    | 000                     | 0.600                        | 0                      | 0.0555                            | 21.65%                      | 45.58%                      |
| 1080   |                                     | 3           |                      | 0.133                    | 3                  | 0.0000                                   | 0.5128                           | 0.1000             | 0.0    | 000                     | 0.300                        | 0                      | 0.0882                            | 114.56%                     | 86.39%                      |
| 2400   |                                     | 3           |                      | 0.000                    | 0                  | 0.0000                                   | 0.0000                           | 0.0000             | 0.0    | 000                     | 0.000                        | 0                      | 0.0000                            |                             | 100.00%                     |
| Angular (Correc                                | cted) Transfo                       | ormed Su    | mm                   | ary                      |                    |  |                                  |                    |        |                         |                              |                        |                                   |                             |                             |
| Conc-µg/L                                      | Code                                | Count       |                      | Mean                     |                    | 95% LCL                                  | 95% UCL                          | Median             | Min    | <u> </u>                | Max                          |                        | Std Err                           | CV%                         | %Effect                     |
| 2.9  | D                                   | 5           |                      | 1.379                    | 0                  | 1.2890                                   | 1.4700                           | 1.4120             | 1.2    | 490                     | 1.412                        | 0                      | 0.0326                            | 5.28%                       | 0.00%                       |
| 119  |                                     | 3           |                      | 1.412                    | 0                  | 1.4110                                   | 1.4130                           | 1.4120             | 1.4    | 120                     | 1.412                        | 0                      | 0.0000                            | 0.00%                       | -2.36%                      |
| 220  |                                     | 3           |                      | 0.810                    | 0                  | 0.9982                                   | 1.4050                           | 0.8861             | 0.6    | 570<br>R47              | 0.886                        | 0<br>1                 | 0.0473                            | 0.62%                       | 40.63%                      |
| 1080   |                                     | 3           |                      | 0.353                    | 4                  | -0.1738                                  | 0.8805                           | 0.3218             | 0.1    | 588                     | 0.579                        | 6                      | 0.1225                            | 60.05%                      | 74.38%                      |
| 2400   |                                     | 3           |                      | 0.158                    | 8                  | 0.1588                                   | 0.1588                           | 0.1588             | 0.1    | 588                     | 0.158                        | 8                      | 0.0000                            | 0.00%                       | 88.49%                      |
|  |                                     |             |                      |                          |                    |  |                                  |                    |        |                         |                              |                        |                                   |                             |                             |

Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS Ana                               | alytical Repo                  | ort                |  |   |                               |                          | Report Date:<br>Test Code/ID:                   | 24 May-24 13:43 (p 2 of 2)<br>24.003.4 Zn / 02-6719-5256 |
|---|--------------------------------|--------------------|--|---|-------------------------------|--------------------------|---|--|
| Daphnia thor                            | nsoni 48-h Acut                | e Survival         | Test                                   |   |                               |                          |   | NIWA Ecotoxicology                                       |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 05-4485-8179<br>24 May-24 13:4 | End<br>1 And<br>MD | dpoint: 48<br>alysis: Pa<br>5 Hash: A1 | h Survival Ra<br>rametric-Mu<br>6B6493E9B | ate<br>Itiple Com<br>F68A2A85 | parison<br>3143B3283FDF/ | CETIS Version:<br>Status Level:<br>A Editor ID: | CETISv2.1.4<br>1   |
| 48h Survival                            | Rate Detail                    |                    |  |   |                               |                          |   |  |
| Conc-µg/L                               | Code                           | Rep 1              | Rep 2                                  | Rep 3                                     | Rep 4                         | Rep 5                    |   |  |
| 2.9                                     | D                              | 1.0000             | 1.0000                                 | 1.0000                                    | 0.9000                        | 1.0000                   |   |  |
| 119                                     |                                | 1.0000             | 1.0000                                 | 1.0000                                    |                               |                          |   |  |
| 225                                     |                                | 0.9000             | 0.9000                                 | 0.8000                                    |                               |                          |   |  |
| 505                                     |                                | 0.6000             | 0.6000                                 | 0.4000                                    |                               |                          |   |  |
| 1080                                    |                                | 0.0000             | 0.1000                                 | 0.3000                                    |                               |                          |   |  |
| 2400                                    |                                | 0.0000             | 0.0000                                 | 0.0000                                    |                               |                          |   |  |
| Angular (Cor                            | rected) Transfor               | med Detail         | <b>D</b>                               | <b>D</b> 0                                |                               | <b>D C</b>               |   |  |
| Conc-µg/L                               | Code                           | Rep 1              | Rep 2                                  | Rep 3                                     | 1 2490                        | Rep 5                    |   |  |
| 119                                     | U                              | 1.4120             | 1.4120                                 | 1.4120                                    | 1.2450                        | 1.4120                   |   |  |
| 225                                     |                                | 1.2490             | 1.2490                                 | 1,1070                                    |                               |                          |   |  |
| 505                                     |                                | 0.8861             | 0.8861                                 | 0.6847                                    |                               |                          |   |  |
| 1080                                    |                                | 0.1588             | 0.3218                                 | 0.5796                                    |                               |                          |   |  |
| 2400                                    |                                | 0.1588             | 0.1588                                 | 0.1588                                    |                               |                          |   |  |
| 48h Survival                            | Rate Binomials                 |                    |  |   |                               |                          |   |  |
| Conc-µg/L                               | Code                           | Rep 1              | Rep 2                                  | Rep 3                                     | Rep 4                         | Rep 5                    |   |  |
| 2.9                                     | D                              | 10/10              | 10/10                                  | 10/10                                     | 9/10                          | 10/10                    |   |  |
| 119                                     |                                | 10/10              | 10/10                                  | 10/10                                     |                               |                          |   |  |
| 225                                     |                                | 9/10               | 9/10                                   | 8/10                                      |                               |                          |   |  |
| 505                                     |                                | 6/10               | 6/10                                   | 4/10                                      |                               |                          |   |  |
| 1080                                    |                                | 0/10               | 1/10                                   | 3/10                                      |                               |                          |   |  |
| 2400                                    |                                | 0/10               | 0/10                                   | 0/10                                      |                               |                          |   |  |
| Graphics                                |                                |                    |  |   |                               |                          |   |  |
| 1.0 T F                                 |                                |                    |  |   |                               |                          |   | ŧ  |
| 0.9 -                                   | •                              |                    |  |   |                               | 0.20 -                   |   |  |
| 0.8 -                                   |                                |                    |  |   | •                             | 0.15 -                   |   |  |
| <b>2</b> 0.7 -                          |                                |                    |  |   |                               | 0.10 -                   |   |  |
| 0.6 -                                   |                                |                    |  |   |                               | <b>8</b> 0.05 -          |   |  |
| 0.5 -                                   |                                |                    | •                                      |   |                               | Y NO                     |   |  |
| S 0.4 -                                 |                                |                    |  |   |                               | 5 0.00 -                 | /   |  |
| <b>6</b> 0.3 -                          |                                |                    | г                                      |   |                               | -0.05 -                  |   |  |
| 0.2 -                                   |                                |                    |  |   |                               | -0.10 -                  |   |  |
| 0.1 -                                   |                                |                    |  | •   |                               | -0.15 -                  | • •   |  |
| 0.0 -                                   |                                |                    |  | <u> </u>                                  | _                             | -0.20 -                  |   |  |
| 2                                       | .9 D 119                       | 225                | 505                                    | 1080 240                                  | 00                            | -1                       | .5 -1.0 -0.5                                    | 0.0 0.5 1.0 1.5  |
|   |                                | Conc-µ             | ig/L                                   |   |                               |                          | R   | ankits   |
|   |                                |                    |  |   |                               |                          |   |  |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS                                     | Analyti   | cal Repo                     | ort                  |   |   |                                 |                   | R<br>T | eport<br>est Co                | Date:<br>ode/ID:                       | 24<br>24.  | May-24 13:4<br>003.4 Zn / 0 | 43 (p 1 of 3)<br>2-6719-5256 |
|---|---|------------------------------|----------------------|---|---|---------------------------------|-------------------|--------|--------------------------------|--|--|-----------------------------|------------------------------|
| Daphni                                    | a thomsoni                                      | i 48-h Acute                 | Surviva              | Test  |   |                                 |                   |        |                                |  |  | NIWA Eco                    | toxicology                   |
| Analysi<br>Analyze<br>Edit Da             | sID: 16-3<br>ed: 24 M<br>te:                    | 085-5703<br>May-24 13:41     | Er<br>I Ar<br>M      | ndpoint: 48h<br>nalysis: Nor<br>D5 Hash: A10      | n Survival Ra<br>nlinear Regr<br>6B6493E9BI | ate<br>ession (NLI<br>F68A2A853 | R)<br>3143B3283FI | DFA    | CET<br>State<br>Edite          | IS Version<br>us Level:<br>or ID:      | n: CETISv<br>1   | 2.1.4                       |                              |
| Batch II<br>Start Da<br>Ending<br>Test Le | D: 13-2<br>ate: 07 M<br>Date: 09 M<br>ngth: 48h | 989-6792<br>Nay-24<br>Nay-24 | Te<br>Pr<br>St<br>Ta | stType:Su<br>otocol:NIV<br>pecies:Da<br>xon:      | rvival (48h)<br>VA SOP 10<br>phnia thoms    | (2022)<br>oni (Water            | flea)             |        | Anal<br>Dilue<br>Brine<br>Sour | yst: Ec<br>ent: Cl<br>e: No<br>rce: Fi | cotox Team<br>utha river<br>ot Applicable<br>eld Collected | 1                           | Age:                         |
| Sample<br>Sample<br>Receipt<br>Sample     | D: 16-8<br>Date: 06 M<br>Date: 06 M<br>Age: 24h | 019-3246<br>Nay-24<br>Nay-24 | Co<br>Mi<br>Ci       | ode: 24.<br>aterial: Zin<br>AS (PC):<br>ient: Hyd | 003.4 Zn<br>c sulfate<br>drotoxy Rese       | earch                           |                   |        | Proje<br>Sour<br>Stati         | ect: Sp<br>rce: So<br>on: La           | becial Studie<br>blution made<br>ab Solution               | s<br>by NIWA                |                              |
| Non-Lir                                   | near Regres                                     | ssion Optio                  | ns                   |   |   |                                 |                   |        |                                |  |  |                             |                              |
| Model                                     | Name and F                                      | unction                      |                      |   |   | Weightin                        | g Function        |        |                                | PTBS F                                 | unction  | X Trans                     | Y Trans                      |
| 3P Log-                                   | Logistic: µ=                                    | α/[1+[x/δ]^γ]                |                      |   |   | Binomial                        | [ω=n/[p·q]]       |        |                                | Off [µ*=                               | μ]   | None                        | None                         |
| Regres                                    | sion Summ                                       | ary                          |                      |   |   |                                 |                   |        |                                |  |  |                             |                              |
| Iters                                     | LL  | AICc                         | BIC                  | Adj R2  | PMSD  | Thresh                          | Optimize          | F St   | tat                            | P-Value                                | Decision   | η(α:5%)                     |                              |
| 10  | -16.95  | 41.4                         | 42.89                | 0.8282  | 3.01%                                       | 0.9855                          | Yes               | 0.78   | 313                            | 0.5238                                 | Non-Sigr   | hificant Lack               | -of-Fit                      |
| Point E                                   | stimates  |                              |                      |   |   |                                 |                   |        |                                |  |  |                             |                              |
| Level                                     | µg/L  | 95% LCL                      | 95% UC               | L   |   |                                 |                   |        |                                |  |  |                             |                              |
| LC5                                       | 186.5   |                              | 246.8                |   |   |                                 |                   |        |                                |  |  |                             |                              |
| LC10                                      | 242.7   | 124.4                        | 311.2                |   |   |                                 |                   |        |                                |  |  |                             |                              |
| LC15                                      | 285.7   | 180.5                        | 360.3                |   |   |                                 |                   |        |                                |  |  |                             |                              |
| 1.025                                     | 323   | 224.3                        | 402.0                |   |   |                                 |                   |        |                                |  |  |                             |                              |
| LC20                                      | 456.3   | 365.5                        | 555.4                |   |   |                                 |                   |        |                                |  |  |                             |                              |
| LC50                                      | 526.4   | 432.1                        | 641.3                |   |   |                                 |                   |        |                                |  |  |                             |                              |
| Regres                                    | sion Param                                      | eters                        |                      |   |   |                                 |                   |        |                                |  |  |                             |                              |
| Parame                                    | ter   | Estimate                     | Std Erro             | or 95% LCL  | 95% UCL                                     | t Stat                          | P-Value           | Dec    | ision                          | (a:5%)                                 |  |                             |                              |
| a   |   | 0.9855                       | 0.01405              | 0.9559  | 1 0 1 5                                     | 70.13                           | <1.0E-05          | Siar   | nifican                        | t Paramet                              | er   |                             |                              |
| v   |   | 2.838                        | 0.4606               | 1.866   | 3.81  | 6.161                           | 1.0E-05           | Siar   | nifican                        | t Paramet                              | er   |                             |                              |
| ō   |   | 526.4                        | 49.1                 | 422.8   | 630   | 10.72                           | <1.0E-05          | Sigr   | nifican                        | t Paramet                              | er   |                             |                              |
| ANOVA                                     | Table   |                              |                      |   |   |                                 |                   |        |                                |  |  |                             |                              |
| Source                                    |   | Sum Squa                     | ares M               | ean Square  | DF  | F Stat                          | P-Value           | Dec    | ision                          | (α:5%)                                 |  |                             |                              |
| Model                                     |   | 4741                         | 15                   | 80  | 3   | 1903                            | <1.0E-05          | Sigr   | nifican                        | t Effect                               |  |                             |                              |
| Lack of                                   | Fit   | 2.024                        | 0.                   | 6748  | 3   | 0.7813                          | 0.5238            | Non    | -Signi                         | ficant Lac                             | k-of-Fit   |                             |                              |
| Pure Er                                   | ror   | 12.09                        | 0.                   | 3636  | 14  |                                 |                   |        |                                |  |  |                             |                              |
| Residua                                   | al  | 14.11                        | 0.                   | 3303  | 17  |                                 |                   |        |                                |  |  |                             |                              |
| Residu                                    | al Analysis                                     |                              |                      |   |   |                                 |                   |        |                                |  |  |                             |                              |
| Attribut                                  | e   | Method                       |                      |   | Test Stat                                   | Critical                        | P-Value           | Dec    | ision                          | (α:5%)                                 |  |                             |                              |
| Model F                                   | it  | Likelihood                   | Ratio GO             | F Test  | 12.98                                       | 27.59                           | 0.7374            | Non    | -Signi                         | ficant Het                             | erogeneity   |                             |                              |
| Varian                                    |   | Pearson C                    | ni-Sq GO             | F Test  | 14.11                                       | 27.59                           | 0.6590            | Non    | -Signi                         | Ticant Hete                            | erogeneity   |                             |                              |
| Variano                                   | e   | Mod Lever                    | ne Equalit           | y or variance                                     | 0.6852                                      | 3.087                           | 0.6481            | Equ    | al var                         | Iances                                 | tion   |                             |                              |
| Distribu                                  | uun   | Shapire W                    | Daning A.            | z rest<br>mality Test                             | 0.098                                       | 2.492                           | 0.0219            | Non    | mal Di                         | iai Distribution                       | non  |                             |                              |
| Overdis                                   | persion   | Tarone C(                    | a) Overdis           | spersion Test                                     | 1.043                                       | 1.645                           | 0.1485            | Non    | -Siani                         | ficant Ove                             | rdispersion  |                             |                              |
|   |   |                              | ,                    |   |   |                                 |                   |        | 2.1                            |  |  |                             |                              |
|   |   |                              |                      |   |   |                                 |                   |        |                                |  |  |                             |                              |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS And  | alytical Repo                   | ort  |   |  |   |   | Report<br>Test C   | Date:<br>ode/ID:   | 24 I<br>24.0                                 | May-24 13:4<br>03.4 Zn / 02                              | 3 (p 2 of 3)<br>-6719-5256                       |
|--|---------------------------------|--|---|--|---|---|--|--|--|--|--|
| Daphnia thor   | nsoni 48-h Acute                | Survival   | Test  |  |   |   |  |  |  | NIWA Ecot  | toxicology                                       |
| Analysis ID:<br>Analyzed:<br>Edit Date:  | 16-3085-5703<br>24 May-24 13:41 | En<br>An<br>MD   | dpoint: 48<br>alysis: No<br>05 Hash: A1   | h Survival R<br>onlinear Reg<br>6B6493E9B  | ate<br>ression (NL<br>F68A2A85  | R)<br>3143B3283F  | CET<br>Stat<br>DFA Edit                                  | IS Version:<br>us Level:<br>or ID:                       | CETISv2<br>1                                 | .1.4   |  |
| 48h Survival   | Rate Summary                    |  |   |  |   | Calculate   | d Variate(A  | /B)  |  |  |  |
| Conc-µg/L  | Code                            | Count  | Mean  | Median   | Min   | Мах   | Std Err  | Std Dev  | CV%  | %Effect  | ΣΑ/ΣΒ  |
| 2.9<br>119<br>225<br>505<br>1080<br>2400<br>48h Survival<br>Conc-µg/L<br>2.9<br>119<br>225 | D<br>Rate Detail<br>Code<br>D   | 5<br>3<br>3<br>3<br>3<br>3<br><b>Rep 1</b><br>1.0000<br>1.0000<br>0.9000 | 0.9800<br>1.0000<br>0.8667<br>0.5333<br>0.1333<br>0.0000<br>Rep 2<br>1.0000<br>1.0000<br>0.9000 | 1.0000<br>1.0000<br>0.9000<br>0.6000<br>0.1000<br>0.0000<br><b>Rep 3</b><br>1.0000<br>1.0000<br>0.9000 | 0.9000<br>1.0000<br>0.8000<br>0.4000<br>0.0000<br>0.0000<br>Rep 4<br>0.9000 | 1.0000<br>1.0000<br>0.9000<br>0.6000<br>0.3000<br>0.0000<br>Rep 5<br>1.0000 | 0.0200<br>0.0000<br>0.0333<br>0.0667<br>0.0882<br>0.0000 | 0.0447<br>0.0000<br>0.0577<br>0.1155<br>0.1528<br>0.0000 | 4.56%<br>0.00%<br>6.66%<br>21.65%<br>114.56% | 0.00%<br>-2.04%<br>11.56%<br>45.58%<br>86.39%<br>100.00% | 49/50<br>30/30<br>26/30<br>16/30<br>4/30<br>0/30 |
| 505<br>1080<br>2400  |                                 | 0.6000<br>0.0000<br>0.0000   | 0.6000<br>0.1000<br>0.0000  | 0.4000<br>0.3000<br>0.0000   |   |   |  |  |  |  |  |
| 48h Survival   | Rate Binomials                  |  |   |  |   |   |  |  |  |  |  |
| Conc-µg/L  | Code                            | Rep 1  | Rep 2   | Rep 3  | Rep 4   | Rep 5   |  |  |  |  |  |
| 2.9<br>119<br>225<br>505<br>1080<br>2400   | D                               | 10/10<br>10/10<br>9/10<br>6/10<br>0/10<br>0/10                           | 10/10<br>10/10<br>9/10<br>6/10<br>1/10<br>0/10  | 10/10<br>10/10<br>8/10<br>4/10<br>3/10<br>0/10   | 9/10  | 10/10   |  |  |  |  |  |

CETIS™ v2.1.4.5 (009-951-268-0)



Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

# Copper

| CETIS Anal   | yti                         | cal Report                   |                              |  |   |                                  |                     | Re<br>Te     | port l<br>st Co                  | Date:<br>de/ID:                         | 27 N<br>24.00   | 1ay-24 13:5<br>)3.4 Cu / 0 | 55 (p 1 of 3)<br>6-7607-7132 |
|--|-----------------------------|------------------------------|------------------------------|--|---|----------------------------------|---------------------|--------------|----------------------------------|---|---|----------------------------|------------------------------|
| Daphnia thoms  | soni                        | 48-h Acute Surv              | vival T                      | est  |   |                                  |                     |              |                                  |   |   | NIWA Eco                   | toxicology                   |
| Analysis ID:<br>Analyzed:<br>Edit Date:                    | 14-2<br>27 N                | 862-3054<br>lay-24 13:55     | End<br>Anal<br>MD5           | point: 48h<br>lysis: Par<br>i Hash: 685    | Survival Ra<br>ametric-Mul<br>60BC405F8 | ate<br>tiple Compa<br>35A2458FD7 | irison<br>72874CB5B | 6A9          | CETI<br>Statu<br>Edito           | S Version<br>s Level:<br>r ID:          | : CETISv2.<br>1   | 1.4                        |                              |
| Batch ID:<br>Start Date:<br>Ending Date:<br>Test Length:   | 17-3<br>02 N<br>04 N<br>48h | 956-0696<br>lay-24<br>lay-24 | Test<br>Prot<br>Spec<br>Taxo | Type: Sur<br>ocol: NIV<br>cies: Dap<br>on: | vival (48h)<br>VA SOP 10<br>ohnia thoms | (2022)<br>oni (Water fi          | lea)                |              | Analy<br>Dilue<br>Brine<br>Sourc | rst: Eco<br>nt: Clu<br>: Noi<br>ce: Fie | otox Team<br>itha river<br>t Applicable<br>Id Collected |                            | Age:                         |
| Sample ID:<br>Sample Date:<br>Receipt Date:<br>Sample Age: | 10-6<br>01 M<br>01 M<br>24h | 877-4232<br>lay-24<br>lay-24 | Cod<br>Mate<br>CAS<br>Clier  | e: 24.0<br>erial: Cop<br>(PC):<br>nt: Hyd  | 003.4 Cu<br>oper<br>Irotoxy Rese        | earch                            |                     |              | Proje<br>Soure<br>Static         | ct: Spo<br>ce: Sol<br>on: Lat           | ecial Studies<br>lution made b<br>o Solution            | y NIWA                     |                              |
| Data Transform   | n                           | Alt                          | Нур                          |  |   |                                  | NOEL                | LOE          | L                                | TOEL                                    | Tox Units   | MSDu                       | PMSD                         |
| Angular (Correc  | cted)                       | C >                          | т                            |  |   |                                  | 22.5                | 50.5         |                                  | 33.71                                   |   | 0.1229                     | 12.54%                       |
| Bonferroni Adj   | j t Te                      | st                           |                              |  |   |                                  |                     |              |                                  |   |   |                            |                              |
| Control  | vs                          | Conc-µg/L                    | df                           | Test Stat                                  | Critical                                | MSD                              | P-Type              | P-Va         | lue                              | Decision                                | (α:5%)  |                            |                              |
| Dilution Water   |                             | 2.2                          | 6                            | 0.2906                                     | 2.624                                   | 0.1962                           | CDF                 | 1.000        | 00                               | Non-Sign                                | ificant Effect  |                            |                              |
|  |                             | 4.4                          | 6                            | -0.436                                     | 2.624                                   | 0.1962                           | CDF                 | 1.000        | 00                               | Non-Sign                                | ificant Effect  |                            |                              |
|  |                             | 10.3                         | 6                            | -0.430                                     | 2.024                                   | 0.1902                           | CDF                 | 0.110        | 0                                | Non-Sign                                | ifficant Effect   |                            |                              |
|  |                             | 50.5*                        | 6                            | 14.87                                      | 2.624                                   | 0.1962                           | CDF                 | <1.0         | 5-05                             | Significar                              | nt Effect   |                            |                              |
|  |                             |                              | -                            |  |   |                                  |                     |              |                                  |   |   |                            |                              |
| ANOVA Table  |                             |                              |                              |  |   |                                  | E 61-1              | <b>D</b> 1/- |                                  |   |   |                            |                              |
| Source   |                             | Sum Squares                  |                              | Mean Squ                                   | lare                                    |                                  | F Stat              | P-Va         | Tue                              | Decision                                | i(α:5%)   |                            |                              |
| Between  |                             | 3.11472                      |                              | 0.022944                                   | ,                                       | 5                                | 59.44               | <1.0         | 2-05                             | Significal                              | IT Effect   |                            |                              |
| Total  |                             | 3.26144                      |                              | 0.0104602                                  |   | 19                               | _                   |              |                                  |   |   |                            |                              |
| ANOVA Assum  | nptio                       | ns Tests                     |                              |  |   |                                  |                     |              |                                  |   |   |                            |                              |
| Attribute  |                             | Test                         |                              |  |   | Test Stat                        | Critical            | P-Va         | lue                              | Decision                                | ı(α:1%)   |                            |                              |
| Variance   |                             | Bartlett Equality            | of Va                        | iance Test                                 |   |                                  |                     |              |                                  | Indeterm                                | inate   |                            |                              |
|  |                             | Levene Equality              | of Va                        | riance Test                                |   | 3.979                            | 4.695               | 0.018        | 37                               | Equal Va                                | riances   |                            |                              |
|  |                             | Mod Levene Eq                | uality o                     | of Variance                                | Test                                    | 1.966                            | 6.632               | 0.188        | 36                               | Equal Va                                | riances   |                            |                              |
| Distribution   |                             | Anderson-Darlin              | ig A2 1                      | Fest                                       |   | 1.608                            | 3.878               | 1.2E-        | -05                              | Non-Norr                                | nal Distributio   | on                         |                              |
|  |                             | D'Agostino Kurt              | osis Te                      | est  |   | 1.78                             | 2.576               | 0.075        | 51                               | Normal D                                | Distribution  |                            |                              |
|  |                             | D'Agostino Skev              | whess<br>rean K              | 1 est<br>2 Omnibue 1                       | Teet                                    | 1.407                            | 2.570               | 0.155        | 94<br>30                         | Normal L                                | Distribution  |                            |                              |
|  |                             | Kolmogorov-Sm                | irnov [                      | ) Test                                     | rest                                    | 0.3                              | 0.2235              | 5.0E         | -05                              | Non-Norr                                | nal Distributio   | on                         |                              |
|  |                             | Shapiro-Wilk W               | Norma                        | ality Test                                 |   | 0.86                             | 0.866               | 0.007        | 79                               | Non-Norr                                | mal Distributio   | on                         |                              |
| 48h Survival R   | ate                         | Summary                      |                              |  |   |                                  |                     |              |                                  |   |   |                            |                              |
| Conc-µg/L  |                             | Code Cou                     | int                          | Mean                                       | 95% LCL                                 | 95% UCL                          | Median              | Min          |                                  | Мах                                     | Std Err   | CV%                        | %Effect                      |
| 0.25   |                             | D 5                          |                              | 0.9800                                     | 0.9245                                  | 1.0000                           | 1.0000              | 0.900        | 00                               | 1.0000                                  | 0.0200  | 4.56%                      | 0.00%                        |
| 2.2  |                             | 3                            |                              | 0.9667                                     | 0.8232                                  | 1.0000                           | 1.0000              | 0.900        | 00                               | 1.0000                                  | 0.0333  | 5.97%                      | 1.36%                        |
| 4.4  |                             | 3                            |                              | 1.0000                                     | 1.0000                                  | 1.0000                           | 1.0000              | 1.000        | 00                               | 1.0000                                  | 0.0000  | 0.00%                      | -2.04%                       |
| 10.3   |                             | 3                            |                              | 1.0000                                     | 1.0000                                  | 1.0000                           | 1.0000              | 1.000        | 00                               | 1.0000                                  | 0.0000  | 0.00%                      | -2.04%                       |
| 22.5   |                             | 3                            |                              | 0.8667                                     | 0.4872                                  | 1.0000                           | 0.9000              | 0.700        | 00                               | 1.0000                                  | 0.0882  | 17.63%                     | 11.56%                       |
| 50.5   |                             | 3                            |                              | 0.0667                                     | 0.0000                                  | 0.2101                           | 0.1000              | 0.000        | 00                               | 0.1000                                  | 0.0333  | 86.60%                     | 93.20%                       |
|  |                             |                              |                              |  |   |                                  |                     |              |                                  |   |   |                            |                              |

Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS Ana                               | alytical Rep                   | ort                |   |  |                                  |                     | Repor<br>Test C       | t Date:<br>Code/ID:                   | 27<br>24.0   | May-24 13:5<br>03.4 Cu / 0 | 56 (p 2 of 3)<br>6-7607-7132 |
|---|--------------------------------|--------------------|---|--|----------------------------------|---------------------|-----------------------|---------------------------------------|--------------|----------------------------|------------------------------|
| Daphnia thor                            | msoni 48-h Acut                | e Survival         | Test                                    |  |                                  |                     |                       |                                       |              | NIWA Eco                   | toxicology                   |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 14-2862-3054<br>27 May-24 13:5 | Eno<br>5 Ana<br>MD | dpoint: 48<br>alysis: Pa<br>95 Hash: 68 | h Survival Ra<br>arametric-Mul<br>560BC405F8 | ate<br>tiple Compa<br>35A2458FD7 | irison<br>72874CB5E | CE<br>Sta<br>16A9 Edi | TIS Version:<br>tus Level:<br>tor ID: | CETISV2<br>1 | 2.1.4                      |                              |
| Angular (Cor                            | rected) Transfor               | med Summ           | nary                                    |  |                                  |                     |                       |                                       |              |                            |                              |
| Conc-µg/L                               | Code                           | Count              | Mean                                    | 95% LCL                                      | 95% UCL                          | Median              | Min                   | Max                                   | Std Err      | CV%                        | %Effect                      |
| 0.25                                    | D                              | 5                  | 1.3790                                  | 1.2890                                       | 1.4700                           | 1.4120              | 1.2490                | 1.4120                                | 0.0326       | 5.28%                      | 0.00%                        |
| 2.2                                     |                                | 3                  | 1.3580                                  | 1.1240                                       | 1.5910                           | 1.4120              | 1.2490                | 1.4120                                | 0.0543       | 6.93%                      | 1.58%                        |
| 4.4                                     |                                | 3                  | 1.4120                                  | 1.4110                                       | 1.4130                           | 1.4120              | 1.4120                | 1.4120                                | 0.0000       | 0.00%                      | -2.36%                       |
| 10.3                                    |                                | 3                  | 1.4120                                  | 1.4110                                       | 1.4130                           | 1.4120              | 1.4120                | 1.4120                                | 0.0000       | 0.00%                      | -2.36%                       |
| 22.0<br>50.5                            |                                | 3                  | 0.2674                                  | 0.0903                                       | 0.5012                           | 0.3218              | 0.9912                | 0.3218                                | 0.1225       | 17.43%<br>35.18%           | 11.75%<br>80.61%             |
| 48h Survival                            | Rate Detail                    | -                  | 0.2014                                  | 0.0007                                       | 0.0012                           | 0.0210              | 0.1000                | 0.0210                                | 0.0040       | 00.1070                    | 00.0170                      |
| Conc-ua/L                               | Code                           | Rep 1              | Rep 2                                   | Rep 3  | Rep 4                            | Rep 5               |                       |                                       |              |                            |                              |
| 0.25                                    | D                              | 1.0000             | 1.0000                                  | 1.0000                                       | 0.9000                           | 1.0000              |                       |                                       |              |                            |                              |
| 2.2                                     | 2                              | 1.0000             | 0.9000                                  | 1.0000                                       | 0.0000                           |                     |                       |                                       |              |                            |                              |
| 4.4                                     |                                | 1 0000             | 1 0000                                  | 1 0000                                       |                                  |                     |                       |                                       |              |                            |                              |
| 10.3                                    |                                | 1 0000             | 1 0000                                  | 1 0000                                       |                                  |                     |                       |                                       |              |                            |                              |
| 22.5                                    |                                | 0.7000             | 1.0000                                  | 0.9000                                       |                                  |                     |                       |                                       |              |                            |                              |
| 50.5                                    |                                | 0.1000             | 0.1000                                  | 0.0000                                       |                                  |                     |                       |                                       |              |                            |                              |
| Angular (Cor                            | rected) Transfor               | med Detai          |   |  |                                  |                     |                       |                                       |              |                            |                              |
| Conc-ug/l                               | Code                           | Rep 1              | Rep 2                                   | Rep 3  | Ren 4                            | Rep 5               |                       |                                       |              |                            |                              |
| 0.25                                    | D                              | 1 4120             | 1 4120                                  | 1 4120                                       | 1 2490                           | 1 4120              |                       |                                       |              |                            |                              |
| 22                                      | 0                              | 1 4120             | 1 2490                                  | 1 4120                                       | 1.2400                           | 1.4120              |                       |                                       |              |                            |                              |
| 4.4                                     |                                | 1 4120             | 1 4120                                  | 1 4120                                       |                                  |                     |                       |                                       |              |                            |                              |
| 10.3                                    |                                | 1 4120             | 1 4120                                  | 1 4120                                       |                                  |                     |                       |                                       |              |                            |                              |
| 22.5                                    |                                | 0.9912             | 1 4120                                  | 1 2490                                       |                                  |                     |                       |                                       |              |                            |                              |
| 50.5                                    |                                | 0.3218             | 0.3218                                  | 0.1588                                       |                                  |                     |                       |                                       |              |                            |                              |
| 48h Survival                            | Rate Binomials                 |                    |   |  |                                  |                     |                       |                                       |              |                            |                              |
| Conc-µg/L                               | Code                           | Rep 1              | Rep 2                                   | Rep 3  | Rep 4                            | Rep 5               |                       |                                       |              |                            |                              |
| 0.25                                    | D                              | 10/10              | 10/10                                   | 10/10  | 9/10                             | 10/10               |                       |                                       |              |                            |                              |
| 2.2                                     |                                | 10/10              | 9/10                                    | 10/10  |                                  |                     |                       |                                       |              |                            |                              |
| 4.4                                     |                                | 10/10              | 10/10                                   | 10/10  |                                  |                     |                       |                                       |              |                            |                              |
| 10.3                                    |                                | 10/10              | 10/10                                   | 10/10  |                                  |                     |                       |                                       |              |                            |                              |
| 22.5                                    |                                | 7/10               | 10/10                                   | 9/10   |                                  |                     |                       |                                       |              |                            |                              |
| 50.5                                    |                                | 1/10               | 1/10                                    | 0/10   |                                  |                     |                       |                                       |              |                            |                              |
| Graphics                                |                                |                    |   |  |                                  |                     |                       |                                       |              |                            |                              |
| 1.0                                     |                                |                    | _                                       |  |                                  | 0.20 -              |                       |                                       |              |                            | ٠                            |
| 0.9 -                                   | •                              | •                  | Ľ                                       |  |                                  | 0.15 -              |                       |                                       |              |                            | /                            |
| 0.8-                                    |                                |                    |   |  | _ ▼                              |                     |                       |                                       |              |                            |                              |
| 0.07                                    |                                |                    |   |  |                                  | 0.10 -              |                       |                                       |              |                            |                              |
| ate                                     |                                |                    |   |  |                                  | 0.05 -              |                       |                                       |              | <b>~••</b>                 | •                            |
| 0.6-                                    |                                |                    |   |  |                                  | 0.00                |                       |                                       |              | •                          |                              |
| · <u>2</u> 0.5 -                        |                                |                    |   |  | Ā                                | 0.00 -              |                       |                                       |              |                            |                              |
| <b>S</b> 0.4 -                          |                                |                    |   |  |                                  | -0.05 -             |                       |                                       |              |                            |                              |
| <b>6</b> 03                             |                                |                    |   |  | 0                                | 0.10                | /                     |                                       |              |                            |                              |
| <b>T</b> 0.5-                           |                                |                    |   |  |                                  | -0.10               | <u> </u>              | •                                     |              |                            |                              |
| 0.2 -                                   |                                |                    |   |  |                                  | -0.15 - 🦯           |                       |                                       |              |                            |                              |
| 0.1 -                                   |                                |                    |   |  |                                  | -0.20 -             |                       |                                       |              |                            |                              |
| 0.0 -                                   |                                |                    |   | ,  |                                  | ۲                   |                       | , <u>,</u>                            |              |                            |                              |
|   | 0.25 D 2.2                     | 4.4                | 10.3 2                                  | 2.5 50.5                                     |                                  |                     | -1.5 -1               | 1.0 -0.5                              | 0.0 0.       | 5 1.0                      | 1.5                          |
|   |                                | Conc-µ             | ıg/L                                    |  |                                  |                     |                       | R                                     | ankits       |                            |                              |
| Convergent Pr                           | ounding (4 sf)                 |                    |   | CETIST                                       | V21450                           | 09-951-269          | 8-01                  |                                       | Analyst:     | 0                          | A-                           |
| - shiring on the                        | (+ or)                         |                    |   | 0L110  | Jan                              | 25 051 200          |                       |                                       |              |                            | · · ·                        |

| CETIS                                    | Analy                                     | tical Rep                           | ort        |                             |                                 |                       |  |                                 |                  | F<br>T | Report<br>Fest Co               | Date:<br>de/ID:             |                                  | 27<br>24.0                                     | May-24 13:5<br>003.4 Cu / 06 | 56 (p 1 of 3)<br>6-7607-7132 |
|--|---|-------------------------------------|------------|-----------------------------|---------------------------------|-----------------------|--|---------------------------------|------------------|--------|---------------------------------|-----------------------------|----------------------------------|--|------------------------------|------------------------------|
| Daphni                                   | a thomso                                  | ni 48-h Acut                        | e Surv     | ival T                      | est                             |                       |  |                                 |                  |        |                                 |                             |                                  |  | NIWA Eco                     | toxicology                   |
| Analysi<br>Analyze<br>Edit Da            | sID: 15<br>ed: 27<br>te:                  | -7079-1881<br>' May-24 13:5         | 5          | End<br>Ana<br>MD5           | point:<br>lysis:<br>6 Hash:     | 48h (<br>Noni<br>6856 | Survival Ra<br>inear Regr<br>i0BC405F8 | ate<br>ession (NLI<br>15A2458FD | R)<br>)72874CB5B | 6A9    | CETI<br>Statu<br>Edito          | S Vers<br>Is Leve<br>or ID: | sion:<br>el:                     | CETISv<br>1                                    | 2.1.4                        |                              |
| Batch I<br>Start Da<br>Ending<br>Test Le | D: 17<br>ate: 02<br>Date: 04<br>ngth: 48  | -3956-0696<br>May-24<br>May-24<br>h |            | Test<br>Prot<br>Spe<br>Taxe | Type:<br>cocol:<br>cies:<br>on: | Surv<br>NIW<br>Dapt   | ival (48h)<br>A SOP 10<br>nnia thoms   | (2022)<br>oni (Water            | flea)            |        | Analy<br>Dilue<br>Brine<br>Sour | yst:<br>ent:<br>e:<br>ce:   | Ecoto<br>Cluth<br>Not A<br>Field | ox Team<br>na river<br>Applicable<br>Collected | 1                            | Age:                         |
| Sample<br>Sample<br>Receipt<br>Sample    | ID: 10<br>Date: 01<br>Date: 01<br>Age: 24 | -6877-4232<br>May-24<br>May-24<br>h |            | Cod<br>Mate<br>CAS<br>Clie  | e:<br>erial:<br>(PC):<br>nt:    | 24.00<br>Copp<br>Hydr | 03.4 Cu<br>ber<br>otoxy Rese           | earch                           |                  |        | Proje<br>Sour<br>Stati          | ect:<br>ce:<br>on:          | Spec<br>Solut<br>Lab \$          | ial Studie:<br>tion made<br>Solution           | s<br>by NIWA                 |                              |
| Non-Lir                                  | near Regr                                 | ession Opti                         | ons        |                             |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| Model                                    | Name and                                  | I Function                          |            |                             |                                 |                       |  | Weightin                        | g Function       |        |                                 | PTB                         | S Fun                            | ction  | X Trans                      | Y Trans                      |
| 3P Log-                                  | Logistic:                                 | u=α/[1+[x/δ]^                       | <b>v</b> ] |                             |                                 |                       |  | Binomial                        | [ω=n/[p·q]]      |        |                                 | Off [l                      | 1*=h]                            |  | None                         | None                         |
| Regres                                   | sion Sum                                  | mary                                |            |                             |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| Iters                                    | LL  | AICc                                | BIC        |                             | Adj R2                          | 2                     | PMSD                                   | Thresh                          | Optimize         | FS     | itat                            | P-Va                        | lue                              | Decision                                       | n(α:5%)                      |                              |
| 5  | -13.36                                    | 34.21                               | 35.7       |                             | 0.8309                          |                       | 2.21%                                  | 0.9856                          | Yes              | 0.5    | 387                             | 0.663                       | 35                               | Non-Sigr                                       | nificant Lack-               | -of-Fit                      |
| Point E                                  | stimates                                  |                                     |            |                             |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| Level                                    | µg/L                                      | 95% LCL                             | . 95%      | UCL                         |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| LC5                                      | 19.16                                     |                                     | 22.7       | 7                           |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| LC10                                     | 21.81                                     |                                     | 25.6       | 2                           |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| LC15                                     | 23.63                                     |                                     | 27.6       | 5                           |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| LC20                                     | 25.1                                      | 16.13                               | 29.3       | 4                           |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| LC25                                     | 26.38                                     | 19                                  | 30.8       | 7                           |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| LC40                                     | 29.75                                     | 23.91                               | 35.2       | 9                           |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| -  | 51.52                                     | 20.30                               | 50.0       | 2                           |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| Regres                                   | sion Para                                 | meters                              |            |                             |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| Parame                                   | eter                                      | Estimate                            | Std        | Error                       | 95% L(                          | CL                    | 95% UCL                                | t Stat                          | P-Value          | Dee    | cision(                         | α:5%)                       |                                  |  |                              |                              |
| α  |   | 0.9856                              | 0.01       | 032                         | 0.9639                          |                       | 1.007                                  | 95.47                           | <1.0E-05         | Sig    | nificant                        | t Paran                     | neter                            |  |                              |                              |
| Ŷ  |   | 5.769                               | 1.19       | 0                           | 3.244                           |                       | 8.294                                  | 4.82                            | 0.0002           | Sig    | nificant                        | t Paran                     | neter                            |  |                              |                              |
| 0  |   | 31.92                               | 2.03       | 8                           | 20.30                           |                       | 37.48                                  | 12.1                            | <1.0E-05         | Sig    | niicani                         | Paran                       | neter                            |  |                              |                              |
| ANOVA                                    | Table                                     |                                     |            |                             |                                 |                       |  |                                 |                  | _      |                                 |                             |                                  |  |                              |                              |
| Source                                   |   | Sum Squ                             | lares      | Mea                         | n Squar                         | e                     | DF                                     | F Stat                          | P-Value          | Dee    | cision(                         | α:5%)                       |                                  |  |                              |                              |
| Model                                    | <b>F</b> #                                | 9621                                |            | 3201                        | 05                              |                       | 3                                      | 3137                            | <1.0E-05         | Sig    | nificani                        | Effect                      |                                  | 6 F.H  |                              |                              |
| Lack of<br>Pure Er                       |   | 1.799                               |            | 0.59                        | 95<br>2                         |                       | 3                                      | 0.5387                          | 0.0030           | NO     | n-Signii                        | iicant L                    | аск-о                            | 11-FIL   |                              |                              |
| Residua                                  | al  | 17.38                               |            | 1.02                        | 2                               |                       | 17                                     |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| Residu                                   | al Analysi                                | ie                                  |            |                             |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
| Attribut                                 |   | Method                              |            |                             |                                 |                       | Toet Stat                              | Critical                        | D Value          | Dec    | cision(                         | a.2%)                       |                                  |  |                              |                              |
| Model F                                  | ie<br>iit                                 | Likelihoo                           | d Ratio    | GOE                         | Test                            |                       | 14.50                                  | 27.50                           | 0.6251           | Nor    | n-Signit                        | ticant F                    | letero                           | neneity  |                              |                              |
| moderr                                   |   | Pearson                             | Chi-Sa     | GOF                         | Test                            |                       | 17.38                                  | 27.59                           | 0.4290           | Nor    | n-Sianit                        | ficant F                    | letero                           | geneity  |                              |                              |
| Varianc                                  | е   | Mod Leve                            | ene Eq     | uality (                    | of Varian                       | ce                    | 0.5962                                 | 3.687                           | 0.7051           | Equ    | ual Vari                        | iances                      |                                  | ,  |                              |                              |
| Distribu                                 | Distribution Anderson-Darling A2 Test     |                                     |            |                             |                                 |                       | 4.186                                  | 2.492                           | <1.0E-05         | Nor    | n-Norm                          | al Dist                     | ributio                          | n  |                              |                              |
|  |   | Shapiro-V                           | Vilk W     | Norm                        | ality Tes                       | t                     | 0.6397                                 | 0.9044                          | <1.0E-05         | Nor    | n-Norm                          | al Dist                     | ributio                          | n  |                              |                              |
| Overdis                                  | persion                                   | Tarone C                            | (α) Ov     | erdisp                      | ersion Te                       | est                   | 0.8299                                 | 1.645                           | 0.2033           | Nor    | n-Signit                        | ficant (                    | Overdi                           | spersion                                       |                              |                              |
|  |   |                                     |            |                             |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |
|  |   |                                     |            |                             |                                 |                       |  |                                 |                  |        |                                 |                             |                                  |  |                              |                              |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS An                                | alytical Repo                   | ort              |   |   | Report<br>Test C                | Date:<br>ode/ID: | 27<br>24.0               | May-24 13:5<br>003.4 Cu / 06       | 6 (p 2 of 3)<br>-7607-7132 |          |            |
|---|---------------------------------|------------------|---|---|---------------------------------|------------------|--------------------------|------------------------------------|----------------------------|----------|------------|
| Daphnia tho                             | msoni 48-h Acute                | e Survival       | Test                                    |   |                                 |                  |                          |                                    |                            | NIWA Eco | toxicology |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 15-7079-1881<br>27 May-24 13:55 | En<br>5 An<br>MC | dpoint: 48<br>alysis: No<br>05 Hash: 68 | 8h Survival R<br>onlinear Reg<br>9560BC405F | ate<br>ression (NL<br>85A2458FE | R)<br>072874CB58 | CET<br>Stat<br>B6A9 Edit | IS Version:<br>us Level:<br>or ID: | CETISv2<br>1               | 2.1.4    |            |
| 48h Survival                            | Rate Summary                    |                  |   |   |                                 | Calculate        | d Variate(A              | /B)                                |                            |          |            |
| Conc-µg/L                               | Code                            | Count            | Mean                                    | Median                                      | Min                             | Мах              | Std Err                  | Std Dev                            | CV%                        | %Effect  | ΣΑ/ΣΒ      |
| 0.25                                    | D                               | 5                | 0.9800                                  | 1.0000                                      | 0.9000                          | 1.0000           | 0.0200                   | 0.0447                             | 4.56%                      | 0.00%    | 49/50      |
| 2.2                                     |                                 | 3                | 0.9667                                  | 1.0000                                      | 0.9000                          | 1.0000           | 0.0333                   | 0.0577                             | 5.97%                      | 1.36%    | 29/30      |
| 4.4                                     |                                 | 3                | 1.0000                                  | 1.0000                                      | 1.0000                          | 1.0000           | 0.0000                   | 0.0000                             | 0.00%                      | -2.04%   | 30/30      |
| 10.3                                    |                                 | 3                | 1.0000                                  | 1.0000                                      | 1.0000                          | 1.0000           | 0.0000                   | 0.0000                             | 0.00%                      | -2.04%   | 30/30      |
| 22.5                                    |                                 | 3                | 0.8667                                  | 0.9000                                      | 0.7000                          | 1.0000           | 0.0882                   | 0.1528                             | 17.63%                     | 11.56%   | 26/30      |
| 50.5                                    |                                 | 3                | 0.0667                                  | 0.1000                                      | 0.0000                          | 0.1000           | 0.0333                   | 0.0577                             | 86.60%                     | 93.20%   | 2/30       |
| 48h Survival                            | Rate Detail                     |                  |   |   |                                 |                  |                          |                                    |                            |          |            |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                   | Rep 3                                       | Rep 4                           | Rep 5            |                          |                                    |                            |          |            |
| 0.25                                    | D                               | 1.0000           | 1.0000                                  | 1.0000                                      | 0.9000                          | 1.0000           |                          |                                    |                            |          |            |
| 2.2                                     |                                 | 1.0000           | 0.9000                                  | 1.0000                                      |                                 |                  |                          |                                    |                            |          |            |
| 4.4                                     |                                 | 1.0000           | 1.0000                                  | 1.0000                                      |                                 |                  |                          |                                    |                            |          |            |
| 10.3                                    |                                 | 1.0000           | 1.0000                                  | 1.0000                                      |                                 |                  |                          |                                    |                            |          |            |
| 22.5                                    |                                 | 0.7000           | 1.0000                                  | 0.9000                                      |                                 |                  |                          |                                    |                            |          |            |
| 50.5                                    |                                 | 0.1000           | 0.1000                                  | 0.0000                                      |                                 |                  |                          |                                    |                            |          |            |
| 48h Survival                            | Rate Binomials                  |                  |   |   |                                 |                  |                          |                                    |                            |          |            |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                   | Rep 3                                       | Rep 4                           | Rep 5            |                          |                                    |                            |          |            |
| 0.25                                    | D                               | 10/10            | 10/10                                   | 10/10                                       | 9/10                            | 10/10            |                          |                                    |                            |          |            |
| 2.2                                     |                                 | 10/10            | 9/10                                    | 10/10                                       |                                 |                  |                          |                                    |                            |          |            |
| 4.4                                     |                                 | 10/10            | 10/10                                   | 10/10                                       |                                 |                  |                          |                                    |                            |          |            |
| 10.3                                    |                                 | 10/10            | 10/10                                   | 10/10                                       |                                 |                  |                          |                                    |                            |          |            |
| 22.5                                    |                                 | 7/10             | 10/10                                   | 9/10  |                                 |                  |                          |                                    |                            |          |            |

50.5

1/10

1/10

0/10

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Analyst:\_\_\_\_\_ QA:\_\_\_\_



CETIS™ v2.1.4.5 (009-951-268-0)

# Appendix J CETIS statistical analyses – Waihou

## Zinc

| CETIS Analyti  | cal Report                   | :                  |                             |   | Re<br>Te                        | eport<br>est Co   | Date:<br>de/ID: |                        | 27 M<br>24.00                  | lay-24 13:3<br>)3.5 Zn / 1 | 35 (p 1 of 2)<br>6-7680-7646           |                |             |
|--|------------------------------|--------------------|-----------------------------|---|---------------------------------|-------------------|-----------------|------------------------|--------------------------------|----------------------------|--|----------------|-------------|
| Daphnia thomson  | i 48-h Acute S               | urvival T          | est                         |   |                                 |                   |                 |                        |                                |                            | I                                      | NIWA Eco       | otoxicology |
| Analysis ID: 20-4<br>Analyzed: 27 M<br>Edit Date:          | 181-6750<br>Nay-24 13:34     | End<br>Ana<br>MDS  | point:<br>lysis:<br>5 Hash: | 48h Survival Ra<br>Parametric-Mul<br>1A253C1D48Bl | ate<br>tiple Compa<br>DA445832E | rison<br>D5DA447D | 9A7B            | CETI<br>Statu<br>Edito | S Versio<br>Is Level<br>or ID: | on:<br>:                   | CETISv2.<br>1                          | 1.4            |             |
| Batch ID: 11-2<br>Start Date: 07 M                         | 219-0889<br>May-24           | Test<br>Prot       | Type:<br>ocol:              | Survival (48h)<br>NIWA SOP 10                     | (2022)                          |                   |                 | Analy<br>Dilue         | yst: E<br>ent: \               | Ecoto<br>Waih              | ox Team<br>ou                          |                |             |
| Ending Date: 09 M<br>Test Length: 48h                      | lay-24                       | Spe<br>Tax         | cies:<br>on:                | Daphnia thoms                                     | oni (Water f                    | lea)              |                 | Brine<br>Sour          | e: N<br>ce: F                  | Not A<br>Field             | pplicable<br>Collected                 |                | Age:        |
| Sample ID: 06-1<br>Sample Date: 06 M<br>Receipt Date: 06 M | 101-1726<br>1ay-24<br>1ay-24 | Cod<br>Mate<br>CAS | e:<br>erial:<br>(PC):       | 24.003.5 Zn<br>Zinc sulfate                       | arab                            |                   |                 | Proje<br>Sour<br>Stati | ect: S<br>ce: S<br>on: L       | Speci<br>Solut<br>Lab S    | ial Studies<br>ion made by<br>Solution | y NIWA         |             |
| Sample Age: 241  |                              | Cile               | nt:                         | Hydrotoxy Rese                                    | earch                           |                   |                 |                        |                                |                            |  |                |             |
| Data Transform   | A                            | lt Hyp             |                             |   |                                 | NOEL              | LOE             | L                      | TOEL                           |                            | Tox Units                              | MSDu<br>0.1242 | PMSD        |
| Angular (Corrected)  |                              | ~1                 |                             |   |                                 | 110               | 230             |                        | 103.5                          |                            |  | 0.1242         | 12.93%      |
| Bonterroni Adj t li  | est                          | -16                | Test C                      | tat Critical                                      | MED                             | Dime              | DV              | -                      | Decisi                         |                            | -E9()                                  |                |             |
| Dilution Water   | 116                          | 6                  | 0.5547                      | 2 466   | 0.1932                          | CDF               | 0.88            | nue<br>69              | Non-Si                         | ianifi                     | cant Effect                            |                |             |
|  | 230*<br>500*                 | 6<br>6             | 3.443<br>8.47               | 2.466<br>2.466                                    | 0.1932<br>0.1932                | CDF               | 0.00<br>1.1E    | 95<br>-05              | Signifi<br>Signifi             | cant<br>cant               | Effect<br>Effect                       |                |             |
| ANOVA Table  |                              |                    |                             |   |                                 |                   |                 |                        |                                |                            |  |                |             |
| Source   | Sum Square                   | s                  | Mean                        | Square  | DF                              | F Stat            | P-Va            | alue                   | Decisi                         | ion(a                      | :5%)                                   |                |             |
| Between  | Between 0.931589             |                    |                             | 3   | 3                               | 26.98             | 4.1E            | -05                    | Signifi                        | cant                       | Effect                                 |                |             |
| Total  |                              | 0.0115             | 087                         | 10  | _                               |                   |                 |                        |                                |                            |  |                |             |
| ANOVA Assumptio  | ons Tests                    |                    |                             |   |                                 |                   |                 |                        |                                |                            |  |                |             |
| Attribute  | Test                         |                    |                             |   | Test Stat                       | Critical          | P-Va            | alue                   | Decisi                         | ion(a                      | (:1%)                                  |                |             |
| Variance   | Bartlett Equa                | lity of Va         | riance Te                   | est   | 0.7195                          | 11.34             | 0.86            | 86                     | Equal                          | Varia                      | inces                                  |                |             |
|  | Levene Equa                  | lity of Va         | riance Te                   | est<br>es Test                                    | 0.8939                          | 6.552             | 0.47            | 76                     | Equal                          | Varia                      | inces                                  |                |             |
| Distribution   | Anderson-Da                  | rling A2           | or variari<br>Test          | ce rest   | 0.09641                         | 9.78<br>3.878     | 0.95            | 92<br>49               | Norma                          | vana<br>al Dis             | tribution                              |                |             |
|  | D'Agostino S                 | kewness            | Test                        |   | 0.6961                          | 2.576             | 0.48            | 64                     | Norma                          | al Dis                     | tribution                              |                |             |
|  | Kolmogorov-                  | Smirnov            | D Test                      |   | 0.2182                          | 0.262             | 0.06            | 96                     | Norma                          | al Dis                     | tribution                              |                |             |
|  | Shapiro-Wilk                 | W Norm             | ality Tes                   | t   | 0.8807                          | 0.8239            | 0.05            | 95                     | Norma                          | al Dis                     | tribution                              |                |             |
| 48h Survival Rate  | Summary                      |                    |                             |   |                                 |                   |                 |                        |                                |                            |  |                |             |
| Conc-µg/L  | Code C                       | ount               | Mean                        | 95% LCL   | 95% UCL                         | Median            | Min             |                        | Max                            |                            | Std Err                                | CV%            | %Effect     |
| 3.25   | D 5                          |                    | 0.9600                      | 0.8920  | 1.0000                          | 1.0000            | 0.90            | 00                     | 1.0000                         | )                          | 0.0245                                 | 5.71%          | 0.00%       |
| 230  | 3                            |                    | 0.9333                      | 0.7699  | 1.0000                          | 0.9000            | 0.90            | 00                     | 0.9000                         | ,<br>)                     | 0.0333                                 | 0.19%          | 2.76%       |
| 500  | 3                            |                    | 0 4000                      | 0.1516  | 0.6484                          | 0 4000            | 0.30            | 00                     | 0.5000                         | ,<br>)                     | 0.0577                                 | 25.00%         | 58.33%      |
| 1110   | 3                            |                    | 0.0000                      | 0.0000  | 0.0000                          | 0.0000            | 0.00            | 00                     | 0.0000                         | )                          | 0.0000                                 |                | 100.00%     |
| 2450   | 3                            |                    | 0.0000                      | 0.0000  | 0.0000                          | 0.0000            | 0.00            | 00                     | 0.0000                         | )                          | 0.0000                                 |                | 100.00%     |
| Angular (Correcte  | d) Transforme                | d Summ             | ary                         |   |                                 |                   |                 |                        |                                |                            |  |                |             |
| Conc-µg/L  | Code C                       | ount               | Mean                        | 95% LCL   | 95% UCL                         | Median            | Min             |                        | Мах                            |                            | Std Err                                | CV%            | %Effect     |
| 3.25   | D 5                          |                    | 1.3470                      | 1.2360  | 1.4580                          | 1.4120            | 1.24            | 90                     | 1.4120                         | )                          | 0.0399                                 | 6.63%          | 0.00%       |
| 116  | 3                            |                    | 1.3030                      | 1.0700  | 1.5370                          | 1.2490            | 1.24            | 90                     | 1.4120                         | )                          | 0.0543                                 | 7.22%          | 3.23%       |
| ∠30<br>500   | 3                            |                    | 1.0770                      | 0./0/3  | 1.44/0                          | 0.9912            | 0.99            | 12                     | 1.2490                         | ,                          | 0.0860                                 | 13.82%         | 20.03%      |
| 1110   | 3                            |                    | 0.0833                      | 0.4277  | 0.9588                          | 0.0047            | 0.57            | 90<br>88               | 0.7804                         | •                          | 0.0094                                 | 0.00%          | 49.27%      |
| 2450   | 2450 3                       |                    | 0.1588                      | 0.1588  | 0.1588                          | 0.1588            | 0 15            | 88                     | 0.1588                         | 3                          | 0.0000                                 | 0.00%          | 88.21%      |
|  | -                            |                    |                             |   |                                 |                   |                 |                        |                                |                            |  |                |             |

Convergent Rounding (4 sf)

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| CETIS And                               | alytical Repo                   | ort                |  |   |                                 | 1                       | Report Date:<br>Test Code/ID:                   | 27 May-24 13:36 (p 2 of 2)<br>24.003.5 Zn / 16-7680-7646 |
|---|---------------------------------|--------------------|--|---|---------------------------------|-------------------------|---|--|
| Daphnia thor                            | msoni 48-h Acute                | Survival 1         | est                                      |   |                                 |                         |   | NIWA Ecotoxicology                                       |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 20-4181-6750<br>27 May-24 13:34 | End<br>4 Ana<br>MD | lpoint: 48h<br>lysis: Par<br>5 Hash: 1A2 | n Survival Ra<br>rametric-Mu<br>253C1D48B | ate<br>Itiple Comp<br>DA4458328 | arison<br>ED5DA447D9A7E | CETIS Version:<br>Status Level:<br>B Editor ID: | CETISv2.1.4<br>1   |
| 48h Survival                            | Rate Detail                     |                    |  |   |                                 |                         |   |  |
| Conc-µg/L                               | Code                            | Rep 1              | Rep 2                                    | Rep 3                                     | Rep 4                           | Rep 5                   |   |  |
| 3.25                                    | D                               | 1.0000             | 0.9000                                   | 1.0000                                    | 0.9000                          | 1.0000                  |   |  |
| 116                                     |                                 | 0.9000             | 1.0000                                   | 0.9000                                    |                                 |                         |   |  |
| 230                                     |                                 | 0.9000             | 0.7000                                   | 0.7000                                    |                                 |                         |   |  |
| 500                                     |                                 | 0.5000             | 0.4000                                   | 0.3000                                    |                                 |                         |   |  |
| 1110                                    |                                 | 0.0000             | 0.0000                                   | 0.0000                                    |                                 |                         |   |  |
| 2450                                    |                                 | 0.0000             | 0.0000                                   | 0.0000                                    |                                 |                         |   |  |
| Angular (Cor                            | rected) Transfor                | med Detail         |  |   |                                 |                         |   |  |
| Conc-µg/L                               | Code                            | Rep 1              | Rep 2                                    | Rep 3                                     | Rep 4                           | Rep 5                   |   |  |
| 3.25                                    | D                               | 1.4120             | 1.2490                                   | 1.4120                                    | 1.2490                          | 1.4120                  |   |  |
| 116                                     |                                 | 1.2490             | 1.4120                                   | 1.2490                                    |                                 |                         |   |  |
| 230                                     |                                 | 1.2490             | 0.9912                                   | 0.9912                                    |                                 |                         |   |  |
| 500                                     |                                 | 0.7854             | 0.6847                                   | 0.5796                                    |                                 |                         |   |  |
| 2450                                    |                                 | 0.1588             | 0.1588                                   | 0.1588                                    |                                 |                         |   |  |
| 2430                                    |                                 | 0.1500             | 0.1300                                   | 0.1300                                    |                                 |                         |   |  |
| 48h Survival                            | Rate Binomials                  |                    |  |   |                                 |                         |   |  |
| Conc-µg/L                               | Code                            | Rep 1              | Rep 2                                    | Rep 3                                     | Rep 4                           | Rep 5                   |   |  |
| 3.25                                    | D                               | 10/10              | 9/10                                     | 10/10                                     | 9/10                            | 10/10                   |   |  |
| 116                                     |                                 | 9/10               | 10/10                                    | 9/10                                      |                                 |                         |   |  |
| 230                                     |                                 | 9/10               | //10                                     | //10                                      |                                 |                         |   |  |
| 1110                                    |                                 | 0/10               | 4/10                                     | 3/10                                      |                                 |                         |   |  |
| 2450                                    |                                 | 0/10               | 0/10                                     | 0/10                                      |                                 |                         |   |  |
| Graphice                                |                                 | 0/10               | 0,10                                     | 0,10                                      |                                 |                         |   |  |
|   |                                 |                    |  |   |                                 |                         |   |  |
| 0.9 -                                   | •                               |                    |  |   |                                 | 0.15 -                  |   | •  |
| 0.8-                                    |                                 |                    |  |   | _ ▼                             |                         |   |  |
| <b>0</b> 07                             |                                 |                    |  |   |                                 | 0.10 -                  |   | •  |
| Rat                                     |                                 |                    |  |   |                                 | 0.05-                   |   | • • •  |
| a                                       |                                 |                    |  |   |                                 | 6.00                    |   |  |
| 0.5-                                    |                                 | Г                  |  |   |                                 | 0.00 -                  | ••  | ••••   |
| <u>v</u> 0.4 –                          |                                 |                    | •  |   | 6                               | 3                       |   |  |
| <b>6</b> 0.3 -                          |                                 | L                  |  |   |                                 | -0.05 -                 | <b>.</b> .                                      |  |
| 0.2 -                                   |                                 |                    |  |   |                                 |                         | <b>.</b> .                                      |  |
| 0.1 -                                   |                                 |                    |  |   |                                 | -0.10 -                 |   |  |
| 0.0 -                                   |                                 |                    |  | <u> </u>                                  |                                 | -0.15 -                 |   |  |
|   | 3.25 D 116                      | 230                | 500 11                                   | 10 2450                                   |                                 | -1.5                    | -1.0 -0.5                                       | 0.0 0.5 1.0 1.5  |
|   |                                 | Conc-u             | g/L                                      |   |                                 |                         | R   | ankits   |
|   |                                 |                    | -  |   |                                 |                         |   |  |
|   |                                 |                    |  |   |                                 |                         |   |  |

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| CETIS                                | S Analyt   | ical Repo                          |        |                            |  |  | R                              | eport E<br>est Cod | )ate:<br>de/ID: |                           | 27<br>24.0                   | May-24 13:3<br>)03.5 Zn / 10 | 36 (p 1 of 3)<br>5-7680-7646           |               |            |
|--------------------------------------|--|------------------------------------|--------|----------------------------|--|--|--------------------------------|--------------------|-----------------|---------------------------|------------------------------|------------------------------|--|---------------|------------|
| Daphni                               | a thomsor  | ni 48-h Acute                      | Surv   | ival T                     | est                                      |  |                                |                    |                 |                           |                              |                              |  | NIWA Eco      | toxicology |
| Analysi<br>Analyzo<br>Edit Da        | is ID: 02-<br>ed: 27<br>ite:   | 3974-0228<br>May-24 13:35          | j      | End<br>Ana<br>MD5          | point: 48h<br>Iysis: Nor<br>5 Hash: 1A2  | n Survival Ra<br>nlinear Regr<br>253C1D48B | ate<br>ession (NL<br>DA4458321 | R)<br>ED5DA447D    | 9 <b>A7B</b>    | CETIS<br>Status<br>Editor | 6 Versio<br>s Level<br>r ID: | on:<br>:                     | CETISv2<br>1                           | 2.1.4         |            |
| Batch I<br>Start D                   | D: 11-<br>ate: 07  | 2219-0889<br>May-24                |        | Test<br>Prot               | Type: Sur                                | vival (48h)<br>VA SOP 10                   | (2022)                         |                    |                 | Analy:<br>Diluer          | st: E<br>nt: \               | Ecoto<br>Naih                | ox Team<br>Iou                         |               |            |
| Ending<br>Test Le                    | Date: 09<br>ength: 48h   | May-24<br>า                        |        | Spe<br>Taxe                | cies: Dap<br>on:                         | phnia thoms                                | oni (Water                     | flea)              |                 | Brine:<br>Sourc           | : N<br>:e: F                 | Not A<br>Field               | Applicable<br>Collected                |               | Age:       |
| Sample<br>Sample<br>Receip<br>Sample | e ID: 06-<br>e Date: 06<br>t Date: 06<br>e Age: 24h                    | 1101-1726<br>May-24<br>May-24<br>า |        | Cod<br>Mate<br>CAS<br>Clie | e: 24.<br>erial: Zin<br>(PC):<br>nt: Hyd | 003.5 Zn<br>c sulfate<br>drotoxy Rese      | earch                          |                    |                 | Projec<br>Sourc<br>Statio | ct: S<br>ce: S<br>n: L       | Spec<br>Solut<br>Lab \$      | ial Studies<br>tion made l<br>Solution | by NIWA       |            |
| Non-Li                               | near Regre<br>Name and   | ession Option                      | ns     |                            |  |  | Weightin                       | a Function         |                 |                           | DTRS                         | Fun                          | ction                                  | ¥ Trans       | V Trans    |
| 3P Log                               | Logistic: µ  | =α/[1+[x/δ]^v]                     |        |                            |  |  | Binomial                       | [ω=n/[p·q]]        |                 |                           | Off [µ*                      | =µ]                          | cuon                                   | None          | None       |
| Regree                               | sion Sum   | mary                               |        |                            |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| Iters                                | 11   | AICo                               | BIC    |                            | Adi R2                                   | PMSD                                       | Thresh                         | Ontimize           | E St            | tat                       | D.Valu                       | 10                           | Decision                               | (a:5%)        |            |
| 9                                    | -17.38   | 42.26                              | 43.7   | 5                          | 0.8533                                   | 4.30%                                      | 0.9529                         | Yes                | 1.94            | 19                        | 0.1682                       | 2                            | Non-Sign                               | ificant Lack- | of-Fit     |
| Point E                              | stimates   |                                    |        |                            |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| Level                                | µg/L   | 95% LCL                            | 95%    | UCL                        |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| LC5                                  | 161.8  |                                    | 206.   | 9                          |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| LC10                                 | 204.1  | 121.3                              | 251.   | 9                          |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| LC15                                 | 235.6  | 162.8                              | 286.   | 6                          |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| LC20                                 | 262.5  | 194.8                              | 316.   | 1                          |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| LC25                                 | 287  | 222.6                              | 343    |                            |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| LC40                                 | 356  | 296.5                              | 419.   | 1<br>0                     |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| LCOU                                 | 403.7  | 344                                | 413.   | 0                          |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| Regres                               | sion Parar   | neters                             |        |                            |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| Parame                               | eter   | Estimate                           | Std    | Error                      | 95% LCL                                  | 95% UCL                                    | t Stat                         | P-Value            | Dec             | ision(a                   | :5%)                         |                              |  |               |            |
| α                                    |  | 0.9529                             | 0.01   | 943                        | 0.9119                                   | 0.9939                                     | 49.05                          | <1.0E-05           | Sigr            | nificant                  | Parame                       | eter                         |  |               |            |
| Ŷ                                    |  | 3.22                               | 0.48   | 4                          | 2.199                                    | 4.241                                      | 0.053                          | <1.0E-05           | Sigr            | nificant                  | Parame                       | eter                         |  |               |            |
| -                                    | Table  | 403.1                              | 51.4   |                            | 331.3                                    | 470  | 12.00                          | <1.0L-03           | Jigi            | incan                     | raiaine                      | etei                         |  |               |            |
| Source                               | Table  | Sum Saus                           | roe    | Моз                        | n Square                                 | DE   | E Stat                         | D Value            | Dec             | ision(a                   |                              |                              |  |               |            |
| Model                                |  | 1601                               | 1105   | 533                        | 7  | 3  | 083.6                          | <1.0E-05           | Sigr            | nificant                  | Effect                       |                              |  |               |            |
| Lack of                              | Fit  | 2 717                              |        | 0.00                       | ,<br>58                                  | 3  | 1 0/10                         | 0.1682             | Non             | -Signifi                  | cant La                      | ick-o                        | f_Fit                                  |               |            |
| Pure Er                              | TOF  | 6.507                              |        | 0.46                       | 48                                       | 14   | 1.040                          | 0.1002             | 1401            | , orginin                 | oant Ea                      |                              |  |               |            |
| Residua                              | al   | 9.224                              |        | 0.54                       | 26                                       | 17   |                                |                    |                 |                           |                              |                              |  |               |            |
| Residu                               | al Analysis  | 8                                  |        |                            |  |  |                                |                    |                 |                           |                              |                              |  |               |            |
| Attribu                              | te   | Method                             |        |                            |  | Test Stat                                  | Critical                       | P-Value            | Dec             | ision(a                   | 1:5%)                        |                              |  |               |            |
| Model F                              | it   | Likelihood                         | Ratio  | GOF                        | Test                                     | 11.78                                      | 27.59                          | 0.8134             | Non             | -Signifi                  | cant He                      | etero                        | geneity                                |               |            |
|                                      |  | Pearson C                          | hi-Sq  | GOF                        | Test                                     | 9.224                                      | 27.59                          | 0.9330             | Non             | -Signifi                  | cant He                      | etero                        | geneity                                |               |            |
| variand                              | e  | Mod Lever                          | ie Equ | ality (                    | or variance                              | 0.5622                                     | 3.687                          | 0.7275             | Equ             | ial Varia                 | Inces                        | hu di c                      |  |               |            |
| Distribu                             | Distribution Anderson-Darling A2 Test<br>Shapiro-Wilk W Normality Test |                                    |        |                            |  | 0.9058                                     | 2.492                          | 0.0150             | Non             | Noma                      | II DISTII                    | DUTIO<br>butic               | 11<br>12                               |               |            |
| Overdis                              | persion  | Tarone C(                          | a) Ove | erdisp                     | ersion Test                              | 0.9324                                     | 1.645                          | 0.0420             | Non             | -Norma<br>I-Signifi       | cant Ov                      | verdi                        | spersion                               |               |            |
|                                      |  |                                    |        |                            |  |  |                                |                    |                 |                           |                              |                              |  |               |            |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS Ana                               | alytical Repo                   | ort              |  |   |                                 |                 | Report<br>Test Co | Date:<br>ode/ID:                   | 27<br>24.0   | May-24 13:3<br>)03.5 Zn / 16 | 6 (p 2 of 3)<br>-7680-7646 |
|---|---------------------------------|------------------|--|---|---------------------------------|-----------------|-------------------|------------------------------------|--------------|------------------------------|----------------------------|
| Daphnia thor                            | nsoni 48-h Acute                | Survival         | Test                                   |   |                                 |                 |                   |                                    |              | NIWA Ecot                    | oxicology                  |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 02-3974-0228<br>27 May-24 13:35 | Enc<br>Ana<br>MD | dpoint: 48<br>alysis: No<br>5 Hash: 1A | h Survival R<br>onlinear Reg<br>253C1D48B | ate<br>ression (NL<br>DA4458321 | R)<br>ED5DA447[ | CET<br>Stat       | IS Version:<br>us Level:<br>or ID: | CETISv2<br>1 | 2.1.4                        |                            |
| 48h Survival                            | Rate Summary                    |                  |  |   |                                 | Calculate       | d Variate(A       | /B)                                |              |                              |                            |
| Conc-µg/L                               | Code                            | Count            | Mean                                   | Median                                    | Min                             | Мах             | Std Err           | Std Dev                            | CV%          | %Effect                      | ΣΑ/ΣΒ                      |
| 3.25                                    | D                               | 5                | 0.9600                                 | 1.0000                                    | 0.9000                          | 1.0000          | 0.0245            | 0.0548                             | 5.71%        | 0.00%                        | 48/50                      |
| 116                                     |                                 | 3                | 0.9333                                 | 0.9000                                    | 0.9000                          | 1.0000          | 0.0333            | 0.0577                             | 6.19%        | 2.78%                        | 28/30                      |
| 230                                     |                                 | 3                | 0.7667                                 | 0.7000                                    | 0.7000                          | 0.9000          | 0.0667            | 0.1155                             | 15.06%       | 20.14%                       | 23/30                      |
| 500                                     |                                 | 3                | 0.4000                                 | 0.4000                                    | 0.3000                          | 0.5000          | 0.0577            | 0.1000                             | 25.00%       | 58.33%                       | 12/30                      |
| 1110                                    |                                 | 3                | 0.0000                                 | 0.0000                                    | 0.0000                          | 0.0000          | 0.0000            | 0.0000                             |              | 100.00%                      | 0/30                       |
| 2450                                    |                                 | 3                | 0.0000                                 | 0.0000                                    | 0.0000                          | 0.0000          | 0.0000            | 0.0000                             |              | 100.00%                      | 0/30                       |
| 48h Survival                            | Rate Detail                     |                  |  |   |                                 |                 |                   |                                    |              |                              |                            |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                  | Rep 3                                     | Rep 4                           | Rep 5           |                   |                                    |              |                              |                            |
| 3.25                                    | D                               | 1.0000           | 0.9000                                 | 1.0000                                    | 0.9000                          | 1.0000          |                   |                                    |              |                              |                            |
| 116                                     |                                 | 0.9000           | 1.0000                                 | 0.9000                                    |                                 |                 |                   |                                    |              |                              |                            |
| 230                                     |                                 | 0.9000           | 0.7000                                 | 0.7000                                    |                                 |                 |                   |                                    |              |                              |                            |
| 500                                     |                                 | 0.5000           | 0.4000                                 | 0.3000                                    |                                 |                 |                   |                                    |              |                              |                            |
| 1110                                    |                                 | 0.0000           | 0.0000                                 | 0.0000                                    |                                 |                 |                   |                                    |              |                              |                            |
| 2450                                    |                                 | 0.0000           | 0.0000                                 | 0.0000                                    |                                 |                 |                   |                                    |              |                              |                            |
| 48h Survival                            | Rate Binomials                  |                  |  |   |                                 |                 |                   |                                    |              |                              |                            |
| Conc-µg/L                               | Code                            | Rep 1            | Rep 2                                  | Rep 3                                     | Rep 4                           | Rep 5           |                   |                                    |              |                              |                            |
| 3.25                                    | D                               | 10/10            | 9/10                                   | 10/10                                     | 9/10                            | 10/10           |                   |                                    |              |                              |                            |
| 116                                     |                                 | 9/10             | 10/10                                  | 9/10                                      |                                 |                 |                   |                                    |              |                              |                            |
| 230                                     |                                 | 9/10             | 7/10                                   | 7/10                                      |                                 |                 |                   |                                    |              |                              |                            |
| 500                                     |                                 | 5/10             | 4/10                                   | 3/10                                      |                                 |                 |                   |                                    |              |                              |                            |
| 1110                                    |                                 | 0/10             | 0/10                                   | 0/10                                      |                                 |                 |                   |                                    |              |                              |                            |

2450

0/10

0/10

0/10

CETIS™ v2.1.4.5 (009-951-268-0)



Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

# Copper

| CETIS Ana  | lyti                        | cal Repo   | ort  |   |   |                    | R  | eport<br>est Co  | Date:<br>de/ID:                                    |  | 27 M<br>24.00                   | ay-24 14:0<br>3.5 Cu / 1                           | )2 (p 1 of 2)<br>5-1536-4540                       |  |                  |            |
|--|-----------------------------|--|--|---|---|--------------------|--|--|--|--|---------------------------------|--|--|--|------------------|------------|
| Daphnia thom   | soni                        | 48-h Acute   | Survi  | ival T  | est   |                    |  |  |  |  |                                 |  |  | I  | NIWA Eco         | toxicology |
| Analysis ID:<br>Analyzed:<br>Edit Date:                                      | 18-8<br>27 M                | 837-2322<br> ay-24 14:01   |  | End<br>Anal<br>MD5  | point:<br>lysis:<br>Hash:                                   | 48h<br>Par<br>0B0  | Survival Ra<br>ametric-Mul<br>7C0191126  | ite<br>tiple Compa<br>9DEBFC61:                        | rison<br>2C1A53753                                 | B59  | CETI<br>Statu<br>Edito          | S Versi<br>Is Leve<br>or ID:                       | on:<br>I:  | CETISv2.<br>1  | 1.4              |            |
| Batch ID:<br>Start Date:<br>Ending Date:<br>Test Length:                     | 20-8<br>04 M<br>06 M<br>48h | 394-3851<br>ay-24<br>ay-24   |  | Test<br>Prot<br>Spec<br>Taxo                              | Type:<br>ocol:<br>cies:<br>on:                              | Sun<br>NIW<br>Dap  | vival (48h)<br>/A SOP 10<br>ohnia thomso | (2022)<br>oni (Water fl                                | ea)  |  | Analy<br>Dilue<br>Brine<br>Sour | yst:<br>ent:<br>e:<br>ce:                          | Ecot<br>Waił<br>Not /<br>Field                     | ox Team<br>nou<br>Applicable<br>I Collected                                |                  | Age:       |
| Sample ID:<br>Sample Date:<br>Receipt Date:<br>Sample Age:                   | 02-5<br>03 M<br>03 M<br>24h | 559-2325<br> ay-24<br> ay-24   |  | Cod<br>Mate<br>CAS<br>Clier                               | e:<br>erial:<br>(PC):<br>nt:                                | 24.0<br>Cop<br>Hyd | 003.5 Cu<br>oper<br>irotoxy Rese         | arch   |  |  | Proje<br>Sour<br>Stati          | ect:<br>ce:<br>on:                                 | Spec<br>Solu<br>Lab                                | cial Studies<br>tion made by<br>Solution                                   | y NIWA           |            |
| Data Transfor  | m                           |  | Alt H  | Нур   |   |                    |  |  | NOEL   | LOE  | EL                              | TOEL   |  | Tox Units  | MSDu             | PMSD       |
| Angular (Corrected) C > T  |                             |  |  |   |   |                    |  |  | 21.1   | 48   |                                 | 31.82  |  |  | 0.2037           | 21.22%     |
| Bonferroni Ad  | lj t Te                     | st   |  |   |   |                    |  |  |  |  |                                 |  |  |  |                  |            |
| Control vs Conc-µg/L df Test Stat Critical MSD P-Type P-Value Decision(α:5%) |                             |  |  |   |   |                    |  |  |  |  |                                 |  |  |  |                  |            |
| Dilution Water   |                             | 9.75   |  | 6   | -0.091  | 64                 | 2.466                                    | 0.2924   | CDF  | 1.00   | 000                             | Non-S  | Signif   | icant Effect   |                  |            |
|  | 21.1                        |  | 6  | 1.387   |   | 2.466              | 0.2924                                   | CDF  | 0.29   | 934  | Non-S                           | Signif   | icant Effect                                       |  |                  |            |
|  |                             | 48.  |  | 0   | 5.04  |                    | 2.400                                    | 0.2924   | CDF  | 0.00   | 103                             | Signili  | cant   | Ellect   |                  |            |
| ANOVA Table  |                             |  |  |   |   |                    |  |  |  |  |                                 |  |  |  |                  |            |
| Source   |                             | Sum Squa   | res  |   | Mean  | Squ                | are                                      | DF   | F Stat   | P-V  | alue                            | Decis  | ion(   | α:5%)  |                  |            |
| Between  |                             | 0.988236   | 988236 0.329412  |   |   |                    |  |  | 12.5   | 0.00   | 010                             | Signif   | icant  | Effect   |                  |            |
| Error 0.263566<br>Total 1.2518   |                             |  |  |   | 0.020   | 3000               |  | 10   | _  |  |                                 |  |  |  |                  |            |
|  | nntio                       | ne Toete   |  |   |   |                    |  |  |  |  |                                 |  |  |  |                  |            |
| Attribute  | npuo                        | Toot   |  |   |   |                    |  | Toot Stat  | Critical   | рv   | alua                            | Desis  |  |  |                  |            |
| Variance   |                             | Bartlett En  | uality   | of Va   | iance T   | oet                |  | 3 722  | 11.34  | 0.20   | 330                             | Equal  | Vari   | ances  |                  |            |
| Distribution   |                             | Levene Eq<br>Mod Leven<br>Anderson-I<br>D'Agostino<br>Kolmogoro<br>Shapiro-W | uality<br>ne Equ<br>Darlin <u>c</u><br>Skew<br>ov-Smi<br>ilk W I | of Vai<br>iality o<br>g A2 T<br>iness<br>irnov [<br>Norm: | riance T<br>of Varia<br>Test<br>Test<br>D Test<br>ality Tes | iest<br>nce i      | Test                                     | 3.001<br>0.9186<br>0.9171<br>1.231<br>0.2914<br>0.8995 | 6.552<br>9.78<br>3.878<br>2.576<br>0.262<br>0.8239 | 0.08<br>0.48<br>0.01<br>0.21<br>0.00<br>0.11 | 317<br>365<br>197<br>184<br>021 | Equal<br>Equal<br>Norma<br>Norma<br>Non-N<br>Norma | Vari<br>Vari<br>al Dis<br>al Dis<br>lorm<br>al Dis | ances<br>ances<br>stribution<br>stribution<br>al Distributio<br>stribution | 'n               |            |
| 48h Survival F   | Rate                        | Summary  |  |   |   |                    |  |  |  |  |                                 |  |  |  |                  |            |
| Conc-µg/L  |                             | Code   | Cour   | nt  | Mean  |                    | 95% LCL                                  | 95% UCL  | Median   | Min  |                                 | Мах  |  | Std Err  | CV%              | %Effect    |
| 0.8  |                             | D  | 5  |   | 0.960   | D                  | 0.8920                                   | 1.0000   | 1.0000   | 0.90   | 000                             | 1.000  | D  | 0.0245   | 5.71%            | 0.00%      |
| 9.75   |                             |  | 3  |   | 0.966   | 7                  | 0.8232                                   | 1.0000   | 1.0000   | 0.90   | 000                             | 1.000  | 0  | 0.0333   | 5.97%            | -0.69%     |
| 21.1   |                             |  | 3  |   | 0.833   | 3<br>N             | 0.3162                                   | 1.0000   | 0.9000   | 0.00   | 000                             | 0.500  | n  | 0.1202   | 24.98%<br>42.20% | 13.19%     |
| 40<br>97   |                             |  | 3  |   | 0.400   | 0                  | 0.0000                                   | 0.0000   | 0.0000   | 0.20   | 000                             | 0.000  | D  | 0.0000   | 43.30%           | 100 00%    |
| 211  |                             |  | 3  |   | 0.000   | D                  | 0.0000                                   | 0.0000   | 0.0000   | 0.00   | 000                             | 0.000  | D  | 0.0000   |                  | 100.00%    |
| Angular (Corr  | ecter                       | I) Transform   | ned S  | umm   | arv   |                    |  |  |  |  |                                 |  |  |  |                  |            |
| Conc-ua/L  |                             | Code   | Cour   | nt  | Mean  |                    | 95% LCL                                  | 95% UCL  | Median   | Min  |                                 | Мах  |  | Std Err  | CV%              | %Effect    |
| 0.8  |                             | D  | 5  | -   | 1.347   | 0                  | 1.2360                                   | 1.4580   | 1.4120   | 1.24   | 190                             | 1.412  | D  | 0.0399   | 6.63%            | 0.00%      |
| 9.75   |                             |  | 3  |   | 1.358   | D                  | 1.1240                                   | 1.5910   | 1.4120   | 1.24   | 190                             | 1.412  | D  | 0.0543   | 6.93%            | -0.81%     |
| 21.1   |                             |  | 3  |   | 1.182   | D                  | 0.5136                                   | 1.8510   | 1.2490   | 0.88   | 361                             | 1.412  | D  | 0.1554   | 22.77%           | 12.21%     |
| 48   |                             |  | 3 0.6781 0.2167  |   |   |                    |  |  | 0.7854   | 0.46   | 636                             | 0.785  | 4  | 0.1073   | 27.39%           | 49.65%     |
| 97<br>211  |                             |  | 3 0.1588 0.1588  |   |   |                    |  |  | 0.1588   | 0.15   | 88<br>588                       | 0.158  | D<br>R   | 0.0000   | 0.00%            | 88.21%     |
| 211  |                             |  |  |   | 0.100   | -                  | 0.1000                                   | 5.1500   | 3.1000   | 0.10   |                                 | 0.100  | -  | 5.0000   | 5.0070           | 50.2170    |

Convergent Rounding (4 sf)

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS Ana                               | alytical Repo                   | ort                |  |  |                                | Report Date:<br>Test Code/ID: | 27 May-24 14:02 (p 2 of 2)<br>24.003.5 Cu / 15-1536-4540 |                    |
|---|---------------------------------|--------------------|--|--|--------------------------------|-------------------------------|--|--------------------|
| Daphnia thor                            | nsoni 48-h Acute                | • Survival         | Fest                                   |  |                                |                               |  | NIWA Ecotoxicology |
| Analysis ID:<br>Analyzed:<br>Edit Date: | 18-8837-2322<br>27 May-24 14:01 | Enc<br>I Ana<br>MD | lpoint: 48<br>Ilysis: Pa<br>5 Hash: 0B | h Survival R<br>rametric-Mu<br>07C019112 | ate<br>Itiple Comp<br>69DEBFC6 | oarison<br>312C1A53753B5      | CETIS Version:<br>Status Level:<br>9 Editor ID:          | CETISv2.1.4<br>1   |
| 48h Survival                            | Rate Detail                     |                    |  |  |                                |                               |  |                    |
| Conc-µg/L                               | Code                            | Rep 1              | Rep 2                                  | Rep 3                                    | Rep 4                          | Rep 5                         |  |                    |
| 0.8                                     | D                               | 1.0000             | 0.9000                                 | 1.0000                                   | 0.9000                         | 1.0000                        |  |                    |
| 9.75                                    |                                 | 1.0000             | 0.9000                                 | 1.0000                                   |                                |                               |  |                    |
| 21.1                                    |                                 | 1.0000             | 0.9000                                 | 0.6000                                   |                                |                               |  |                    |
| 48                                      |                                 | 0.5000             | 0.2000                                 | 0.5000                                   |                                |                               |  |                    |
| 97<br>211                               |                                 | 0.0000             | 0.0000                                 | 0.0000                                   |                                |                               |  |                    |
| Angular (Cor                            | rected) Transfor                | med Detail         |  |  |                                |                               |  |                    |
| Conc-µg/L                               | Code                            | Rep 1              | Rep 2                                  | Rep 3                                    | Rep 4                          | Rep 5                         |  |                    |
| 0.8                                     | D                               | 1.4120             | 1.2490                                 | 1.4120                                   | 1.2490                         | 1.4120                        |  |                    |
| 9.75                                    |                                 | 1.4120             | 1.2490                                 | 1.4120                                   |                                |                               |  |                    |
| 21.1                                    |                                 | 1.4120             | 1.2490                                 | 0.8861                                   |                                |                               |  |                    |
| 48                                      |                                 | 0.7854             | 0.4636                                 | 0.7854                                   |                                |                               |  |                    |
| 97<br>211                               |                                 | 0.1588             | 0.1588                                 | 0.1588                                   |                                |                               |  |                    |
| 48h Survival                            | Rate Binomials                  |                    |  |  |                                |                               |  |                    |
| Conc.ua/l                               | Code                            | Rep 1              | Ren 2                                  | Rep 3                                    | Ren 4                          | Ren 5                         |  |                    |
| 0.8                                     | D                               | 10/10              | 9/10                                   | 10/10                                    | 9/10                           | 10/10                         |  |                    |
| 9.75                                    | 2                               | 10/10              | 9/10                                   | 10/10                                    | 0.10                           | 10/10                         |  |                    |
| 21.1                                    |                                 | 10/10              | 9/10                                   | 6/10                                     |                                |                               |  |                    |
| 48                                      |                                 | 5/10               | 2/10                                   | 5/10                                     |                                |                               |  |                    |
| 97                                      |                                 | 0/10               | 0/10                                   | 0/10                                     |                                |                               |  |                    |
| 211                                     |                                 | 0/10               | 0/10                                   | 0/10                                     |                                |                               |  |                    |
| Graphics                                |                                 |                    |  |  |                                |                               |  |                    |
| 1.0-                                    | -                               |                    |  |  |                                | 0.2 -                         |  | -                  |
| 0.9 -                                   |                                 | •                  |  |  |                                |                               |  |                    |
| 0.8 -                                   |                                 |                    |  |  | <b>— •</b>                     | 0.1 -                         |  | ••                 |
| - 7.0 gt                                |                                 |                    |  |  |                                |                               |  |                    |
| 0.6-                                    |                                 |                    |  |  |                                | 0.0 -                         |  |                    |
| € 0.5-                                  |                                 | ſ                  |  |  |                                | A .                           |  |                    |
| 0.4 -                                   |                                 |                    |  |  |                                | 0.1 -                         |  |                    |
| <b>6</b> 0.3 -                          |                                 |                    |  |  |                                | -                             | /  |                    |
| 0.2 -                                   |                                 | l                  |  |  |                                |                               |  |                    |
| 0.1 -                                   |                                 |                    |  |  |                                |                               | •  |                    |
| 0.0 -                                   |                                 |                    | _                                      |  |                                | .03.                          |  |                    |
| 0.0                                     | 0.8 D 9.75                      | 21.1               | 48 9                                   | 97 211                                   |                                | -1.5                          | 5 -1.0 -0.5  | 0.0 0.5 1.0 1.5    |
|   |                                 | Conc-              | a/I                                    |  |                                |                               | R  | ankits             |
|   |                                 | conc-p             | 9 L                                    |  |                                |                               | K  |                    |
|   |                                 |                    |  |  |                                |                               |  |                    |

CETIS™ v2.1.4.5 (009-951-268-0)

| CETIS                                   | S Analyt  | ical Repo                     | ort              |   |   |                                | R                | leport<br>est Co | Date:<br>de/ID:                 |                             | 27<br>24.0                   | May-24 14:0<br>003.5 Cu / 15                 | 04 (p 1 of 3)<br>5-1536-4540 |            |
|---|---|-------------------------------|------------------|---|---|--------------------------------|------------------|------------------|---------------------------------|-----------------------------|------------------------------|--|------------------------------|------------|
| Daphn                                   | ia thomson  | ii 48-h Acute                 | Surviva          | al Test                                   |   |                                |                  |                  |                                 |                             |                              |  | NIWA Eco                     | toxicology |
| Analys<br>Analyz<br>Edit Da             | is ID: 15⊰<br>ed: 27 I<br>nte:  | 5899-0665<br>May-24 14:02     | E<br>2 A<br>M    | ndpoint:<br>nalysis:<br>1D5 Hash:         | 48h Survival Ra<br>Nonlinear Regr<br>0B07C0191126 | ate<br>ression (NL<br>69DEBFC6 | R)<br>12C1A53753 | B59              | CETI<br>Statu<br>Edito          | S Vers<br>Is Leve<br>or ID: | ion:<br>el:                  | CETISV.<br>1                                 | 2.1.4                        |            |
| Batch I<br>Start D<br>Ending<br>Test Le | D: 20-4<br>ate: 04  <br>  Date: 06  <br>ength: 48h  | 8394-3851<br>May-24<br>May-24 | T<br>P<br>S<br>T | est Type:<br>rotocol:<br>pecies:<br>axon: | Survival (48h)<br>NIWA SOP 10<br>Daphnia thoms    | (2022)<br>soni (Water          | flea)            |                  | Analy<br>Dilue<br>Brine<br>Sour | yst:<br>ent:<br>e:<br>ce:   | Ecot<br>Waii<br>Not<br>Field | tox Team<br>hou<br>Applicable<br>I Collected | I                            | Age:       |
| Sample<br>Sample<br>Receip<br>Sample    | e ID: 02-4<br>e Date: 03  <br>t Date: 03  <br>e Age: 24h  | 5559-2325<br>May-24<br>May-24 | C<br>M<br>C<br>C | code:<br>laterial:<br>AS (PC):<br>lient:  | 24.003.5 Cu<br>Copper<br>Hydrotoxy Rese           | earch                          |                  |                  | Proje<br>Sour<br>Stati          | ect:<br>ce:<br>on:          | Spec<br>Solu<br>Lab          | cial Studie:<br>tion made<br>Solution        | s<br>by NIWA                 |            |
| Non-Li                                  | near Regre  | ssion Optio                   | ns               |   |   |                                | _                |                  |                                 |                             |                              |  |                              |            |
| Model                                   | Name and  | Function                      |                  |   |   | Weightin                       | ng Function      |                  |                                 | PTBS                        | Fun                          | nction                                       | X Trans                      | Y Trans    |
| 3P Log                                  | -Logistic: µ:   | =α/[1+[x/o]^γ]                |                  |   |   | Binomiai                       | [ω=n/[p·q]]      |                  |                                 | ΟΠ[μ                        | r=h]                         |  | None                         | None       |
| Regres                                  | sion Sumr   | nary                          |                  |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Iters                                   | LL  | AICc                          | BIC              | Adj Ra                                    | 2 PMSD  | Thresh                         | Optimize         | FS               | tat                             | P-Val                       | ue                           | Decisior                                     | n(α:5%)                      |            |
| 10                                      | -18.87  | 45.23                         | 46.72            | 0.8551                                    | 5.32%   | 0.9576                         | Yes              | 0.78             | 865                             | 0.521                       | 2                            | Non-Sigr                                     | nificant Lack-               | -of-Fit    |
| Point E                                 | stimates  |                               |                  |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Level                                   | µg/L  | 95% LCL                       | 95% U(           | CL  |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| LC5                                     | 18.78   | 24.99                         |                  |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| LC10                                    | 22.83   |                               | 29.64            |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| LC15                                    | 25.78   | 12 10                         | 33.09            |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| 1.025                                   | 20.24   | 18.71                         | 38.55            |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| LC40                                    | 36.51   | 27.81                         | 45.61            |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| LC50                                    | 40.6  | 32.61                         | 50.55            |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Regres                                  | sion Parar  | neters                        |                  |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Daram                                   | ator  | Eetimato                      | Std Err          | or 05% 1                                  | 05% 1101  | t Stat                         | D Value          | Dec              | ricion/                         | a:5%)                       |                              |  |                              |            |
| Farann                                  |   | 0.0576                        | 0.02413          | 0 0067                                    | 1 008   | 20.60                          | <1.0E_05         | Sig              | nificant                        | t Param                     | otor                         |  |                              |            |
| v                                       |   | 3 818                         | 0.8905           | 1 939                                     | 5 697   | 4 287                          | 0.0005           | Sig              | nificant                        | t Param                     | neter                        |  |                              |            |
| ō                                       |   | 40.6                          | 4.128            | 31.89                                     | 49.31   | 9.836                          | <1.0E-05         | Sig              | nificant                        | t Param                     | neter                        |  |                              |            |
| ANOVA                                   | Table   |                               |                  |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Source                                  |   | Sum Squa                      | ares M           | lean Squar                                | e DF  | F Stat                         | P-Value          | Dec              | cision(                         | α:5%)                       |                              |  |                              |            |
| Model                                   |   | 1989                          | 6                | 62.9                                      | 3   | 615.5                          | <1.0E-05         | Sig              | nificant                        | t Effect                    |                              |  |                              |            |
| Lack of                                 | Fit   | 2.641                         | 0                | .8803                                     | 3   | 0.7865                         | 0.5212           | Nor              | n-Signif                        | ficant L                    | ack-o                        | of-Fit                                       |                              |            |
| Pure El                                 | ror   | 15.67                         | 1.               | .119                                      | 14  |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Residu                                  | ai  | 10.31                         | 1.               | .077                                      | 17  |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Residu                                  | al Analysis   | ;                             |                  |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |
| Attribu                                 | te  | Method                        |                  |   | Test Stat   | Critical                       | P-Value          | Dec              | cision(                         | α:5%)                       |                              |  |                              |            |
| Model I                                 | Fit   | Likelihood                    | Ratio GO         | OF Test                                   | 19.38   | 27.59                          | 0.3073           | Nor              | n-Signif                        | ficant H                    | letero                       | ogeneity                                     |                              |            |
| Variana                                 |   | Pearson C                     | ni-Sq GC         | JF lest<br>itu of Vorion                  | 18.31   | 27.59                          | 0.3695           | Nor              | 1-Signi<br>Iol Vori             | ficant H                    | etero                        | ogeneity                                     |                              |            |
| Distribu                                | tion.   | Anderson-                     | Darling A        | 10 Ur variari<br>12 Tost                  | 0 7253  | 2 492                          | 0.4114           | Nor              | mal Di                          | etributir                   | n                            |  |                              |            |
| Distribu                                | and the second se | Shapiro-W                     | ilk W No         | t 0.893                                   | 0.9044  | 0.0305                         | Nor              | n-Norm           | al Distr                        | ibutio                      | on                           |  |                              |            |
| Overdis                                 | persion   | Tarone C(                     | a) Overdi        | ispersion Te                              | est 1.394   | 1.645                          | 0.0816           | Nor              | n-Signif                        | ficant C                    | verd                         | ispersion                                    |                              |            |
|   |   |                               |                  |   |   |                                |                  |                  |                                 |                             |                              |  |                              |            |

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| CETIS And   | alytical Repo                   | ort  |   |   | Report<br>Test C  | Date:<br>ode/ID:  | 27<br>24.0   | May-24 14:0<br>03.5 Cu / 15                              | 4 (p 2 of 3)<br>-1536-4540             |   |  |
|---|---------------------------------|--|---|---|---|---|--|--|--|---|--|
| Daphnia thor  | nsoni 48-h Acute                | Survival   | Test  |   |   |   |  |  |  | NIWA Ecot   | oxicology  |
| Analysis ID:<br>Analyzed:<br>Edit Date:                             | 15-5899-0665<br>27 May-24 14:02 | En<br>Ana<br>MD  | dpoint: 48<br>alysis: No<br>95 Hash: 0B                           | h Survival R<br>onlinear Reg<br>07C019112                         | ate<br>ression (NL<br>69DEBFC6                                    | R)<br>12C1A5375   | CET<br>Stat<br>3B59 Edit                                 | IS Version:<br>us Level:<br>or ID:                       | CETISv2<br>1                           | 2.1.4   |  |
| 48h Survival  | Rate Summary                    |  |   |   |   | Calculate   | d Variate(A  | 'B)  |  |   |  |
| Conc-µg/L   | Code                            | Count  | Mean  | Median  | Min   | Мах   | Std Err  | Std Dev  | CV%                                    | %Effect   | ΣΑ/ΣΒ  |
| 0.8<br>9.75<br>21.1<br>48<br>97<br>211<br>48h Survival<br>Conc-µg/L | D<br>Rate Detail<br>Code        | 5<br>3<br>3<br>3<br>3<br>3<br>3<br>Rep 1                 | 0.9600<br>0.9667<br>0.8333<br>0.4000<br>0.0000<br>0.0000<br>Rep 2 | 1.0000<br>1.0000<br>0.9000<br>0.5000<br>0.0000<br>0.0000<br>Rep 3 | 0.9000<br>0.9000<br>0.6000<br>0.2000<br>0.0000<br>0.0000<br>Rep 4 | 1.0000<br>1.0000<br>0.5000<br>0.0000<br>0.0000<br>Rep 5 | 0.0245<br>0.0333<br>0.1202<br>0.1000<br>0.0000<br>0.0000 | 0.0548<br>0.0577<br>0.2082<br>0.1732<br>0.0000<br>0.0000 | 5.71%<br>5.97%<br>24.98%<br>43.30%<br> | 0.00%<br>-0.69%<br>13.19%<br>58.33%<br>100.00%<br>100.00% | 48/50<br>29/30<br>25/30<br>12/30<br>0/30<br>0/30 |
| 0.8<br>9.75<br>21.1<br>48<br>97<br>211                              | D                               | 1.0000<br>1.0000<br>1.0000<br>0.5000<br>0.0000<br>0.0000 | 0.9000<br>0.9000<br>0.9000<br>0.2000<br>0.0000<br>0.0000          | 1.0000<br>1.0000<br>0.6000<br>0.5000<br>0.0000<br>0.0000          | 0.9000  | 1.0000  |  |  |  |   |  |
| 48h Survival  | Rate Binomials                  |  |   |   |   |   |  |  |  |   |  |
| Conc-µg/L   | Code                            | Rep 1  | Rep 2   | Rep 3   | Rep 4   | Rep 5   |  |  |  |   |  |
| 0.8<br>9.75<br>21.1<br>48<br>97<br>211                              | D                               | 10/10<br>10/10<br>10/10<br>5/10<br>0/10<br>0/10          | 9/10<br>9/10<br>9/10<br>2/10<br>0/10<br>0/10                      | 10/10<br>10/10<br>6/10<br>5/10<br>0/10<br>0/10                    | 9/10  | 10/10   |  |  |  |   |  |

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Convergent Rounding (4 sf)

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## Appendix K Physico-chemical data for zinc tests

Table K-1:Summary of physico-chemical measures from acute *D. thomsoni* zinc toxicity testing withMahurangi Stream water.Values shown are the measurements taken at test initiation ( $T_0$ ) and testtermination ( $T_{48}$ ).Shaded cells indicate concentrations used in statistical analyses.

| Nominal concentration    | Ŗ              | н           | Dissolved O | kygen (mg/L) | Condu<br>(µՏյ | uctivity<br>/cm) | Tempera | iture (°C)  |
|--------------------------|----------------|-------------|-------------|--------------|---------------|------------------|---------|-------------|
| (µg/L Zn <sup>2+</sup> ) | T <sub>0</sub> | <b>T</b> 48 | To          | <b>T</b> 48  | To            | <b>T</b> 48      | To      | <b>T</b> 48 |
| 0 Control                | 7.8            | 7.9         | 8.8         | 8.8          | 241           | 254              | 20      | 20          |
| 100                      | 7.9            | 8.0         | 9.2         | 9.2          | 249           | 259              | 20      | 20          |
| 220                      | 7.9            | 8.1         | 9.2         | 9.1          | 245           | 257              | 20      | 20          |
| 484                      | 7.9            | 8.0         | 9.2         | 9.1          | 248           | 254              | 20      | 20          |
| 1,065                    | 7.9            | 8.0         | 9.2         | 9.0          | 251           | 257              | 20      | 20          |
| 2,343                    | 7.7            | 7.9         | 9.2         | 9.1          | 252           | 259              | 20      | 20          |
| 5,154                    | 7.5            | 7.4         | 9.2         | 9.0          | 259           | 254              | 20      | 20          |
| 11,000                   | 7.2            | 7.1         | 9.1         | 9.0          | 275           | 244              | 20      | 20          |

#### Table K-2: Summary of physico-chemical measures from acute D. thomsoni zinc toxicity testing with

**Hoteo River water.** Values shown are the measurements taken at test initiation ( $T_0$ ) and test termination ( $T_{48}$ ). Shaded cells indicate concentrations used in statistical analyses.

| Nominal<br>concentration | р   | н           | Dissolved O | xygen (mg/L) | Conc<br>(μ | ductivity<br>S/cm) | Tempera | iture (°C)  |
|--------------------------|-----|-------------|-------------|--------------|------------|--------------------|---------|-------------|
| (µg/L Zn <sup>2+</sup> ) | To  | <b>T</b> 48 | To          | <b>T</b> 48  | To         | <b>T</b> 48        | To      | <b>T</b> 48 |
| 0 Control                | 7.6 | 7.6         | 8.2         | 8.8          | 198        | 225                | 20      | 20          |
| 100                      | 7.8 | 7.9         | 9.3         | 9.0          | 258        | 211                | 20      | 20          |
| 220                      | 7.8 | 8.0         | 9.1         | 9.0          | 205        | 205                | 20      | 20          |
| 484                      | 7.9 | 8.0         | 9.1         | 9.0          | 205        | 212                | 20      | 20          |
| 1065                     | 7.8 | 7.9         | 9.1         | 9.0          | 206        | 217                | 20      | 20          |
| 2343                     | 7.7 | 7.8         | 9.1         | 9.1          | 220        | 231                | 20      | 20          |
| 5154                     | 7.6 | 7.9         | 9.2         | 9.0          | 218        | 218                | 20      | 20          |
| 11000                    | 7.3 | 7.9         | 9.0         | 9.0          | 236        | 218                | 20      | 20          |

Table K-3:Summary of physico-chemical measures from acute *D. thomsoni* zinc toxicity testing with pHadjusted Okutua Creek water.Values shown are the measurements taken at test initiation  $(T_0)$  and testtermination  $(T_{48})$ , nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal<br>concentration | рН  |             | Dissolved O | Dissolved Oxygen (mg/L) |      | Conductivity<br>(μS/cm) |    | ature (°C)  |
|--------------------------|-----|-------------|-------------|-------------------------|------|-------------------------|----|-------------|
| (µg/L Zn <sup>2+</sup> ) | To  | <b>T</b> 48 | To          | <b>T</b> 48             | To   | T <sub>48</sub>         | To | <b>T</b> 48 |
| 0 Control                | 6.0 | 6.5         | 8.4         | 8.8                     | 32.0 | 34.6                    | 19 | 20          |
| 100                      | 5.9 | 6.3         | 8.3         | 8.7                     | 31.3 | 34.8                    | 19 | 20          |
| 220                      | 5.8 | 6.3         | 8.2         | 8.7                     | 30.6 | 40.7                    | 20 | 20          |
| 484                      | 5.8 | 6.3         | 8.2         | 8.7                     | 32.5 | 35.9                    | 19 | 20          |
| 1,065                    | 5.8 | 6.3         | 8.0         | 8.7                     | 30.8 | 36.0                    | 20 | 20          |

| Nominal<br>concentration | рН  |             | Dissolved O    | Dissolved Oxygen (mg/L) |      | Conductivity<br>(μS/cm) |    | Temperature (°C)       |  |
|--------------------------|-----|-------------|----------------|-------------------------|------|-------------------------|----|------------------------|--|
| (µg/L Zn <sup>2+</sup> ) | To  | <b>T</b> 48 | T <sub>0</sub> | <b>T</b> <sub>48</sub>  | To   | <b>T</b> <sub>48</sub>  | To | <b>T</b> <sub>48</sub> |  |
| 2,343                    | 5.7 | 6.1         | 8.3            | 8.7                     | 32.9 | 40.4                    | 20 | 20                     |  |
| 5,154                    | 5.5 | nm          | 8.5            | nm                      | 47.1 | nm                      | 20 | nm                     |  |
| 11,000                   | 5.4 | nm          | 8.5            | nm                      | 69.3 | nm                      | 19 | nm                     |  |

Table K-4:Summary of physico-chemical measures from acute D. thomsoni zinc toxicity testing withClutha River water.Values shown are the measurements taken at test initiation (T<sub>0</sub>) and test termination (T<sub>48</sub>),nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal concentration    | рН             |                        | Dissolved O | Dissolved Oxygen (mg/L) |      | Conductivity<br>(μS/cm) |    | Temperature (°C)       |  |
|--------------------------|----------------|------------------------|-------------|-------------------------|------|-------------------------|----|------------------------|--|
| (µg/L Zn <sup>2+</sup> ) | T <sub>0</sub> | <b>T</b> <sub>48</sub> | To          | <b>T</b> <sub>48</sub>  | To   | <b>T</b> <sub>48</sub>  | To | <b>T</b> <sub>48</sub> |  |
| 0 Control                | 7.5            | 7.5                    | 8.3         | 9.0                     | 76.2 | 85.4                    | 19 | 20                     |  |
| 45                       | 7.6            | nm                     | 9.0         | nm                      | 79.3 | nm                      | 19 | nm                     |  |
| 100                      | 7.6            | 7.5                    | 8.9         | 9.1                     | 78.0 | 84.5                    | 19 | 20                     |  |
| 220                      | 7.6            | 7.6                    | 9.0         | 9.0                     | 77.2 | 89.9                    | 19 | 20                     |  |
| 484                      | 7.6            | 7.6                    | 9.0         | 8.9                     | 78.3 | 86.9                    | 19 | 20                     |  |
| 1,065                    | 7.5            | 7.5                    | 8.8         | 8.9                     | 78.0 | 92.4                    | 20 | 20                     |  |
| 2,343                    | 7.3            | 7.4                    | 8.9         | 9.0                     | 84.2 | 93.3                    | 20 | 20                     |  |
| 5,154                    | 7.1            | nm                     | 8.9         | nm                      | 95.7 | nm                      | 19 | nm                     |  |

Table K-5:Summary of physico-chemical measures from acute *D. thomsoni* zinc toxicity testing withWaihou River water.Values shown are the measurements taken at test initiation ( $T_0$ ) and test termination( $T_{48}$ ), nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal<br>concentration | рН  |             | Dissolved O | Dissolved Oxygen (mg/L) |      | Conductivity<br>(µS/cm) |                | Temperature (°C) |  |
|--------------------------|-----|-------------|-------------|-------------------------|------|-------------------------|----------------|------------------|--|
| (µg/L Zn <sup>2+</sup> ) | To  | <b>T</b> 48 | To          | <b>T</b> 48             | To   | <b>T</b> 48             | T <sub>0</sub> | <b>T</b> 48      |  |
| 0 Control                | 7.5 | 7.9         | 8.4         | 8.6                     | 89.1 | 100                     | 19             | 20               |  |
| 100                      | 7.7 | 7.7         | 8.8         | 8.9                     | 90.1 | 99.6                    | 19             | 20               |  |
| 220                      | 7.6 | 7.7         | 8.8         | 8.9                     | 88.6 | 101                     | 20             | 20               |  |
| 484                      | 7.7 | 7.7         | 8.8         | 8.9                     | 91.3 | 102                     | 19             | 20               |  |
| 1,065                    | 7.6 | 7.7         | 8.7         | 8.8                     | 93.8 | 100                     | 19             | 20               |  |
| 2,343                    | 7.5 | 7.5         | 8.8         | 8.9                     | 97.7 | 106                     | 19             | 20               |  |
| 5,154                    | 7.2 | nm          | 8.9         | nm                      | 107  | nm                      | 19             | nm               |  |

## Appendix L Physico-chemical data for copper tests

Table L-1:Summary of physico-chemical measures from acute D. thomsoni copper toxicity testing withMahurangi Stream water.Values shown are the measurements taken at test initiation  $(T_0)$  and testtermination  $(T_{48})$ , nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal concentration    | рН             |             | Dissolved O | Dissolved Oxygen (mg/L) |     | uctivity<br>/cm) | Temperature (°C) |             |
|--------------------------|----------------|-------------|-------------|-------------------------|-----|------------------|------------------|-------------|
| (µg/L Cu <sup>2+</sup> ) | T <sub>0</sub> | <b>T</b> 48 | To          | <b>T</b> 48             | To  | <b>T</b> 48      | To               | <b>T</b> 48 |
| 0 Control                | 7.8            | 7.9         | 8.8         | 8.8                     | 241 | 254              | 20               | 20          |
| 1                        | 7.7            | nm          | 8.7         | nm                      | 242 | nm               | 20               | nm          |
| 2.2                      | 7.8            | nm          | 8.6         | nm                      | 244 | nm               | 20               | nm          |
| 4.8                      | 7.8            | nm          | 9.8         | nm                      | 243 | nm               | 20               | nm          |
| 10.6                     | 7.8            | nm          | 8.8         | nm                      | 242 | nm               | 20               | nm          |
| 23.4                     | 7.7            | 8.0         | 8.7         | 8.8                     | 242 | 253              | 20               | 20          |
| 51.5                     | 7.8            | 8.0         | 8.3         | 8.7                     | 244 | 253              | 20               | 20          |
| 154.5                    | 7.7            | 7.6         | 9.7         | 8.6                     | 250 | 253              | 20               | 20          |
| 463.5                    | 7.6            | 7.8         | 9.5         | 8.7                     | 250 | 250              | 20               | 20          |
| 1,390.5                  | 7.2            | 7.8         | 8.9         | 8.7                     | 250 | 251              | 20               | 20          |

# Table L-2:Summary of physico-chemical measures from acute *D. thomsoni* copper toxicity testing withHoteo River water.Values shown are the measurements taken at test initiation ( $T_0$ ) and test termination ( $T_{48}$ ),nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal concentration    | рН             |             | Dissolved O | Dissolved Oxygen (mg/L) |     | ıctivity<br>/cm) | Temperature (°C) |             |
|--------------------------|----------------|-------------|-------------|-------------------------|-----|------------------|------------------|-------------|
| (µg/L Cu <sup>2+</sup> ) | T <sub>0</sub> | <b>T</b> 48 | To          | <b>T</b> 48             | To  | <b>T</b> 48      | T <sub>0</sub>   | <b>T</b> 48 |
| 0 Control                | 7.6            | 7.6         | 8.2         | 8.8                     | 198 | 225              | 20               | 20          |
| 1                        | 7.6            | nm          | 9.3         | nm                      | 196 | nm               | 20               | nm          |
| 2.2                      | 7.6            | nm          | 8.7         | nm                      | 199 | nm               | 20               | nm          |
| 4.8                      | 7.7            | nm          | 8.7         | nm                      | 199 | nm               | 20               | nm          |
| 10.6                     | 7.6            | nm          | 8.9         | nm                      | 200 | nm               | 20               | nm          |
| 23.4                     | 7.6            | 7.7         | 8.6         | 8.8                     | 199 | 218              | 20               | 20          |
| 51.5                     | 7.6            | 7.8         | 9.0         | 8.8                     | 200 | 207              | 19               | 20          |
| 154.5                    | 7.6            | 7.8         | 9.7         | 8.7                     | 210 | 212              | 20               | 20          |
| 463.5                    | 7.6            | 7.8         | 9.2         | 8.7                     | 205 | 208              | 20               | 20          |
| 1,390.5                  | 7.5            | 7.8         | 8.2         | 8.7                     | 207 | 211              | 20               | 20          |

Table L-3:Summary of physico-chemical measures from acute *D. thomsoni* copper toxicity testing with pHadjusted Okutua Creek water.Values shown are the measurements taken at test initiation (T<sub>0</sub>) and testtermination (T<sub>48</sub>), nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal concentration    | рН  |             | Dissolved O | Dissolved Oxygen (mg/L) |      | Conductivity<br>(μS/cm) |    | Temperature (°C) |  |
|--------------------------|-----|-------------|-------------|-------------------------|------|-------------------------|----|------------------|--|
| (µg/L Cu <sup>2+</sup> ) | To  | <b>T</b> 48 | To          | <b>T</b> 48             | To   | T <sub>48</sub>         | To | <b>T</b> 48      |  |
| 0 Control                | 6.0 | 6.5         | 8.4         | 8.8                     | 32.0 | 34.6                    | 19 | 20               |  |

| Nominal concentration    | рН             |             | Dissolved O | Dissolved Oxygen (mg/L) |      | ıctivity<br>/cm)       | Temperature (°C) |                        |
|--------------------------|----------------|-------------|-------------|-------------------------|------|------------------------|------------------|------------------------|
| (µg/L Cu <sup>2+</sup> ) | T <sub>0</sub> | <b>T</b> 48 | To          | <b>T</b> 48             | To   | <b>T</b> <sub>48</sub> | To               | <b>T</b> <sub>48</sub> |
| 1                        | 6.0            | nm          | 8.5         | nm                      | 32.2 | nm                     | 20               | nm                     |
| 2.2                      | 6.0            | nm          | 8.5         | nm                      | 32.5 | nm                     | 20               | nm                     |
| 4.8                      | 6.0            | nm          | 8.3         | nm                      | 33.4 | nm                     | 19               | nm                     |
| 10.6                     | 6.0            | 6.6         | 8.3         | 8.9                     | 34.5 | 41.7                   | 20               | 20                     |
| 23.4                     | 5.9            | 6.4         | 8.3         | 8.8                     | 32.3 | 32.9                   | 20               | 20                     |
| 51.5                     | 5.9            | 6.3         | 8.2         | 8.8                     | 31.9 | 37.0                   | 19               | 20                     |
| 113.4                    | 5.9            | 6.3         | 8.4         | 8.8                     | 32.6 | 36.2                   | 19               | 20                     |
| 249                      | 5.6            | 6.3         | 8.5         | 8.8                     | 33.9 | 38.8                   | 19               | 20                     |

Table L-4:Summary of physico-chemical measures from acute D. thomsoni copper toxicity testing withClutha River water.Values shown are the measurements taken at test initiation ( $T_0$ ) and test termination ( $T_{48}$ ),nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal<br>concentration | рН  |                 | Dissolved O | Dissolved Oxygen (mg/L) |      | ıctivity<br>/cm) | Temperature (°C) |                        |
|--------------------------|-----|-----------------|-------------|-------------------------|------|------------------|------------------|------------------------|
| (µg/L Cu <sup>2+</sup> ) | To  | T <sub>48</sub> | To          | <b>T</b> 48             | To   | <b>T</b> 48      | To               | <b>T</b> <sub>48</sub> |
| 0 Control                | 7.5 | 7.5             | 8.3         | 9.0                     | 76.2 | 85.4             | 19               | 20                     |
| 1                        | 7.8 | nm              | 8.7         | nm                      | 74.8 | nm               | 19               | nm                     |
| 2.2                      | 7.8 | 7.6             | 8.7         | 9.1                     | 74.9 | 90.6             | 19               | 20                     |
| 4.8                      | 7.8 | 7.6             | 8.7         | 9.2                     | 74.9 | 93.0             | 19               | 20                     |
| 10.6                     | 7.9 | 7.6             | 8.8         | 9.1                     | 75.2 | 90.3             | 19               | 20                     |
| 23.4                     | 7.9 | 7.7             | 8.8         | 9.1                     | 75.4 | 95.7             | 19               | 20                     |
| 51.5                     | 8.0 | 7.7             | 9.1         | 9.1                     | 75.6 | 90.7             | 20               | 20                     |

Table L-5:Summary of physico-chemical measures from acute D. thomsoni copper toxicity testing withWaihou River water.Values shown are the measurements taken at test initiation  $(T_0)$  and test termination  $(T_{48})$ , nm = not measured.Shaded cells indicate concentrations used in statistical analyses.

| Nominal concentration    | рН  |             | Dissolved O    | Dissolved Oxygen (mg/L) |      | ıctivity<br>/cm)       | Temperature (°C) |             |
|--------------------------|-----|-------------|----------------|-------------------------|------|------------------------|------------------|-------------|
| (µg/L Cu <sup>2+</sup> ) | To  | <b>T</b> 48 | T <sub>0</sub> | <b>T</b> <sub>48</sub>  | To   | <b>T</b> <sub>48</sub> | To               | <b>T</b> 48 |
| 0 Control                | 7.5 | 7.9         | 8.4            | 8.6                     | 89.1 | 100                    | 19               | 20          |
| 1                        | 7.7 | nm          | 9.1            | nm                      | 92.8 | nm                     | 20               | nm          |
| 2.2                      | 7.5 | nm          | 9.1            | nm                      | 89.2 | nm                     | 20               | nm          |
| 4.8                      | 7.6 | 7.8         | 8.9            | 8.7                     | 89.3 | 93.6                   | 20               | 20          |
| 10.6                     | 7.6 | 7.8         | 8.9            | 8.7                     | 88.9 | 98.5                   | 20               | 20          |
| 23.4                     | 7.6 | 7.8         | 8.7            | 8.6                     | 88.8 | 95.9                   | 20               | 20          |
| 51.5                     | 7.5 | 7.8         | 8.5            | 8.6                     | 88.8 | 102                    | 20               | 20          |
| 113                      | 7.8 | 7.7         | 9.4            | 8.8                     | 94.7 | 99.4                   | 20               | 20          |
| 249                      | 7.8 | 7.7         | 9.2            | 8.8                     | 93.1 | 96.4                   | 20               | 20          |

# Appendix M NIWA's unpublished zinc and copper toxicity data

Table M-1:Summary of NIWA's unpublished or publicly unavailable New Zealand native species acute zinctoxicity testing data.Shaded values are nominal concentrations. na = not available.

| Notes incl. source   | Duration     | Temp.<br>°C | pH mean (min<br>max.) | Hardness<br>mg/L<br>CaCO <sub>3</sub> | DOC<br>mg/L | EC <sub>50</sub> ª (95% CL or ± 2<br>SD)<br>µg/L Zn <sup>2+</sup> |
|--|--------------|-------------|-----------------------|---------------------------------------|-------------|---|
| Fish - Smelt - Retropinna retro  | pinna        |             |                       |                                       |             |   |
| Juvenile wet weight 1.8 g;<br>Head-tail base length 57 mm.<br>NIWA reference toxicant data<br>base (unpublished). Zinc<br>stock concentration verified,<br>test dilutions not. | 96 h         | 15          | 7.4-7.7               | ≈ 40                                  | <0.5        | 5,601 n=1   |
| Fish - Common bully - Gobiom   | orphus cotid | ianus       |                       |                                       |             |   |
| NIWA reference toxicant data<br>base (unpublished). Zinc<br>stock concentration verified,<br>test dilutions not.   | 96 h         | 15          | 7.4-7.7               | ≈ 40                                  | <0.5        | 3,060 (684-5,435)<br>n=12   |
| Juvenile wet weight 0.45 g;<br>Head-tail base length 32 mm.<br>Ouwerkerk (2017)  | 96 h         | 16          | 7.24 (6.93-7.54)      | 14.2                                  | 0.3ª        | 1,868 (1,581-2,207)<br>n=1  |
| Juvenile wet weight 0.75 g;<br>Head-tail base length 37.5<br>mm. Ouwerkerk (2017)  | 96 h         | 16          | 7.05 (6.65-7.38)      | 14.2                                  | 1.3ª        | 3,888 (3,655-4,136)<br>n=1  |
| Juvenile wet weight 0.63 g;<br>Head-tail base length 37 mm.<br>Ouwerkerk (2017)  | 96 h         | 16          | 7.21 (6.87-7.58)      | 14.2                                  | 4.3ª        | 2,171 (1,654-2,848)<br>n=1  |
| Juvenile wet weight 0.45 g;<br>Head-tail base length 32 mm.<br>Ouwerkerk (2017)  | 10 d         | 16          | 7.24 (6.93-7.54)      | 14.2                                  | 0.3ª        | 382 (284-514) n=1   |
| Juvenile wet weight 0.75 g;<br>Head-tail base length 37.5<br>mm. Ouwerkerk (2017)  | 10 d         | 16          | 7.05 (6.65-7.38)      | 14.2                                  | 1.3ª        | 244 (121-493) n=1   |
| Juvenile wet weight 0.63 g;<br>Head-tail base length 37 mm.<br>Ouwerkerk (2017)  | 10 d         | 16          | 7.21 (6.87-7.58)      | 14.2                                  | 4.3ª        | 166 (94-293) n=1  |
| Fish - Inanga - <i>Galaxias maculo</i>   | ntus         |             |                       |                                       |             |   |
| Juvenile wet weight 0.3 g,<br>length 40 mm. NIWA<br>reference toxicant data base<br>(unpublished). Zinc stock<br>concentration verified, test<br>dilutions not.                | 96 h         | 15          | 7.4-7.7               | ≈ 40                                  | <0.5        | 3,493 (3,073-3,969<br>n=1   |
| Invertebrate - Snail - Potamop   | yrgus antipo | darum       |                       |                                       |             |   |
| NIWA reference toxicant data<br>base (unpublished). Zinc<br>stock concentration verified,<br>test dilutions not. Survival.   | 96 h         | 20          | 7.4-7.7               | ≈ 40                                  | <0.5        | 1,561 (1,345-1,812)<br>n=1  |
| NIWA reference toxicant data<br>base (unpublished). Zinc<br>stock concentration verified,<br>test dilutions not. Morbidity<br>(mobility).                                      | 96 h         | 20          | 7.4-7.7               | ≈ 40                                  | <0.5        | 936 (796-1,101) n=1   |

| Notes incl. source   | Duration      | Temp.<br>°C | pH mean (min<br>max.) | Hardness<br>mg/L<br>CaCO3 | DOC<br>mg/L       | EC <sub>50</sub> ª (95% CL or ± 2<br>SD)<br>μg/L Zn <sup>2+</sup> |
|--|---------------|-------------|-----------------------|---------------------------|-------------------|---|
| Ouwerkerk (2017). Survival.  | 96 h          | 19          | 7.06 (6.94-7.18)      | 14.2                      | 0.3ª              | 532 (474-597) n=1   |
| Ouwerkerk (2017). Survival.  | 96 h          | 20          | 7.02 (6.86-7.10)      | 14.2                      | 1.3ª              | 819 (694-968) n=1   |
| Ouwerkerk (2017). Survival.  | 96 h          | 21          | 7.03 (6.86-7.13)      | 14.2                      | 4.3ª              | 1,004 (896-1,125) n=1   |
| Ouwerkerk (2017). Survival.  | 96 h          | 20          | 6.99 (6.94-7.06)      | 14.2                      | 9.3ª              | 1,372 (1,222-1,541)<br>n=1  |
| Ouwerkerk (2017). Morbidity<br>(mobility).   | 96 h          | 19          | 7.06 (6.94-7.18)      | 14.2                      | 0.3ª              | 446 (403-492) n=1   |
| Ouwerkerk (2017). Morbidity<br>(mobility).   | 96 h          | 20          | 7.02 (6.86-7.10)      | 14.2                      | 1.3ª              | 459 (401-525) n=1   |
| Ouwerkerk (2017). Morbidity<br>(mobility).   | 96 h          | 21          | 7.03 (6.86-7.13)      | 14.2                      | 4.3ª              | 723 (655-771) n=1   |
| Ouwerkerk (2017). Morbidity<br>(mobility).   | 96 h          | 20          | 6.99 (6.94-7.06)      | 14.2                      | 9.3ª              | 993 (905-1089) n=1  |
| Invertebrate - Amphipod - Pare   | acalliope flu | viatilis    |                       |                           |                   |   |
| NIWA reference toxicant data<br>base (unpublished). Zinc<br>stock concentration verified,<br>test dilutions not. | 48 h          | 20          | 7.4                   | 48                        | na                | 887 (564-1,210) n=5   |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h          | 20          | 7.32 (7.21-7.37)      | 14.2                      | 0.3ª              | 482 (403-577) n=1   |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h          | 20          | 7.21 (7.16-7.25)      | 14.2                      | 1.3ª              | 293 (242-353) n=1   |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h          | 20          | 7.37 (7.21-7.48)      | 14.2                      | 4.3ª              | 579 (500-672) n=1   |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h          | 20          | 7.43 (7.13-7.58)      | 14.2                      | 9.3ª              | 823 (671-950) n=1   |
| Invertebrate – Water flea – Da   | phnia thoms   | ioni        | 7 5 (7 2 7 7)         | 14.0                      | 0.42 h            | 270 (200 248) -1  |
| NIVVA SLUDY FWWQ2009   | 48 11         | 20          | 7.5 (7.2-7.7)         | 14.0                      | 0.42 °            | 270 (209-348) N=1   |
| NIWA study FWWQ2009  | 48 h          | 20          | 7.4 (7.1-7.6)         | 14.0                      | 1.76 <sup>b</sup> | 240 (202-303) n=1   |
| NIWA study FWWQ2009<br>NIWA reference toxicant data  | 48 h          | 20          | 7.4 (7.0-7.8)         | 14.0                      | 3.66⁵             | 856 (na-na) n=1   |
| stock concentration verified,<br>test dilutions not.   | 48 h          | 20          | 7.8                   | 48                        | na                | 998 (812-1,226) n=1   |
| Bivalve – Freshwater mussel ju   | venile - Echy | ridella me  | enziesii              |                           |                   |   |
| NIWA reference toxicant data<br>base (unpublished). Zinc<br>stock concentration verified,<br>test dilutions not. | 48 h          | 20          | 7.4-7.7               | ≈ 40                      | <0.5              | 354 (212-518) n=1   |
| NIWA reference toxicant data<br>base (unpublished). Zinc<br>stock concentration verified,<br>test dilutions not. | 96 h          | 20          | 7.4-7.7               | ≈ 40                      | <0.5              | 261 (172-341) n=1   |

<sup>a</sup> Humic acid reference carbon source. <sup>b</sup> Manuka leaf extract carbon source.

| Notes incl. source   | Duration | Temp.<br>°C | pH mean (min<br>max.) | Hardness<br>mg/L<br>CaCO₃ | DOC<br>mg/L       | EC <sub>50</sub> ª (95% CL)<br>μg/L Cu <sup>2+</sup> |
|--|----------|-------------|-----------------------|---------------------------|-------------------|--|
| Fish - Common bully - Gobiomorphus cotidianus                                    |          |             |                       |                           |                   |  |
| Juvenile wet weight 0.45g;<br>Head-tail base length 32mm.<br>Ouwerkerk (2017)    | 96 h     | 16          | 7.29 (7.05-7.80)      | 14.2                      | 0.3ª              | 476 (289-565) n=1                                    |
| Juvenile wet weight 0.75g;<br>Head-tail base length 37.5<br>mm. Ouwerkerk (2017) | 96 h     | 16          | 7.22 (6.84-7.52)      | 14.2                      | 1.3ª              | 601 (477-757) n=1                                    |
| Juvenile wet weight 0.63 g;<br>Head-tail base length 37 mm.<br>Ouwerkerk (2017)  | 96 h     | 16          | 7.18 (6.87-7.58)      | 14.2                      | 4.3ª              | 1,789 (1,000-3,200)<br>n=1                           |
| Juvenile wet weight 0.45 g;<br>Head-tail base length 32 mm.<br>Ouwerkerk (2017)  | 10 d     | 16          | 7.29 (7.05-7.80)      | 14.2                      | 0.3ª              | 125 (93-163) n=1                                     |
| Juvenile wet weight 0.75g;<br>Head-tail base length 37.5<br>mm. Ouwerkerk (2017) | 10 d     | 16          | 7.22 (6.84-7.52)      | 14.2                      | 1.3ª              | 534 (496-574) n=1                                    |
| Juvenile wet weight 0.63 g;<br>Head-tail base length 37 mm.<br>Ouwerkerk (2017)  | 10 d     | 16          | 7.18 (6.87-7.58)      | 14.2                      | 4.3ª              | 1,006 (797-1,270)<br>n=1                             |
| Invertebrate - Snail - Potamopyrgus antipodarum                                  |          |             |                       |                           |                   |  |
| Ouwerkerk (2017). Survival   | 96 h     | 20          | 6.86 (6.65-7.1)       | 14.2                      | 0.3ª              | 17 (14-20) n=1                                       |
| Ouwerkerk (2017). Survival   | 96 h     | 20          | 7.06 (7.0-7.14)       | 14.2                      | 1.3ª              | 36 (31-41) n=1                                       |
| Ouwerkerk (2017). Survival   | 96 h     | 20          | 7.06 (7.03-7.16)      | 14.2                      | 4.3ª              | 77 (67-89) n=1                                       |
| Ouwerkerk (2017). Survival   | 96 h     | 20          | 6.89 (6.78-7.06)      | 14.2                      | 9.3ª              | 110 (102-120) n=1                                    |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 96 h     | 20          | 6.86 (6.65-7.1)       | 14.2                      | 0.3ª              | 14 (13-15) n=1                                       |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 96 h     | 20          | 7.06 (7.0-7.14)       | 14.2                      | 1.3ª              | 34 (30-38) n=1                                       |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 96 h     | 20          | 7.06 (7.03-7.16)      | 14.2                      | 4.3ª              | 52 (47-58) n=1                                       |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 96 h     | 20          | 6.89 (6.78-7.06)      | 14.2                      | 9.3ª              | 91 (83-100) n=1                                      |
| Invertebrate - Amphipod - Paracalliope fluviatilis                               |          |             |                       |                           |                   |  |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h     | 21          | 7.37 (7.31-7.44)      | 14.2                      | 0.3ª              | 70 (58-84) n=1                                       |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h     | 20          | 7.29 (7.18-7.35)      | 14.2                      | 1.3ª              | 92 (80-108) n=1                                      |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h     | 20          | 7.48 (7.35-53)        | 14.2                      | 4.3ª              | 263 (184-308) n=1                                    |
| Ouwerkerk (2017). Morbidity<br>(mobility)  | 48 h     | 20          | 7.53 (7.47-7.59)      | 14.2                      | 9.3ª              | 629 (564-700) n=1                                    |
| Invertebrate – Water flea – Daphnia thomsoni                                     |          |             |                       |                           |                   |  |
| NIWA study FWWQ2009  | 48 h     | 20          | 7.6 (7.3-7.8)         | 14.0                      | 0.42 <sup>b</sup> | 28 (na-na) n=1                                       |
| NIWA study FWWQ2009  | 48 h     | 20          | 7.7 (7.5-7.8)         | 14.0                      | 1.70 <sup>b</sup> | 215 (164-246) n=1                                    |
| NIWA study FWWQ2009  | 48 h     | 20          | 7.7 (7.5-7.8)         | 14.0                      | 2.52 ⁵            | 597 (558-614) n=1                                    |

Table M-2:Summary of NIWA's unpublished or publicly unavailable New Zealand native species acutecopper toxicity testing data.Shaded values are nominal concentrations. na = not available.

<sup>a</sup> Humic acid reference carbon source.

## Appendix D Calculation of acute algal toxicity data

#### D.1 Introduction

Algal toxicity tests with zinc had been undertaken in a variety of waters for development of the chronic zinc guideline values from studies funded by IZA.<sup>1</sup> Those tests were undertaken under an exposure duration of 72 hours, however in accordance with the test protocols, algal cell counts were also measured at 24 hours – an acceptable time frame to be considered an acute toxicity test. The raw data from those tests were therefore used to generate toxicity data for use in deriving acute guideline values.

#### D.2 Methods

The original report<sup>2</sup> should be referred to for a full description of the methods including locations of natural water samples, toxicity test methods and analysis of water chemistry during and after tests. Raw data were obtained for six tests with *Raphidocelis subcapitata* from NIWA (K Thompson, pers. comm) and for multiple tests with *Chlorella* sp. (PNG isolate) from Gwilym Price. These each included data for 24 hour cell yields and measured zinc concentrations at day 0 and day 3.

The 24 hour growth rates were calculated as:

Growth rate = log(24-h cell yield) - log(0-h cell yield/inoculum)

The growth rate was calculated as a percentage of the control for each test replicate as follows:

 $Growth \, rate \, inhibition = 100 \times \frac{Growth \, rate \, _{test, replicate}}{Growth \, rate \, _{control}}$ 

For all tests, zinc concentrations were measured at 0 hours and at 72 hours but not at 24 hours. The measured zinc concentrations at 0 hours were used for the zinc concentration in concentration-response regressions as these would be more reflective of the 24 hour concentrations than those measured at 72-hours.

Non-linear regression models were fitted in the R studio environment with the extension package drc.<sup>3</sup> Multiple models were fitted including log-logistic and Weibull (type 1 and type 2) with fixed and varying parameters, and AIC was used to select the model with the best fit, along with visual assessments of the fitted curves.

Several tests, especially those with *R. subcapitata* resulted in concentration-response curves that reached an asymptote around 20% growth rate (as a percentage of control). In these situations, models were fitted with varying lower limits (instead of zero) to enable the model to best fit the curve. As the ED function in drc calculates effect concentrations between the upper and lower limits of the model (i.e., an EC50 will represent the midpoint of the upper and lower asymptotes, not the 50% response relative to the control, an absolute EC50 was calculated – that is, the concentration where a line at 50% of control growth rate intersects with the fitted model.

#### D.3 Results

The results for the six waters tested with *R. subcapitata* (Figure D.1) indicate some variation in the response between the waters tested, with EC50 values ranging from 50 to  $165 \mu g/L$ .

<sup>&</sup>lt;sup>1</sup> J Stauber et al., 2022. Towards bioavailability-based guideline values for zinc in Australian and New Zealand natural waters. Report to the International Zinc Association (CSIRO, Australia, January 2022).

<sup>&</sup>lt;sup>2</sup> Stauber et al., 2022.

<sup>&</sup>lt;sup>3</sup> R: A Language and Environment for Statistical Computing. , R Foundation for Statistical Computing, Vienna, Austria. C Ritz et al., 2015. Dose-response analysis using R. *PLoS ONE* 10, 12: e0146021.
The Chlorella tests indicate a much wider range in the EC50 response, from 27  $\mu$ g/L (at pH 8.5, hardness 90 mg/L as CaCO<sub>3</sub> and DOC 0.5 mg/L) to >1000  $\mu$ g/L in natural waters with high DOC (20-25 mg/L) and lower pH.



Figure D.I: Concentration-response curves for *R. subcapitata* after exposure to **zinc** for 24 hours. Note that for most of the waters tested growth rate did not reduce to zero after 24 hours of exposure. EC50 calculated as 50% of control growth rate.

Appendix Table D.1: Acute (24 hour) algal (*R. subcapitata*) growth rate inhibition EC50 values for **zinc**. Model type 3pLL= 3-parameter log-logistic; 4pLL= 4-parameter log-logistic.

| River     | EC50<br>(95% confidence interval,<br>µg Zn/L) | Model | рН  | Hardness (mg/L<br>as CaCO₃) | DOC (mg/L) |
|-----------|---|-------|-----|-----------------------------|------------|
| Control   | 50 (48-53)                                    | 4pLL  | 7.2 | 8.7                         | 0.15       |
| Clutha    | 57 (55-60)                                    | 4pLL  | 8   | 50                          | 0.4        |
| Waihou    | 69 (67-72)                                    | 4pLL  | 7.9 | 39                          | 0.15       |
| Mahurangi | 81 (77-84)                                    | 4pLL  | 8.2 | 110                         | 2.4        |
| Hoteo     | 76 (69-83)                                    | 3pLL  | 7.6 | 72                          | 4.6        |
| Okutua    | 165 (156-173)                                 | 3pLL  | 7.2 | 26                          | 7.3        |

| Test             | рН   | Hardness | DOC  | EC50 value (CI)  | Model |
|------------------|------|----------|------|------------------|-------|
| Synthetic waters |      |          |      |                  |       |
| 200302_H5_pH6.5  | 6.5  | 4.8      | 0.75 | 240 (230-260)    | W     |
| 200302_H5_pH8.5  | 8.5  | 4.8      | 0.75 | 47 (38-55)       | LL    |
| 200609_H30_pH6.5 | 6.5  | 32       | 0.50 | 800 (770-840)    | LL    |
| 200609_H30_pH7.5 | 7.5  | 32       | 0.50 | 330 (300-350)    | W     |
| 200601_H30_pH7.5 | 7.5  | 30       | 0.50 | 170 (140-200)    | LL    |
| 200601_H30_pH8.5 | 8.5  | 30       | 0.50 | 45 (39-51)       | W     |
| 200609_H30_pH8.5 | 8.5  | 32       | 0.50 | 47 (44-50)       | LL    |
| 190923_pH8.5     | 8.3  | 93       | 0.54 | 27 (24-30)       | LL    |
| 191021_pH8.5     | 8.3  | 94       | 1.1  | 40 (36-44)       | W     |
| Natural waters   |      |          |      |                  |       |
| WR buffered      | 7.11 | 18       | 5.3  | 870 (350-660)    | LL    |
| BW buffered      | 8.05 | 355      | 4.2  | 300 (240-380)    | W     |
| OR buffered      | 7.47 | 11       | <1   | 380 (360-400)    | LL    |
| MC buffered      | 6.38 | 3        | 6.0  | 300 (290-320)    | W     |
| LC buffered      | 7.42 | 89       | 20   | 1400 (1200-1600  | LL    |
| TC unbuffered    | 6.1  | 13       | 25   | 1800 (1600-1900) | LL    |

Appendix Table D.2: Acute (24 hour) algal (*Chlorella* sp. (PNG isolate)) growth rate inhibition EC50 values for **zinc** in synthetic and natural waters. Model type W = Weibull type 1; LL= 3-parameter log-logistic.



Figure D.2: Concentration-response curves for *Chlorella* sp. after exposure to **zinc** for 24 hours. This includes tests in synthetic waters at various pH and hardness values and in natural waters that varied by pH, hardness and DOC.

# Appendix E Evaluation of metal bioavailability models for plants and algae

#### E.1 Models and the evaluation process

There is limited use of models for assessing bioavailability of algal or plant acute toxicity data internationally, particularly within the context of deriving guideline values. Therefore, models that could potentially be used for this purpose were evaluated. This was undertaken independently of model evaluation for the complete toxicity dataset to identify whether models for fish and invertebrates would perform adequately for plants and algae, or whether specific models would be required.

Appendix Table E.1 sets out the models available for both copper and zinc. No algal MLRs have been developed for copper to date. Although the European risk assessment for copper uses a chronic toxicity algal BLM, that model was not available for evaluation.

### Appendix Table E.I: Models available for normalising acute **copper** and **zinc** toxicity data for plants and algae. Additional information on each model including the applicable TMF ranges and taxonomic groups included in their development is available in the excel files associated with this project.

| Model  | Use internationally                             | TMFs included                    |  |
|--|---|----------------------------------|--|
| Copper   |   |                                  |  |
| Plant BLM <sup>4</sup>                         | Canadian copper GVs <sup>5</sup>                | pH, DOC, multiple cations/anions |  |
| Zinc   |   |                                  |  |
| <i>R. subcapitata</i> chronic MLR <sup>6</sup> | Chronic zinc DGVs for Australia & NZ $^{\rm 8}$ | pH, DOC                          |  |
| Chlorella sp. chronic MLR <sup>7</sup>         | Chronic zinc DGVs for Australia & NZ $^{\rm 8}$ | pH, DOC, hardness                |  |
| <i>R. subcapitata</i> BLM <sup>6</sup>         | No use to date                                  | pH, DOC, multiple cations/anions |  |

There were few data available for model evaluation. For copper, one study tested the macrophyte *Ceratophyllum demersum* at varying hardness values from 35 to 335 mg CaCO<sub>3</sub>/L.<sup>9</sup> However, there was no variation in pH or DOC for these tests, or any other tests with this species. Further, there were no other toxicity data identified for other species where pH, DOC and hardness were varied. Without suitable data, no evaluation of the models for copper could be made.

For zinc, algal data were available at a 24 hour time period from the work undertaken to support the derivation of chronic zinc guideline values,<sup>10</sup> where pH, DOC and/or hardness varied (see Appendix D). These data were used to evaluate each of the models listed in Appendix Table E.2, as well as the models used for fish and invertebrates (see section 6), using the quantitative assessment method outlined in section 5.4.3.

<sup>&</sup>lt;sup>4</sup> X Wang, L Hua, and Y Ma, 2012. A biotic ligand model predicting acute copper toxicity for barley (Hordeum vulgare): Influence of calcium, magnesium, sodium, potassium and pH. Chemosphere 89, 1: 89-95.

<sup>&</sup>lt;sup>5</sup> ECCC, 2021; B.C. Ministry of Environment and Climate Change Strategy, 2019.

<sup>6</sup> DeForest et al., 2023.

<sup>7</sup> Price et al., 2023.

<sup>&</sup>lt;sup>8</sup> ANZG, 2024.

<sup>&</sup>lt;sup>9</sup> Markich, King, and Wilson, 2006.

<sup>&</sup>lt;sup>10</sup> Stauber et al., 2023; GAV Price et al., 2021. The influence of pH on zinc lability and toxicity to a tropical freshwater microalga. *Environmental Toxicology and Chemistry* 40, 10: 2836-45; GAV Price et al., 2022. The influence of hardness at varying pH on zinc toxicity and lability to a freshwater microalga, *Chlorella* sp. *Environmental Science-Processes & Impacts* 24, 5: 783-93.

Appendix Table E.2: Toxicity data used to evaluate models for normalising acute **copper** and **zinc** toxicity data for plants and algae. The full set of data used for this evaluation is available in the excel files associated with this project.

| Species                | Test duration & effect      | TMF ranges   | Metal (no.<br>data points) |
|------------------------|-----------------------------|--|----------------------------|
| Cerotophyllum demersum | 96-h biomass                | pH 7.0<br>Hardness 35-335 mg CaCO₃/L<br>DOC 0.1 mg/L*          | Copper (3)                 |
| Chlorella sp.          | 24-h growth rate inhibition | pH 6.1-8.3<br>Hardness 3-355 mg CaCO₃/L<br>DOC 0.69-20 mg/L    | Zinc (15)                  |
| R. subcapitata         | 24-h growth rate inhibition | pH 7.2-8.2<br>Hardness 9-110 mg CaCO₃/L<br>DOC 0.15**-7.3 mg/L | Zinc (6)                   |

Note: DOC values were reported as below the limit of detection (\*<0.2 mg/L and \*\*<0.3 mg/L).

#### E.2 Performance of algal models for zinc

Plots of predicted EC50 values versus observed (Figure E.1) indicated poor performance for predicting toxicity to *Chlorella* sp. in synthetic waters for all models except the *R. subcapitata* chronic MLR, which had a model performance score of 0.84, compared to 0.41–0.57 for the remaining models (Appendix Table E.3). If these synthetic water tests are excluded, there was much less difference between the models.

Overall, and surprisingly the *R. subcapitata* chronic BLM did not predict the toxicity *R. subcapitata* or *Chlorella* sp. very well, over-predicting the EC50 values for most tests. As this means the model underpredicts toxicity, this would not be a conservative model to use for guideline value derivation.

Based on the above assessment, if the synthetic water *Chlorella* sp. data are excluded, the fish/invertebrate MLR or BLMs could be adopted. Otherwise, the *R. subcapitata* chronic MLR model performed the best and could be used for adjusting the zinc algal data for the SSD and guideline value derivation.



Species • Chlorella sp. (PNG isolate) • Chlorella sp. (PNG isolate, nat. water) • Raphidocelis subcapitata

Figure E.I: Observed EC50 values for acute **zinc** toxicity to algal species, compared to EC50 values predicted with various models. Solid line is line of perfect agreement between observed and predicted EC50 values. Dashed lines indicate a factor of ±2 difference. The closest agreement is shown using the *R. subcapitata* chronic MLR model, for both algal species tested.

Appendix Table E.3: Model performance metrics for plant/algal models. See plots in Annex for additional information.

| Model                 | R <sup>2</sup> | <b>RF</b> <sub>x,2.0</sub> <sup>†</sup> | Residual<br>scores <sup>‡</sup> | Overall score<br>(average of 3 metrics) |
|-----------------------|----------------|---|---------------------------------|---|
| Hardness              | 0.22           | 0.47                                    | 0.62                            | 0.44                                    |
| Fish/invertebrate MLR | 0.39           | 0.53                                    | 0.71                            | 0.56                                    |
| Fish/invertebrate BLM | 0.47           | 0.38                                    | 0.75                            | 0.53                                    |
| Chlorella MLR         | 0.45           | 0.47                                    | 0.74                            | 0.57                                    |
| R. subcapitata MLR    | 0.77           | 0.80                                    | 0.94                            | 0.84                                    |
| R. subcapitata BLM    | 0.32           | 0.24                                    | 0.67                            | 0.41                                    |

Notes: <sup>†</sup> Proportion of predictions within a factor of two. <sup>‡</sup> Residual score based on linear regressions between residuals (observed/predicted) and log(observed EC50), pH, log(hardness) and log(DOC). Each is calculated as 2/(1+10^|(slope × (1-p-value))|)<sup>11</sup> and all are averaged to calculate the overall residual score. See plots in next section for relationships between residuals and each of these variables.

#### Annex to Appendix E: Additional details for model evaluation

This section provides the plots of residuals versus log EC50, pH, log hardness and log DOC, from which the slopes and p-values were calculated for the residual scores.

<sup>&</sup>lt;sup>11</sup> Besser et al., 2021..



Figure E.2: Model evaluation plots for **zinc** plant/algae data with hardness regression. Green points are *R*. *subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.



Figure E.3: Model evaluation plots for **zinc** plant/algae data with pooled fish/invertebrate MLR. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.



Figure E.4: Model evaluation plots for **zinc** plant/algae data with pooled fish/invertebrate BLM. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.



Figure E.5: Model evaluation plots for **zinc** plant/algae data with *Chlorella* sp. MLR. Green points are *R*. *subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.



Figure E.6: Model evaluation plots for **zinc** plant/algae data with *R. subcapitata*. MLR. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.

| Medel                     | Slopes (p-values) of observed/predicted EC50 versus: |               |                |              |  |  |  |  |
|---------------------------|--|---------------|----------------|--------------|--|--|--|--|
| Woder                     | Observed EC50  | рН            | Log (Hardness) | Log (DOC)    |  |  |  |  |
| Hardness                  | 0.46 (0.07)  | -0.67 (<0.01) | -0.38 (<0.01)  | 0.01 (0.91)  |  |  |  |  |
| Fish/invertebrate MLR     | 0.33 (0.1)   | -0.45 (<0.01) | -0.29 (<0.01)  | -0.05 (0.54) |  |  |  |  |
| Fish/invertebrate BLM     | te BLM 0.39 (0.02)                                   |               | -0.16 (0.04)   | -0.07 (0.31) |  |  |  |  |
| Chlorella MLR             | 0.22 (0.29)  | -0.37 (0.02)  | -0.3 (<0.01)   | -0.12 (0.12) |  |  |  |  |
| <i>R. subcapitata</i> MLR | 0.17 (0.11)  | 0.01 (0.89)   | 0.06 (0.22)    | -0.03 (0.51) |  |  |  |  |
| R. subcapitata BLM        | 0.61 (<0.01)   | -0.47 (<0.01) | -0.17 (0.04)   | 0.04 (0.61)  |  |  |  |  |

## Appendix F Additional results of bioavailability model evaluation

#### F.1 Copper cross-species validation

|                                     | Correlation                   |                              | Slopes (p-values) of observed/predicted EC50 versus: ‡ |              |                   |               |                     |
|-------------------------------------|-------------------------------|------------------------------|--|--------------|-------------------|---------------|---------------------|
| Model                               | coefficient<br>(pred.vs obs.) | <b>RF</b> x,2.0 <sup>†</sup> | Log (obs.<br>EC50)*                                    | рН           | Log<br>(hardness) | Log (DOC)     | Log<br>(Alkalinity) |
| Hardness regression                 | 0.45                          | 0.48                         | 0.58 (<0.01)   | 0 (0.97)     | -0.21 (0.014)     | 0.37 (<0.01)  | -0.12 (0.15)        |
| Pooled fish/<br>invertebrate<br>MLR | 0.64                          | 0.67                         | 0.2 (<0.01)  | 0 (0.98)     | -0.08 (0.16)      | -0.1 (0.02)   | -0.01 (0.93)        |
| Trophic MLR                         | 0.58                          | 0.67                         | 0.27 (<0.01)   | -0.01 (0.84) | -0.13 (0.04)      | -0.02 (0.70)  | -0.05 (0.41)        |
| Fish/<br>invertebrate<br>BLM        | 0.69                          | 0.62                         | 0.12 (0.055)   | 0.05 (0.2)   | -0.01 (0.92)      | -0.22 (<0.01) | 0.05 (0.39)         |

#### Appendix Table F.I: Cross-species model performance metrics for **copper**.

Notes: <sup>†</sup> Proportion of predictions within a factor of two. <sup>‡</sup> Linear regressions between residuals (observed/predicted) and log(observed EC50), pH, log(hardness) and log(DOC). Each is calculated as 2/(1+10^|(slope × (1-p-value))|)<sup>12</sup> and all are averaged to calculate the overall residual score. See plots below for relationships between residuals and each of these variables. \* Observed EC50 concentration.

<sup>&</sup>lt;sup>12</sup> Besser et al., 2021..







Figure F.2: Model evaluation plots for **copper** cross-validation of pooled fish/invertebrate MLR model.







Figure F.4: Model evaluation plots for copper cross-validation of BLM. .

#### F.2 Zinc cross-species validation plots

|                                     | Correlation                   |   | Slopes (p-values) of observed/predicted EC50 versus: <sup>‡</sup> |               |                   |               |                     |  |
|-------------------------------------|-------------------------------|---|---|---------------|-------------------|---------------|---------------------|--|
| Model                               | coefficient<br>(pred.vs obs.) | <b>RF</b> <sub>x,2.0</sub> <sup>†</sup> | Log<br>(obs. EC50)*   | рН            | Log<br>(hardness) | Log (DOC)     | Log<br>(Alkalinity) |  |
| Hardness<br>regression              | 0.59                          | 0.76                                    | 0.32 (<0.01)  | -0.35 (<0.01) | -0.31 (0.001)     | 0.17 (0.014)  | -0.42 (<0.01)       |  |
| Pooled fish/<br>invertebrate<br>MLR | 0.73                          | 0.81                                    | 0.26 (<0.01)  | -0.21 (<0.01) | -0.18 (0.019)     | 0.05 (0.403)  | -0.27 (<0.01)       |  |
| Trophic MLR                         | 0.65                          | 0.66                                    | 0.07 (0.429)  | 0.05 (0.501)  | 0.04 (0.734)      | -0.3 (<0.01)  | 0.1 (0.35)          |  |
| Fish/<br>invertebrate<br>BLM        | 0.61                          | 0.61                                    | 0.28 (<0.01)  | -0.19 (0.006) | -0.21 (0.027)     | -0.09 (0.198) | -0.21 (0.035)       |  |

#### Appendix Table F.2: Cross-species model performance metrics for **zinc**.

Notes: <sup>†</sup> Proportion of predictions within a factor of two. <sup>‡</sup> Linear regressions between residuals (observed/predicted) and log(observed EC50), pH, log(hardness) and log(DOC). Each is calculated as 2/(1+10^|(slope × (1-p-value))|)<sup>13</sup> and all are averaged to calculate the overall residual score. See plots below for relationships between residuals and each of these variables. \* Observed EC50 concentration.



Figure F.5: Model evaluation plots for **zinc** cross-validation of hardness regression model.

<sup>&</sup>lt;sup>13</sup> Besser et al., 2021..



Figure F.6: Model evaluation plots for **zinc** cross-validation of pooled fish/invertebrate MLR.



Figure F.7: Model evaluation plots for zinc cross-validation of trophic fish and invertebrate MLRs.



Figure F.8: Model evaluation plots for **zinc** cross-validation of pooled fish/invertebrate BLM.

#### F.3 Copper native species validation

Appendix Table F.3: Native-species model performance metrics for copper.

|                                     | Correlation                                       |      | Slopes (p-values) of observed/predicted EC50 versus: <sup>‡</sup> |              |                   |               |                     |  |  |
|-------------------------------------|---|------|---|--------------|-------------------|---------------|---------------------|--|--|
| Model                               | coefficient<br>(pred.vs obs.) RF <sub>x,2.0</sub> |      | Log (obs.<br>EC50)*   | рН           | Log<br>(hardness) | Log (DOC)     | Log<br>(Alkalinity) |  |  |
| Hardness<br>regression              | 0.36  | 0.48 | 0.8 (<0.01)   | 0.19 (0.17)  | -1.23 (<0.01)     | 0.7 (<0.01)   | -0.67 (<0.01)       |  |  |
| Pooled fish/<br>invertebrate<br>MLR | 0.94  | 0.84 | 0.1 (0.02)  | -0.04 (0.47) | -0.38 (<0.01)     | 0.03 (0.39)   | -0.16 (0.034)       |  |  |
| Trophic MLR                         | 0.89  | 0.77 | 0.23 (<0.01)  | -0.07 (0.32) | -0.59 (<0.01)     | 0.19 (<0.01)  | -0.34 (<0.01)       |  |  |
| Fish/<br>invertebrate<br>BLM        | 0.94  | 0.73 | -0.1 (0.03)   | -0.06 (0.27) | 0.23 (0.03)       | -0.15 (<0.01) | 0.27 (<0.01)        |  |  |

Notes: <sup>†</sup> Proportion of predictions within a factor of two. <sup>‡</sup> Linear regressions between residuals (observed/predicted) and log(observed EC50), pH, log(hardness) and log(DOC). Each is calculated as 2/(1+10^|(slope × (1-p-value))|)<sup>14</sup> and all are averaged to calculate the overall residual score. See plots below for relationships between residuals and each of these variables. \* Observed EC50 concentration.

<sup>&</sup>lt;sup>14</sup> Besser et al., 2021..



Figure F.9: Model evaluation plots for **copper** native-species validation of hardness regression.



Figure F.10: Model evaluation plots for **copper** native-species validation of pooled fish/invertebrate MLR.



Figure F.11: Model evaluation plots for **copper** native-species validation of trophic-level MLRs.



Figure F.12: Model evaluation plots for **copper** native-species validation of BLM.

#### F.4 Zinc native species validation

|                                     | Correlation                   |   | Slop                | s: ‡          |                   |               |                     |
|-------------------------------------|-------------------------------|---|---------------------|---------------|-------------------|---------------|---------------------|
| Model                               | coefficient<br>(pred.vs obs.) | <b>RF</b> <sub>x,2.0</sub> <sup>†</sup> | Log<br>(obs. EC50)* | рН            | Log<br>(hardness) | Log (DOC)     | Log<br>(Alkalinity) |
| Hardness regression                 | 0.52                          | 0.70                                    | 0.15 (0.33)         | -0.4 (<0.01)  | -0.22 (0.14)      | 0.09 (0.26)   | -0.45 (0.03)        |
| Pooled fish/<br>invertebrate<br>MLR | 0.71                          | 0.85                                    | 0.2 (0.031)         | -0.21 (<0.01) | -0.2 (0.03)       | 0.05 (0.33)   | -0.29 (0.03)        |
| Trophic MLR                         | 0.48                          | 0.46                                    | -0.05 (0.79)        | 0.07 (0.59)   | 0.1 (0.6)         | -0.47 (<0.01) | 0.27 (0.32)         |
| Fish/<br>invertebrate<br>BLM        | 0.59                          | 0.64                                    | 0.22 (0.072)        | -0.04 (0.66)  | -0.07 (0.54)      | -0.05 (0.49)  | 0.12 (0.47)         |

#### Appendix Table F.4: Native-species model performance metrics for **zinc**.

Notes: <sup>†</sup> Proportion of predictions within a factor of two. <sup>‡</sup> Linear regressions between residuals (observed/predicted) and log(observed EC50), pH, log(hardness) and log(DOC). Each is calculated as 2/(1+10^|(slope × (1-p-value))|)<sup>15</sup> and all are averaged to calculate the overall residual score. See plots below for relationships between residuals and each of these variables. \* Observed EC50 concentration.



Figure F.13: Model evaluation plots for **zinc** native-species validation of hardness regression.

<sup>&</sup>lt;sup>15</sup> Besser et al., 2021..



Figure F.14: Model evaluation plots for **zinc** native-species validation of pooled fish/invertebrate MLR.



Figure F.15: Model evaluation plots for **zinc** native-species validation of trophic-level MLRs.



Figure F.16: Model evaluation plots for **zinc** native-species validation of BLM.

### Appendix G Acute toxicity data used in the derivations

Appendix Table G.I: **Copper** acute toxicity values used to derive acute guideline values in freshwater. Reported EC50 value shown here is geometric mean of all reported values. Single values for each species, toxicity values normalised to index water chemistry of pH 7.5, hardness 30 mg CaCO<sub>3</sub>/L and 0.5 mg/L DOC.

| Tax Group  | Species                     | Effect    | Organism<br>life stage | Exposure duration | N. data<br>this<br>species | Reported<br>EC50 * | Norma-<br>lised<br>EC50 | Converted<br>EC10 | Percentile<br>rank<br>(1-100) |
|------------|-----------------------------|-----------|------------------------|-------------------|----------------------------|--------------------|-------------------------|-------------------|-------------------------------|
| Amphibian  | Lithobates<br>clamitans     | Mortality | Embryo                 | 96                | 1                          | 160                | 46                      | 25                | 63                            |
| Amphibian  | Lithobates<br>catesbeianus  | Mortality | Larva                  | 96                | 1                          | 2400               | 7600                    | 4200              | 99                            |
| Annelid    | Lumbriculus<br>variegatus   | Mortality | Adult                  | 48                | 25                         | 110                | 59                      | 36                | 74                            |
| Crustacean | Scapholeberis<br>mucronata  | Mortality | Juvenile               | 48                | 1                          | 5.3                | 1.5                     | 0.9               | 0                             |
| Crustacean | Ceriodaphnia dubia          | Mortality | Neonate                | 48                | 46                         | 45                 | 4                       | 2.5               | 9                             |
| Crustacean | Alona<br>quadrangularis     | Mortality | Juvenile               | 48                | 2                          | 54                 | 4.2                     | 2.6               | 10                            |
| Crustacean | Daphnia longispina          | Mortality | Juvenile               | 48                | 11                         | 7.9                | 5.7                     | 3.5               | 12                            |
| Crustacean | Alona sp.                   | Mortality | Juvenile               | 48                | 1                          | 23                 | 6.4                     | 4                 | 15                            |
| Crustacean | Daphnia pulex               | Mortality | Neonate                | 48                | 31                         | 13                 | 6.7                     | 4.2               | 16                            |
| Crustacean | Simocephalus<br>exspinosus  | Mortality | Juvenile               | 48                | 11                         | 27                 | 7.6                     | 4.7               | 19                            |
| Crustacean | Daphnia galeata             | Mortality | Juvenile               | 48                | 6                          | 14                 | 7.9                     | 4.9               | 20                            |
| Crustacean | Ceriodaphnia<br>reticulata  | Mortality | Neonate                | 48                | 422                        | 41                 | 8.4                     | 5.2               | 21                            |
| Crustacean | Daphnia magna               | Mortality | Neonate                | 48                | 7                          | 16                 | 8.6                     | 5.3               | 22                            |
| Crustacean | Disparalona<br>rostrata     | Mortality | Juvenile               | 48                | 2                          | 99                 | 11                      | 6.6               | 27                            |
| Crustacean | Daphnia obtusa              | Mortality | Neonate                | 48                | 53                         | 23                 | 11                      | 6.7               | 29                            |
| Crustacean | Simocephalus<br>vetulus     | Mortality | Juvenile               | 48                | 18                         | 12                 | 11                      | 6.8               | 30                            |
| Crustacean | Hyalella azteca             | Mortality | Juvenile               | 96                | 7                          | 20                 | 11                      | 6.9               | 33                            |
| Crustacean | Eurycercus<br>Iamellatus    | Mortality | Juvenile               | 48                | 2                          | 18                 | 13                      | 8.2               | 37                            |
| Crustacean | Pleuroxus<br>truncatus      | Mortality | Juvenile               | 48                | 2                          | 160                | 17                      | 11                | 42                            |
| Crustacean | Acroperus harpae            | Mortality | Juvenile               | 48                | 2                          | 26                 | 17                      | 11                | 43                            |
| Crustacean | Chydorus<br>sphaericus      | Mortality | Juvenile               | 48                | 9                          | 39                 | 22                      | 14                | 49                            |
| Crustacean | Caridina sp.                | Mortality | Adult                  | 48                | 1                          | 4.5                | 23                      | 14                | 51                            |
| Crustacean | Daphnia carinata            | Mortality | Neonate                | 48                | 1                          | 40                 | 28                      | 17                | 53                            |
| Crustacean | Paracalliope<br>fluviatilis | Mortality | NA                     | 48                | 1                          | 61                 | 41                      | 26                | 65                            |
| Crustacean | Daphnia thomsoni            | Mortality | Neonate                | 48                | 7                          | 100                | 57                      | 35                | 72                            |

| Tax Group  | Species                        | Effect    | Organism<br>life stage | Exposure<br>duration | N. data<br>this<br>species | Reported<br>EC50 * | Norma-<br>lised<br>EC50 | Converted<br>EC10 | Percentile<br>rank<br>(1-100) |
|------------|--------------------------------|-----------|------------------------|----------------------|----------------------------|--------------------|-------------------------|-------------------|-------------------------------|
| Crustacean | Paratya<br>australiensis       | Mortality | Adult                  | 96                   | 11                         | 140                | 63                      | 39                | 78                            |
| Fish       | Acipenser<br>transmontanus     | Growth    | Larva                  | 96                   | 1                          | 5.3                | 2.1                     | 1.1               | 1                             |
| Fish       | Prosopium<br>williamsoni       | Mortality | Juvenile               | 96                   | 2                          | 5.5                | 2.8                     | 1.5               | 2                             |
| Fish       | Cottus bairdii                 | Mortality | Larva                  | 96                   | 1                          | 17                 | 4.9                     | 2.7               | 11                            |
| Fish       | Ptychocheilus<br>oregonensis   | Mortality | Juvenile               | 96                   | 2                          | 20                 | 12                      | 6.7               | 28                            |
| Fish       | Etheostoma<br>rubrum           | Mortality | NA                     | 96                   | 1                          | 58                 | 12                      | 6.8               | 31                            |
| Fish       | Oncorhynchus<br>clarkii        | Mortality | Adult/<br>Juvenile     | 96                   | 2                          | 67                 | 14                      | 7.9               | 34                            |
| Fish       | Oncorhynchus<br>mykiss         | Mortality | Juvenile               | 96                   | 56                         | 23                 | 15                      | 8.1               | 36                            |
| Fish       | Oncorhynchus<br>apache         | Mortality | NA                     | 96                   | 1                          | 67                 | 17                      | 9.2               | 39                            |
| Fish       | Oncorhynchus<br>tshawytscha    | Mortality | Juvenile               | 96                   | 152                        | 75                 | 19                      | 10                | 40                            |
| Fish       | Oncorhynchus<br>kisutch        | Mortality | Juvenile               | 96                   | 3                          | 15                 | 21                      | 12                | 46                            |
| Fish       | Pimephales<br>promelas         | Mortality | Larva                  | 96                   | 5                          | 71                 | 22                      | 12                | 48                            |
| Fish       | Perca flavescens               | Mortality | Adult/<br>Juvenile     | 96                   | 2                          | 86                 | 33                      | 18                | 55                            |
| Fish       | Poeciliopsis<br>occidentalis   | Mortality | NA                     | 96                   | 1                          | 160                | 40                      | 22                | 57                            |
| Fish       | Galaxias maculatus             | Mortality | Adult                  | 96                   | 1                          | 59                 | 41                      | 22                | 58                            |
| Fish       | Gila elegans                   | Mortality | NA                     | 96                   | 1                          | 190                | 45                      | 25                | 62                            |
| Fish       | Salvelinus<br>confluentus      | Mortality | Juvenile               | 96                   | 5                          | 100                | 52                      | 29                | 67                            |
| Fish       | Scaphirhynchus<br>platorynchus | Mortality | NA                     | 96                   | 1                          | 160                | 55                      | 30                | 69                            |
| Fish       | Etheostoma<br>Iepidum          | Mortality | NA                     | 96                   | 1                          | 250                | 63                      | 34                | 71                            |
| Fish       | Macquaria<br>ambigua           | Mortality | Juvenile               | 96                   | 1                          | 94                 | 65                      | 36                | 73                            |
| Fish       | Acrocheilus<br>alutaceus       | Mortality | Juvenile               | 96                   | 1                          | 140                | 68                      | 38                | 75                            |
| Fish       | Entosphenus<br>tridentatus     | Mortality | Egg                    | 96                   | 1                          | 46                 | 69                      | 38                | 76                            |
| Fish       | Gasterosteus<br>aculeatus      | Mortality | Adult                  | 96                   | 5                          | 330                | 82                      | 45                | 79                            |
| Fish       | Pseudomugil<br>tenellus        | Mortality | Adult                  | 96                   | 1                          | 120                | 95                      | 52                | 82                            |

| Tax Group      | Species                            | Effect    | Organism<br>life stage | Exposure duration | N. data<br>this<br>species | Reported<br>EC50 * | Norma-<br>lised<br>EC50 | Converted<br>EC10 | Percentile<br>rank<br>(1-100) |
|----------------|------------------------------------|-----------|------------------------|-------------------|----------------------------|--------------------|-------------------------|-------------------|-------------------------------|
| Fish           | Etheostoma<br>flabellare           | Mortality | NA                     | 96                | 4                          | 340                | 98                      | 54                | 83                            |
| Fish           | Ptychocheilus<br>Iucius            | Mortality | NA                     | 96                | 2                          | 410                | 120                     | 66                | 84                            |
| Fish           | Etheostoma<br>nigrum               | Mortality | NA                     | 96                | 4                          | 510                | 150                     | 81                | 85                            |
| Fish           | Cyprinus carpio                    | Mortality | Juvenile               | 96                | 4                          | 420                | 160                     | 89                | 87                            |
| Fish           | Melanotaenia<br>nigrans            | Mortality | Adult                  | 96                | 1                          | 230                | 180                     | 100               | 88                            |
| Fish           | Mogurnda<br>mogurnda               | Mortality | Larva                  | 96                | 5                          | 21                 | 230                     | 130               | 89                            |
| Fish           | Hypseleostris<br>compressus        | Mortality | Adult                  | 96                | 1                          | 330                | 260                     | 140               | 91                            |
| Fish           | Denariusa bandata                  | Mortality | Adult                  | 96                | 1                          | 520                | 360                     | 200               | 92                            |
| Fish           | Gobiomorphus<br>cotidianus         | Mortality | Juvenile               | 96                | 1                          | 77                 | 390                     | 220               | 93                            |
| Fish           | Porochilus rendahli                | Mortality | Adult                  | 72                | 1                          | 85                 | 430                     | 240               | 94                            |
| Fish           | Lepomis<br>macrochirus             | Mortality | Juvenile               | 96                | 2                          | 1700               | 550                     | 300               | 96                            |
| Fish           | Melanotaenia<br>splendida inornata | Mortality | Adult                  | 96                | 2                          | 350                | 700                     | 380               | 97                            |
| Fish           | Notemigonus<br>crysoleucas         | Mortality | NA                     | 96                | 1                          | 81200              | 22600                   | 12400             | 100                           |
| Insect         | Rhithrogena hageni                 | Mortality | Larva                  | 96                | 1                          | 140                | 73                      | 45                | 80                            |
| Insect         | Deleatidium spp.                   | Mortality | Larva                  | 48                | 1                          | 86                 | 210                     | 130               | 90                            |
| Insect         | Chironomus<br>decorus              | Mortality | NA                     | 48                | 1                          | 740                | 650                     | 410               | 98                            |
| Macrophyt<br>e | Ceratophyllum<br>demersum          | Biomass   | Juvenile               | 96                | 3                          | 9                  | 20                      | 12                | 47                            |
| Macrophyt<br>e | Lemna<br>aequinoctialis            | Growth    | Mature                 | 96                | 1                          | 16                 | 43                      | 24                | 61                            |
| Mollusc        | Venustaconcha<br>ellipsiformis     | Mortality | Glochidia              | 24                | 1                          | 10                 | 2.5                     | 1.6               | 3                             |
| Mollusc        | Epioblasma<br>capsaeformis         | Mortality | Juvenile               | 48                | 2                          | 14                 | 3.2                     | 2                 | 4                             |
| Mollusc        | Villosa iris                       | Mortality | Juvenile               | 96                | 5                          | 35                 | 3.5                     | 2.2               | 6                             |
| Mollusc        | Potamilus ohiensis                 | Mortality | Glochidia              | 24                | 1                          | 14                 | 3.6                     | 2.2               | 7                             |
| Mollusc        | Lymnaea stagnalis                  | Mortality | Juvenile               | 96                | 2                          | 28                 | 4                       | 2.5               | 8                             |
| Mollusc        | Lampsilis<br>siliquoidea           | Mortality | Juvenile               | 96                | 38                         | 51                 | 6                       | 3.7               | 13                            |
| Mollusc        | Leptodea leptodon                  | Mortality | Juvenile               | 48                | 1                          | 29                 | 6.8                     | 4.2               | 17                            |
| Mollusc        | Lithoglyphus<br>virens             | Mortality | NA                     | 96                | 1                          | 7.7                | 6.9                     | 4.3               | 18                            |
| Mollusc        | Lampsilis abrupta                  | Mortality | Glochidia              | 24                | 1                          | 34                 | 8.6                     | 5.4               | 24                            |

| Tax Group | Species                       | Effect                       | Organism<br>life stage | Exposure duration | N. data<br>this<br>species | Reported<br>EC50 * | Norma-<br>lised<br>EC50 | Converted<br>EC10 | Percentile<br>rank<br>(1-100) |
|-----------|-------------------------------|------------------------------|------------------------|-------------------|----------------------------|--------------------|-------------------------|-------------------|-------------------------------|
| Mollusc   | Villosa fabalis               | Mortality                    | Glochidia              | 24                | 1                          | 6.9                | 9.2                     | 5.7               | 25                            |
| Mollusc   | Lampsilis<br>rafinesqueana    | Mortality                    | Glochidia              | 24                | 1                          | 41                 | 10                      | 6.5               | 26                            |
| Mollusc   | Juga plicifera                | Mortality                    | Adult                  | 96                | 1                          | 15                 | 13                      | 8                 | 35                            |
| Mollusc   | Epioblasma<br>triquetra       | Mortality                    | Glochidia              | 24                | 4                          | 24                 | 14                      | 9                 | 38                            |
| Mollusc   | Obovaria<br>subrotunda        | Mortality                    | Glochidia              | 24                | 1                          | 13                 | 17                      | 11                | 44                            |
| Mollusc   | Epioblasma<br>rangiana        | Mortality                    | Glochidia              | 24                | 1                          | 13                 | 18                      | 11                | 45                            |
| Mollusc   | Lampsilis fasciola            | Mortality                    | Glochidia              | 24                | 3                          | 34                 | 26                      | 16                | 52                            |
| Mollusc   | Pomacea paludosa              | Mortality                    | Adult/<br>Juvenile     | 96                | 19                         | 45                 | 28                      | 17                | 54                            |
| Mollusc   | Potamopyrgus<br>antipodarum   | Mobility                     | NA                     | 96                | 4                          | 39                 | 34                      | 21                | 56                            |
| Mollusc   | Echyridella<br>menziesii      | Mortality                    | Juvenile               | 48                | 1                          | 33                 | 37                      | 23                | 60                            |
| Mollusc   | Actinonaias<br>ligamentina    | Mortality                    | Larva                  | 24                | 1                          | 31                 | 41                      | 26                | 64                            |
| Mollusc   | Ptychobranchus<br>fasciolaris | Mortality                    | Glochidia              | 24                | 2                          | 34                 | 46                      | 29                | 66                            |
| Mollusc   | Ligumia recta                 | Mortality                    | Glochidia              | 24                | 1                          | 35                 | 52                      | 32                | 70                            |
| Mollusc   | Hyridella depressa            | Duration<br>valve<br>opening | Adult/<br>Juvenile     | 48                | 11                         | 100                | 80                      | 50                | 81                            |

Appendix Table G.2: **Zinc** acute toxicity values used to derive acute guideline values in freshwater. Reported EC50 value shown here is geometric mean of all reported values. Single values for each species, toxicity values normalised to index water chemistry of pH 7.5, hardness 30 mg CaCO<sub>3</sub>/L and 0.5 mg/L DOC.

| Tax Group  | Species                       | Effect    | Organism<br>life stage | Exposure duration | N. data<br>this<br>species | Reported<br>EC50* | Norma-<br>lised<br>EC50 | Converted<br>EC10 | Percen<br>tile<br>rank<br>(1-100) |
|------------|-------------------------------|-----------|------------------------|-------------------|----------------------------|-------------------|-------------------------|-------------------|-----------------------------------|
| Amphibian  | Bufo boreas                   | Mortality | Larva                  | 96                | 2                          | 840               | 480                     | 260               | 50                                |
| Amphibian  | Bufo gargarizan               | Mortality | Larva                  | 96                | 2                          | 19000             | 5000                    | 2700              | 84                                |
| Amphibian  | Bufo melanostictus            | Mortality | Larva                  | 96                | 1                          | 20000             | 5700                    | 3100              | 87                                |
| Fish       | Oncorhynchus mykiss           | Mortality | Fry                    | 96                | 41                         | 150               | 110                     | 57                | 12                                |
| Fish       | Cottus bairdi                 | Mortality | Juvenile               | 96                | 2                          | 380               | 140                     | 71                | 15                                |
| Fish       | Oncorhynchus clarkii          | Mortality | Juvenile               | 96                | 6                          | 150               | 160                     | 81                | 16                                |
| Fish       | Prosopium<br>williamsoni      | Mortality | Fry                    | 96                | 3                          | 420               | 270                     | 140               | 29                                |
| Fish       | Cottus confusus               | Mortality | Juvenile               | 96                | 1                          | 300               | 280                     | 140               | 31                                |
| Fish       | Acipenser<br>transmontanus    | Mortality | Larva                  | 96                | 4                          | 680               | 340                     | 180               | 37                                |
| Fish       | Salmo trutta                  | Mortality | Juvenile               | 96                | 16                         | 940               | 400                     | 210               | 40                                |
| Fish       | Pimephales promelas           | Mortality | Larva                  | 96                | 10                         | 920               | 450                     | 230               | 46                                |
| Fish       | Salvelinus fontinalis         | Mortality | Juvenile               | 96                | 2                          | 930               | 560                     | 290               | 56                                |
| Fish       | Rhinichthys<br>cataractae     | Mortality | Fry                    | 96                | 1                          | 1900              | 1100                    | 560               | 63                                |
| Fish       | Retropinna retropinna         | Mortality | Juvenile               | 96                | 1                          | 1500              | 1400                    | 700               | 68                                |
| Fish       | Platygobio gracilis           | Mortality | Juvenile               | 96                | 1                          | 2600              | 1500                    | 770               | 69                                |
| Fish       | Gobiomorphus<br>cotidianus    | Mortality | Juvenile               | 96                | 1                          | 2300              | 2100                    | 1100              | 72                                |
| Fish       | Lepomis macrochirus           | Mortality | Juvenile               | 96                | 1                          | 3200              | 2200                    | 1100              | 74                                |
| Fish       | Cyprinus carpio               | Mortality | Juvenile               | 96                | 1                          | 9700              | 3100                    | 1600              | 76                                |
| Fish       | Pseudorasbora parva           | Mortality | n.r.                   | 96                | 2                          | 19000             | 5000                    | 2600              | 82                                |
| Fish       | Galaxias maculatus            | Mortality | Adult                  | 96                | 1                          | 5500              | 5500                    | 2800              | 85                                |
| Fish       | Misgurnus<br>anguillicaudatus | Mortality | n.r.                   | 96                | 2                          | 29000             | 7800                    | 4000              | 88                                |
| Fish       | Macquaria ambigua             | Mortality | Adult                  | 96                | 1                          | 7900              | 7900                    | 4100              | 90                                |
| Fish       | Anguilla dieffenbachii        | Mortality | Juvenile               | 96                | 1                          | 8900              | 8400                    | 4300              | 93                                |
| Fish       | Anguilla australis            | Mortality | Juvenile               | 96                | 1                          | 11000             | 11000                   | 5400              | 96                                |
| Fish       | Gambusia affinis              | Mortality | Adult                  | 96                | 3                          | 74000             | 26000                   | 13000             | 97                                |
| Crustacean | Hyalella azteca               | Mortality | Juvenile               | 96                | 2                          | 140               | 68                      | 37                | 6                                 |
| Crustacean | Ceriodaphnia dubia            | Mortality | Neonate                | 48                | 11                         | 310               | 200                     | 110               | 19                                |
| Crustacean | Daphnia carinata              | Mortality | Neonate                | 48                | 1                          | 340               | 200                     | 110               | 21                                |
| Crustacean | Paratya australiensis         | Mortality | Juvenile               | 48                | 2                          | 240               | 250                     | 130               | 26                                |
| Crustacean | Ceriodaphnia<br>reticulata    | Mortality | Neonate                | 48                | 1                          | 940               | 280                     | 150               | 32                                |
| Crustacean | Simocephalus vetulus          | Mortality | <48 hr                 | 48                | 2                          | 940               | 280                     | 160               | 34                                |

| Tax Group  | Species                           | Effect    | Organism<br>life stage | Exposure duration | N. data<br>this<br>species | Reported<br>EC50* | Norma-<br>lised<br>EC50 | Converted<br>EC10 | Percen<br>tile<br>rank<br>(1-100) |
|------------|-----------------------------------|-----------|------------------------|-------------------|----------------------------|-------------------|-------------------------|-------------------|-----------------------------------|
| Crustacean | Daphnia galeata                   | Mortality | <48 hr                 | 48                | 1                          | 1000              | 300                     | 170               | 35                                |
| Crustacean | Simocephalus<br>exspinosus        | Mortality | <48 hr                 | 48                | 2                          | 1200              | 350                     | 190               | 38                                |
| Crustacean | Ceriodaphnia<br>pulchella         | Mortality | Neonate                | 48                | 1                          | 1300              | 380                     | 210               | 41                                |
| Crustacean | Daphnia magna                     | Mortality | Neonate                | 48                | 16                         | 960               | 390                     | 210               | 43                                |
| Crustacean | Chydorus sphaericus               | Mortality | <48 hr                 | 48                | 1                          | 1300              | 400                     | 220               | 44                                |
| Crustacean | Daphnia pulex                     | Mortality | Neonate                | 48                | 25                         | 500               | 420                     | 230               | 47                                |
| Crustacean | Daphnia thomsoni                  | Mortality | Neonate                | 48                | 7                          | 470               | 450                     | 250               | 49                                |
| Crustacean | Chydorus ovalis                   | Mortality | less than<br>48 h      | 48                | 1                          | 1600              | 490                     | 270               | 51                                |
| Crustacean | Daphnia longispina                | Mortality | <48 hr                 | 48                | 1                          | 1700              | 520                     | 280               | 54                                |
| Crustacean | Paracalliope fluviatilis          | Mortality | NA                     | 48                | 4                          | 510               | 660                     | 360               | 57                                |
| Crustacean | Macrobrachium<br>nipponense       | Mortality | n.r.                   | 96                | 2                          | 2700              | 720                     | 400               | 59                                |
| Crustacean | Paratya curvirostris              | Mortality | Adult                  | 96                | 1                          | 14000             | 8200                    | 4500              | 94                                |
| Insect     | Neocloeon triangulifer            | Mortality | Neonate                | 96                | 1                          | 69                | 30                      | 16                | 0                                 |
| Insect     | Deleatidium spp.                  | Mortality | Juvenile               | 48                | 1                          | 570               | 500                     | 280               | 53                                |
| Insect     | Rhithrogena sp.                   | Mortality | Larva                  | 96                | 1                          | 1400              | 920                     | 500               | 62                                |
| Insect     | Chironomus riparius               | Mortality | n.r.                   | 96                | 2                          | 11000             | 3000                    | 1700              | 78                                |
| Insect     | <i>Capnia</i> sp.                 | Mortality | Larva                  | 96                | 2                          | 5700              | 3300                    | 1800              | 79                                |
| Insect     | Baetis tricaudatus                | Mortality | Larva                  | 96                | 3                          | 11000             | 7800                    | 4300              | 91                                |
| Insect     | Rhithrogena hageni                | Mortality | Larva                  | 96                | 2                          | 44000             | 30000                   | 16000             | 99                                |
| Insect     | <i>Cinygmula</i> sp.              | Mortality | Larva                  | 96                | 2                          | 69000             | 43000                   | 24000             | 100                               |
| Mollusc    | Leptoxis ampla                    | Mortality | Juvenile               | 96                | 1                          | 67                | 58                      | 32                | 4                                 |
| Mollusc    | Lampsilis<br>rafinesqueana        | Mortality | Juvenile               | 48                | 1                          | 130               | 120                     | 67                | 13                                |
| Mollusc    | Villosa vibex                     | Mortality | Juvenile               | 96                | 1                          | 200               | 170                     | 94                | 18                                |
| Mollusc    | Pomacea paludosa                  | Mortality | Juvenile               | 96                | 8                          | 520               | 210                     | 120               | 22                                |
| Mollusc    | Actinonaias pectorosa             | Mortality | Juvenile               | 96                | 1                          | 360               | 220                     | 120               | 24                                |
| Mollusc    | Epioblasma<br>capsaeformis        | Mortality | Juvenile               | 96                | 1                          | 370               | 240                     | 130               | 25                                |
| Mollusc    | Lampsilis straminea<br>claibornen | Mortality | Juvenile               | 96                | 1                          | 290               | 250                     | 140               | 28                                |
| Mollusc    | Cipangopaludina<br>cathayensis    | Mortality | n.r.                   | 96                | 2                          | 3300              | 870                     | 480               | 60                                |
| Mollusc    | Potamopyrgus<br>antipodarum       | Mortality | Adult                  | 96                | 9                          | 1000              | 1100                    | 630               | 65                                |
| Mollusc    | Lampsilis siliquoidea             | Mortality | Juvenile               | 96                | 1                          | 1700              | 1500                    | 800               | 71                                |
| Mollusc    | Gyraulus sp.                      | Mortality | NA                     | 96                | 1                          | 3300              | 2100                    | 1200              | 75                                |

| Tax Group      | Species                            | Effect            | Organism<br>life stage | Exposure duration | N. data<br>this<br>species | Reported<br>EC50* | Norma-<br>lised<br>EC50 | Converted<br>EC10 | Percen<br>tile<br>rank<br>(1-100) |
|----------------|------------------------------------|-------------------|------------------------|-------------------|----------------------------|-------------------|-------------------------|-------------------|-----------------------------------|
| Mollusc        | Lymnaea luteola                    | Mortality         | Adult                  | 48                | 3                          | 11000             | 3600                    | 2000              | 81                                |
| Annelid        | Limnodrilus<br>hoffmeisteri        | Mortality         | n.r.                   | 96                | 2                          | 100               | 56                      | 31                | 9                                 |
| Annelid        | Nais elinguis                      | Mortality         | Adult                  | 96                | 1                          | 120               | 69                      | 38                | 66                                |
| Rotifer        | Euchlanis dilatata                 | Mortality         | Neonate                | 24                | 1                          | 300               | 80                      | 44                | 3                                 |
| Rotifer        | Lecane quadridentata               | Mortality         | Neonate                | 48                | 1                          | 890               | 1100                    | 630               | 7                                 |
| Green<br>algae | Raphidocelis<br>subcapitata        | Population growth | exponential<br>growth  | 24                | 5                          | 83                | 54                      | 26                | 1                                 |
| Green<br>algae | <i>Chlorella</i> sp. (PNG isolate) | Population growth | exponential<br>growth  | 24                | 10                         | 180               | 120                     | 56                | 10                                |