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[a]	Relevant endpoints[b]	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[f], larval development/ metamorphosis)	<7 d
	Embryos/larvae	Larval development/ metamorphosis	<48 h
Microinvertebrates	Adults/juveniles/larvae	All (except fertilisation, larval development/ 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 ≥2 mm long (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 ≥2 mm long). 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 ≥ 1 h and are considered chronic.

Appendix B Further details of bioavailability models

Appendix Table B.1: Regression-based models currently used or proposed for acute copper criteria/guideline value derivation.

Model type	Key References	Species/group coefficients derived from	TMFs included	Predicted R^2	Slopes		
					pH	Log(Hardness)	Log(DOC)
Hardness	US EPA (1984)	Pooled model based on *Daphnia magna, D. pulicaria, Oncorhynchus clarkii, O. mykiss, O. tshawytscha, Pimephales promelas, Poecilia reticulata, Lepomis macrochirus*	Hardness	Not 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

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

Model type	Key References	Species/group coefficients derived from	TMFs included	Binding coefficients (logK)						
				Cu-BL*	CuOH-BL	$CuCO_3$-BL	H-BL	Ca-BL	Mg-BL	Na-BL
Fish BLM	Di Toro et al. 2001	*P. promelas* and *O. mykiss*	Temp., pH, DOC, humic acid, Ca, Mg, Na, K, Cl, 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 acid, Ca, Mg, Na, K, Cl, 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. (2001)	Temp., pH, DOC, humic acid, Ca, Mg, Na, K, Cl, SO4, alkalinity	7.4	-1.3		5.4	3.6	3.6	3
Fish/invertebrate BLM	BC (2019) & ECCC (2021)	Not specifically reported by BC or ECCC but model files state “derived from fathead minnow” and changed based on an “updated database”.	Temp., pH, DOC, humic acid, Ca, Mg, Na, K, Cl, SO4, alkalinity	7.4	-0.8		6.4	4.4	4.4	4
Plant BLM	ECCC (2021)	Not reported by ECCC, but model files suggest adopted from BLM for barley & soils.	Temp., pH, DOC, humic acid, Ca, Mg, Na, K, Cl, SO4, alkalinity	5.4	21.2	-3.3	7.2	4	3.5	

Note: * BL = Biotic ligand

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

	Key References	Species/group coefficients derived from	TMFs included	Adjusted R^2	Slopes		
Model					**Hardness**	**pH**	**DOC**
Hardness	US EPA (1987)	*D. magna, Physa heterostropha, O. mykiss, P. promelas, Salvelinus fontinalis, P. reticulata, 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 *Daphnia* (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

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 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, 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, Cl, SO4, alkalinity	5.3			3.3	3.1	
Fish BLM	De Schamphelaere et al. (2004)	*O. mykiss*	Temp., pH, DOC, humic acid, Ca, Mg, Na, K, 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, 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, Cl, SO4, alkalinity	5.6	-3.8	Not reported	4.9	4.4	
Fish/ invertebrate BLM	DeForest et al. (2012)	Unified/pooled model based on *D. magna, D. pulex, P. promelas, O. mykiss*	Temp., pH, DOC, humic acid, Ca, Mg, Na, K, Cl, SO4, alkalinity	5.4	-3.8	6.4	3.8	3.3	
Fish/ invertebrate BLM	Windward (2019)	Not specified, model files state based on pooled data, presumably same as earlier HydroQual version	Temp., pH, DOC, humic acid, Ca, Mg, Na, K, 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, 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

Climate, Freshwater & Ocean Science

Prepared by:
Karen Thompson

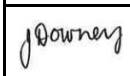
For any information regarding this report please contact:

Karen Thompson
Aquatic Ecology and Ecotoxicology Technician
Chemistry and Ecotoxicology
+64 7 859 1895
karen.thompson@niwa.co.nz

National Institute of Water & Atmospheric Research Ltd
PO Box 11115
Hamilton 3251

Phone +64 7 856 7026

NIWA CLIENT REPORT No: 2024299HN
Report date: September 2024
NIWA Project: HYR24201

Quality Assurance Statement		
	Reviewed by:	Dr. Chris Hickey, RMA Science
	Formatting checked by:	Jo Downey
	Approved for release by:	Michael Bruce

Contents

Tables

Figures

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 $CaCO_3$), 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 (≥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.

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

Natural water	pH	Hardness mg/L $CaCO_3$	DOC mg/L	Zinc		Copper	
				EC_{10}[a] (95% CL) µg/L Zn^{2+}	EC_{50}[a] (95% CL) µg/L Zn^{2+}	EC_{10}[a] (95% CL) µg/L Cu^{2+}	EC_{50}[a] (95% CL) µg/L Cu^{2+}
Mahurangi	7.58	73.5	2.18	511 (459-551)	826 (782-873)	89 (65-150)[c]	211 (159-255)
Hoteo	7.49	58.1	3.73	374 (279-442)	751 (661-853)	180 (178-183)[c]	272 (261-284)
Okutua	6.03[b]	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)

[a] EC_x: The statistically determined test concentration causing a X% effect on the endpoint after the specified exposure period. [b] As pH adjusted for testing. [c] EC_{15} value as EC_{10} 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)[1]. 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 Laboratories). The natural water site locations, characteristics and collection dates are summarised in Table 2-1.

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

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

[a] 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).

[1] 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 1st 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 µL YTC (yeast-trout chow mixture) and 150 µL of green alga *Raphidocellis subcapitata* (concentration of 1 x 10^8 cells/mL). Culture room temperature, nominally 20°C, was monitored periodically.

Table 2-2: Laboratory *Daphnia* culture water composition from NIWA SOP 11.0 (NIWA 2022).

Ingredient or parameter	Amount or Value	Units
Fernhollow Spring Water (GF/C filtered)	18.5	%
Aged UV nanopure water	81.5	%
$NaHCO_3$	48	mg/L
$CaSO_4$	30	mg/L
$MgSO_4$	30	mg/L
KCl	2	mg/L
Vitamin B_{12}	0.01	mg/L
Se	0.002	mg/L
pH	7.8 ± 0.2	pH units
Conductivity	0.23	mS/m
Hardness	40 to 50	mg $CaCO_3$/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 µm 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.

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

Test protocol:	NIWA SOP 10.0 (NIWA 2022b)
Reference method:	OECD (2004)
Test organisms:	*Daphnia thomsoni* (previously known as *D. carinata*, Burns 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 oxygen, zinc or copper of selected test treatments. Hardness (as dissolved calcium and magnesium) and dissolved organic carbon of natural waters.
Observation:	24- and 48-hour survival
Effect measured:	Survival
Test acceptability criteria:	Mean control survival ≥90%

Tests were performed at 20 ± 2°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 µm) for dissolved metals analysis at the start (Time zero, T_0) and end (Time 48, T_{48}) of each test. At test initiation (T_0) the subsamples were taken from the prepared solutions prior to aliquoting into test chambers and at test termination (T_{48}) 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_0 and T_{48}) 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™ v2.1.4.5 (Comprehensive Environmental Toxicity Information System) software and corresponding user manual by Tidepool Scientific (2001-2022). CETIS™ is a statistical application designed for analysing and reporting dose-response 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_{50} and EC_{10}) with associated 95% confidence intervals (α=0.05). When linear interpolation was applied, EC_{15} values were calculated rather than EC_{10} 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 toxicity testing due to low survival in test water.

Natural water	NIWA laboratory ID	Temp.[a] °C	pH	Conductivity µS cm^{-1}	Dissolved Oxygen (DO) mg/L 0_2	Dissolved calcium/ magnesium mg/L	Hardness mg/L $CaCO_3$	DOC[b] mg/L	Dissolved zinc[c] µg/L	Dissolved copper[c] µg/L
Mahurangi Stream	24.003.1	18.4	7.58	242	10.7	16/ 8.1	73.5	2.18	3.2	<0.5[d]
Hoteo River	24.003.2	18.3	7.49	201	10.7	14/ 5.7	58.1	3.73	4.4	1.1
Okutua Creek	24.003.3	18.0	4.96	27	10.9	0.48/ 0.37	2.74	11.9	4.4	1.3
Clutha River	24.003.4	18.1	7.17	72.4	11.2	12/ 0.74	33.7	0.38	2.9	<0.5[d]
Waihou River	24.003.5	19.1	7.26	89.9	8.9	3.3/ 1.8	15.7	<0.3	3.3	0.8

[a] At time of measurements. [b] Measured as dissolved non-purgeable organic carbon (DNPOC). [c] Test initiation and test termination mean measured concentrations. [d] 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 ± 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_2. At the test termination the dissolved oxygen was 8.6-9.2 mg/L O_2, 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 (≤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^{2+} (± 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 µg/L Zn^{2+}) subsampled at test termination (T_{48}). These two measurements were not used in statistical analysis as are considered as outliers, only the test initiation (T_0) 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_0) and end (T_{48}) of the Mahurangi Stream *Daphnia* survival toxicity test. Measured by Hill Labs, nm = not measured. Percentage differences calculated for nominal and mean measured concentrations, and initial and final measured test concentrations. Shaded cells indicate concentrations used in statistical analyses.

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

Treatment	Nominal concentration (µg/L Zn^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Zn^{2+})	Measured T_{48} concentration (µg/L Zn^{2+})	Mean measured concentration (µg/L Zn^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 2	220	100	230	250	240	9	8
Concentration 3	484	90	490	490	490	4	0
Concentration 4	1,065	27	1,080	1,030	1,055	0	5
Concentration 5	2,343	0	2,400	2,300	2,350	0	4
Concentration 6	5,154	0	nm	nm	-	-	-
Concentration 7	11,000	0	nm	nm	-	-	-

[a] Considered an outlier so not used in statistical analysis. [b] Based on T_0 measured concentration only.

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

Treatment	Nominal concentration (µg/L Zn^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Zn^{2+})	Measured T_{48} concentration (µg/L Zn^{2+})	Mean measured concentration (µg/L Zn^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	100	4.2	4.5	4.35	200	7
Concentration 1	100	100	105	510[a]	105[b]	5[b]	-
Concentration 2	220	100	230	240	235	9	4
Concentration 3	484	70	520	490	505	1	6
Concentration 4	1,065	33	1,080	1,030	1,055	2	5
Concentration 5	2,343	0	2,400	2,400	2,400	2	0
Concentration 6	5,154	0	nm	nm	-	-	-
Concentration 7	11,000	0	nm	nm	-	-	-

[a] Considered an outlier so not used in statistical analysis. [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 Creek *Daphnia* survival toxicity test. Measured by Hill Labs, nm = not measured. Percentage differences calculated for nominal and mean measured concentrations, and initial and final measured test concentrations. Shaded cells indicate concentrations used in statistical analyses.

Treatment	Nominal concentration (µg/L Zn^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Zn^{2+})	Measured T_{48} concentration (µg/L Zn^{2+})	Mean measured concentration (µg/L Zn^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	98	3.9	4.4	4.4	200	12
Concentration 1	100	93	110	108	109	9	2
Concentration 2	220	70	250	220	235	7	13
Concentration 3	484	33	500	470	485	0	6
Concentration 4	1,065	0	1,070	1,090	1,080	1	2
Concentration 5	2,343	0	2,300	2,300	2,300	2	0
Concentration 6	5,154	0	nm	nm	-	-	-
Concentration 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 River *Daphnia* survival toxicity test. Measured by Hill Labs, nm = not measured. Percentage differences calculated for nominal and mean measured concentrations, and initial and final measured test concentrations. Shaded cells indicate concentrations used in statistical analyses.

Treatment	Nominal concentration (µg/L Zn^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Zn^{2+})	Measured T_{48} concentration (µg/L Zn^{2+})	Mean measured concentration (µg/L Zn^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	98	2.7	3.0	2.85	200	11
Concentration 1	45	100	nm	nm	-	-	-
Concentration 2	100	100	106	132	119	17	22
Concentration 3	220	87	230	220	225	4	4
Concentration 4	484	53	520	490	505	4	6
Concentration 5	1,065	13	1,120	1,040	1,080	3	7

Treatment	Nominal concentration (µg/L Zn^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Zn^{2+})	Measured T_{48} concentration (µg/L Zn^{2+})	Mean measured concentration (µg/L Zn^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 6	2,343	0	2,500	2,300	2,400	2	8
Concentration 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 River *Daphnia* survival toxicity test. Measured by Hill Labs, nm = not measured. Percentage differences calculated for nominal and mean measured concentrations, and initial and final measured test concentrations. Shaded cells indicate concentrations used in statistical analyses.

Treatment	Nominal concentration (µg/L Zn^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Zn^{2+})	Measured T_{48} concentration (µg/L Zn^{2+})	Mean measured concentration (µg/L Zn^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	96	3.1	3.4	3.25	200	9
Concentration 1	100	93	109	123	116	12	12
Concentration 2	220	77	240	220	230	2	9
Concentration 3	484	40	520	480	500	0	8
Concentration 4	1,065	0	1,130	1,090	1,110	3	4
Concentration 5	2,343	0	2,500	2,400	2,450	2	4
Concentration 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^{2+} after 48 hours when compared to the negative control. However, exposure to 490 mg/L Zn^{2+} 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^{2+}). The acute toxicity test for *Daphnia* resulted in an EC_{10} of 511 µg/L Zn^{2+}, with a 95% confidence interval of 459-551 µg/L Zn^{2+} and an EC_{50} of 826 µg/L Zn^{2+}, with a 95% confidence interval of 782-873 µg/L Zn^{2+} (Figure 3-1, Table 3-7 and Appendix F).

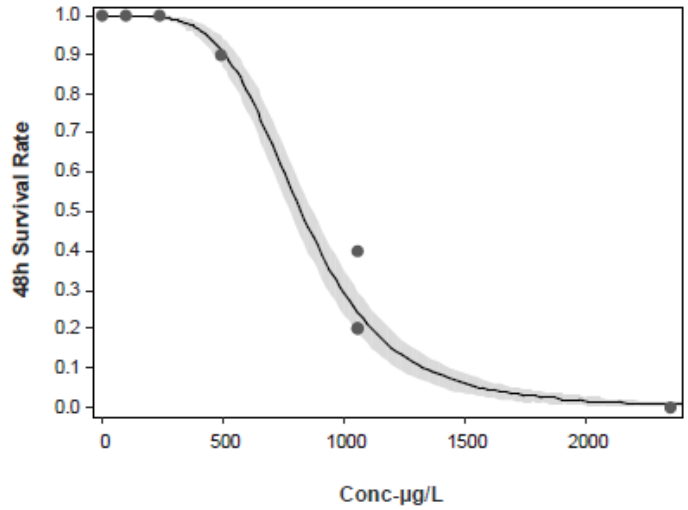


Figure 3-1: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Mahurangi Stream natural water sample. 3P Log-Logistic fitted.

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^{2+} after 48 hours when compared to the negative control. However, exposure to 505 mg/L Zn^{2+} 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^{2+}). The acute toxicity test for *Daphnia* resulted in an EC_{10} of 374 µg/L Zn^{2+}, with a 95% confidence interval of 279-442 µg/L Zn^{2+} and an EC_{50} of 751 µg/L Zn^{2+}, with a 95% confidence interval of 661-853 µg/L Zn^{2+} (Figure 3-2, Table 3-7 and Appendix G).

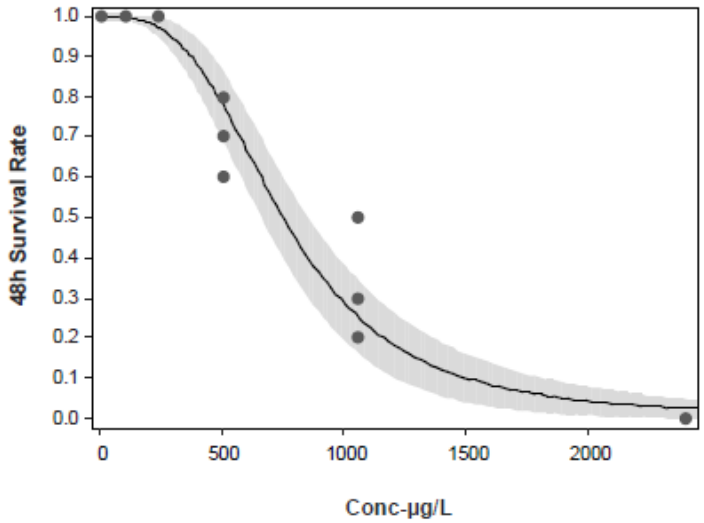


Figure 3-2: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Hoteo River natural water sample. 3P Log-Logistic fitted.

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^{2+} after 48 hours when compared to the negative control. However, exposure to 235 mg/L Zn^{2+} 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^{2+}).
The acute toxicity test for *Daphnia* resulted in an EC_{10} of 162 µg/L Zn^{2+}, with a 95% confidence interval of 78-208 µg/L Zn^{2+} and an EC_{50} of 343 µg/L Zn^{2+}, with a 95% confidence interval of 282-416 µg/L Zn^{2+} (Figure 3-3, Table 3-7 and Appendix H).

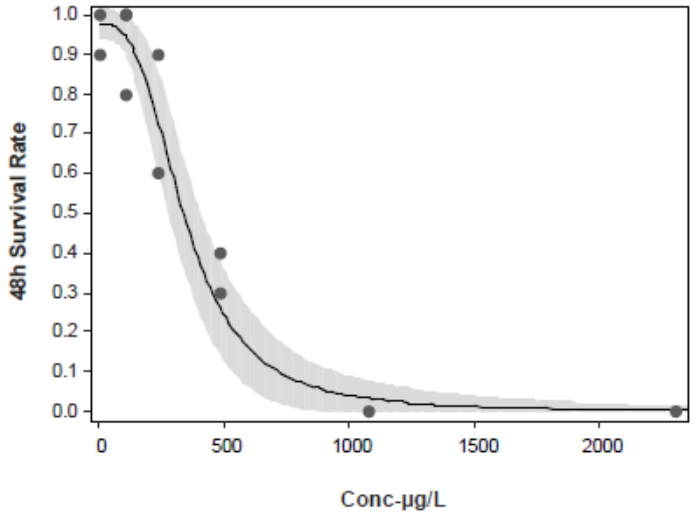


Figure 3-3: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for pH adjusted Okutua Creek natural water sample. 3P Log-Logistic fitted.

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^{2+} after 48 hours when compared to the negative control. However, exposure to 505 mg/L Zn^{2+} 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^{2+}). The acute toxicity test for *Daphnia* resulted in an EC_{10} of 243 µg/L Zn^{2+}, with a 95% confidence interval of 124-311 µg/L Zn^{2+} and an EC_{50} of 526 µg/L Zn^{2+}, with a 95% confidence interval of 432-641 µg/L Zn^{2+} (Figure 3-4, Table 3-7 and Appendix I).

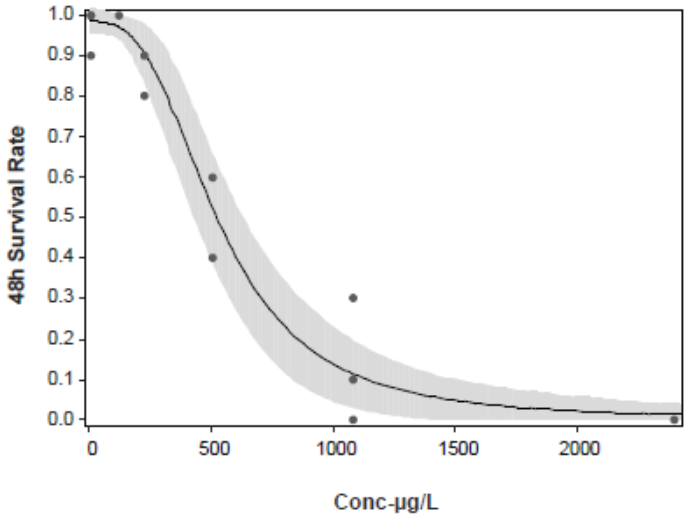


Figure 3-4: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Clutha River natural water sample. 3P Log-Logistic fitted.

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^{2+} after 48 hours when compared to the negative control. However, exposure to 230 mg/L Zn^{2+} 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^{2+}). The acute toxicity test for *Daphnia* resulted in an EC_{10} of 204 µg/L Zn^{2+}, with a 95% confidence interval of 121-252 µg/L Zn^{2+} and an EC_{50} of 404 µg/L Zn^{2+}, with a 95% confidence interval of 344-474 µg/L Zn^{2+} (Figure 3-5, Table 3-7 and Appendix J).

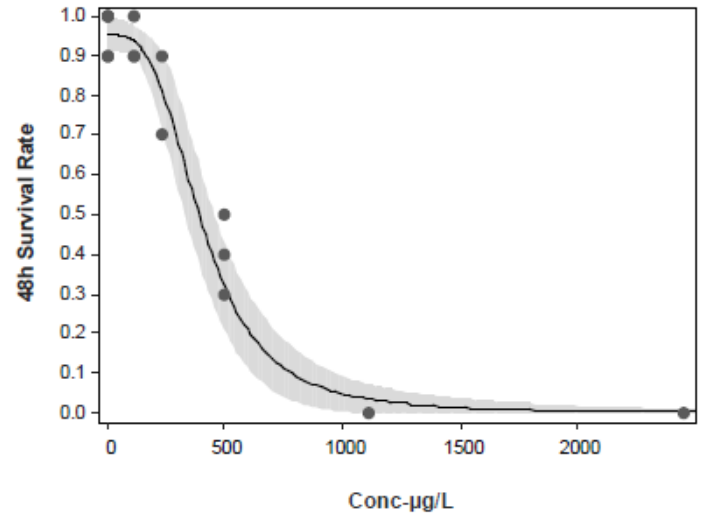


Figure 3-5: Survival of *D. thomsoni* (compared to control) versus measured zinc concentrations for Waihou River natural water sample. 3P Log-Logistic fitted.

Table 3-7: Toxicity statistics as statistically derived by CETIS™ (µg/L zinc) for *D. thomsoni* 48-hour survival in each natural water tested. Values in parentheses are the EC_{50} value 95% confidence intervals.

Natural water	EC_{10}[a] (95% CL) µg/L Zn^{2+}	EC_{50}[a] (95% CL) µg/L Zn^{2+}	NOEC[b] µg/L Zn^{2+}	LOEC[c] µg/L Zn^{2+}	TEC[d] µg/L Zn^{2+}
Mahurangi	511 (459-551)	826 (782-873)	240	490	343
Hoteo	374 (279-442)	751 (661-853)	235	505	345
Okutua[e]	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

[a] EC_x: The statistically determined test Concentration causing a X% Effect on the endpoint after the specified exposure period. [b] NOEC: The highest tested Concentration causing No Observed Effect relative to the controls. [c] LOEC: The Lowest tested Concentration causing an Observed Effect relative to the controls. [d] TEC: Threshold Effect Concentration, the geometric mean of NOEC and LOEC. [e] 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 µg/L Cu^{2+}) and the mean measured (14 µg/L Cu^{2+}) 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_0) and end (T_{48}) of the Mahurangi Stream *Daphnia* survival toxicity test. Measured by Hill Labs, nm = not measured. Percentage differences calculated for nominal and mean measured concentrations, and initial and final measured test concentrations. Shaded cells indicate concentrations used in statistical analyses.

Treatment	Nominal concentration (µg/L Cu^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Cu^{2+})	Measured T_{48} concentration (µg/L Cu^{2+})	Mean measured concentration (µg/L Cu^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	100	<0.5 [a]	<0.5 [a]	<0.5 [a]	0	0
Concentration 1	1	100	nm	nm	-	-	-
Concentration 2	2.2	100	nm	nm	-	-	-
Concentration 3	4.8	100	nm	nm	-	-	-
Concentration 4	10.6	100	nm	nm	-	-	-
Concentration 5	23.4	100	25	21	23	2	17

Treatment	Nominal concentration (µg/L Cu^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Cu^{2+})	Measured T_{48} concentration (µg/L Cu^{2+})	Mean measured concentration (µg/L Cu^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 6	51.5	100	52	50	51	1	4
Concentration 7	154.5	70	156	155	155.5	1	1
Concentration 8	463.5	0	470	430	450	3	9
Concentration 9	1390.5	0	1440	1200	1320	5	18

[a] Less than detection limit.

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

Treatment	Nominal concentration (µg/L Cu^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Cu^{2+})	Measured T_{48} concentration (µg/L Cu^{2+})	Mean measured concentration (µg/L Cu^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	100	1.1	1	1.05	200	10
Concentration 1	1	100	nm	nm	-	-	-
Concentration 2	2.2	100	nm	nm	-	-	-
Concentration 3	4.8	100	nm	nm	-	-	-
Concentration 4	10.6	100	nm	nm	-	-	-
Concentration 5	23.4	100	24	25	24.5	5	4
Concentration 6	51.5	100	53	53	53	3	0
Concentration 7	154.5	100	149	153	151	2	3
Concentration 8	463.5	33	470	470	470	1	0
Concentration 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 Okutua Creek *Daphnia* survival toxicity test. Measured by Hill Labs, nm = not measured. Percentage differences calculated for nominal and mean measured concentrations, and initial and final measured test concentrations. Shaded cells indicate concentrations used in statistical analyses.

Treatment	Nominal concentration (µg/L Cu^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Cu^{2+})	Measured T_{48} concentration (µg/L Cu^{2+})	Mean measured concentration (µg/L Cu^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	98	1.3	1.3	1.3	200	0
Concentration 1	1	100	nm	nm	-	-	-
Concentration 2	2.2	100	nm	nm	-	-	-
Concentration 3	4.8	97	nm	nm	-	-	-
Concentration 4	10.6	100	14.3	13.7	14	28	4
Concentration 5	23.4	100	28	28	28	18	0
Concentration 6	51.5	97	60	58	59	14	3
Concentration 7	113.4	13	136	136	136	18	0
Concentration 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 River *Daphnia* survival toxicity test. Measured by Hill Labs, nm = not measured. Percentage differences calculated for nominal and mean measured concentrations, and initial and final measured test concentrations. Shaded cells indicate concentrations used in statistical analyses.

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

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

[a] Less than detection limit.

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

Treatment	Nominal concentration (µg/L Cu^{2+})	Mean 48-hour survival (%)	Measured T_0 concentration (µg/L Cu^{2+})	Measured T_{48} concentration (µg/L Cu^{2+})	Mean measured concentration (µg/L Cu^{2+})	Difference between nominal and mean measured concentrations (%)	Difference between T_0 and T_{48} measured concentrations (%)
Concentration 0 - Control	0	96	0.9	0.7	0.8	200	25
Concentration 1	1	100	nm	nm	-	-	-
Concentration 2	2.2	100	nm	nm	-	-	-
Concentration 3	4.8	100	nm	nm	-	-	-
Concentration 4	10.6	97	11.3	8.2	9.75	8	32
Concentration 5	23.4	83	23	19.1	21.05	11	19
Concentration 6	51.5	40	53	43	48	7	21
Concentration 7	113	0	102	92	97	15	10
Concentration 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^{2+} after 48 hours when compared to the negative control. However, exposure to 156 mg/L Cu^{2+} 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^{2+}). The acute toxicity test for

Daphnia resulted in an EC_{15} of 89 µg/L Cu^{2+}, with a 95% confidence interval of 65-150 µg/L Cu^{2+} and an EC_{50} of 211 µg/L Cu^{2+}, with a 95% confidence interval of 159-255 µg/L Cu^{2+} (Figure 3-6, Table 3-13 and Appendix F).

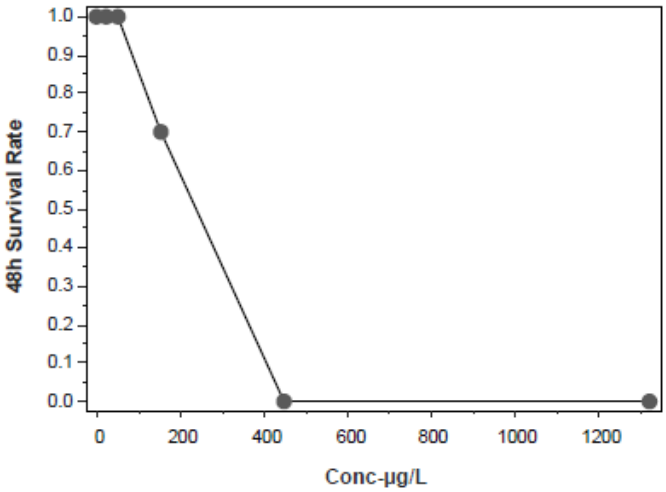


Figure 3-6: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Mahurangi Stream natural water sample. Linear interpolation.

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^{2+} after 48 hours when compared to the negative control. However, exposure to 470 mg/L Cu^{2+} 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^{2+}). The acute toxicity test for *Daphnia* resulted in an EC_{15} of 180 µg/L Cu^{2+}, with a 95% confidence interval of 178-183 µg/L Cu^{2+} and an EC_{50} of 272 µg/L Cu^{2+}, with a 95% confidence interval of 261-284 µg/L Cu^{2+} (Figure 3-7, Table 3-13 and Appendix G).

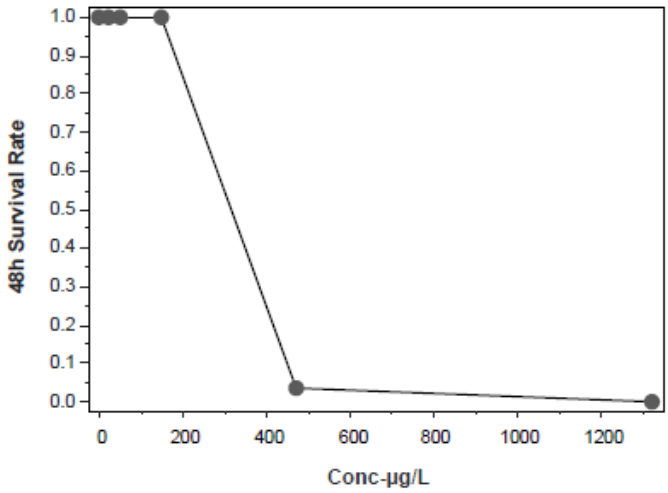


Figure 3-7: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Hoteo River natural water sample. Linear interpolation.

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^{2+} after 48 hours when compared to the negative control. However, exposure to 136 mg/L Cu^{2+} 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^{2+}). The acute toxicity test for *Daphnia* resulted in an EC_{10} of 74 µg/L Cu^{2+}, with a upper 95% confidence limit of 93 µg/L Cu^{2+} and an EC_{50} of 103 µg/L Cu^{2+}, with a 95% confidence interval of 78-136 µg/L Cu^{2+} (Figure 3-8, Table 3-13 and Appendix H).

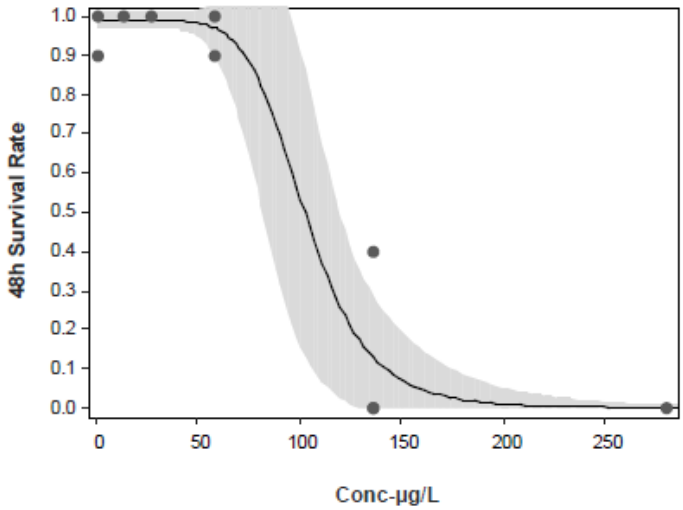


Figure 3-8: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for pH adjusted Okutua Creek natural water sample. 3P Log-Logistic fitted.

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^{2+} after 48 hours when compared to the negative control. However, exposure at the highest tested concentration, 50.5 mg/L Cu^{2+} 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 µg/L Cu2+, with a upper 95% confidence limit of 26 µg/L Cu2+ and an EC50 of 32 µg/L Cu2+, with a 95% confidence interval of 26-39 µg/L Cu2+ (Figure 3-9, Table 3-13 and Appendix I).

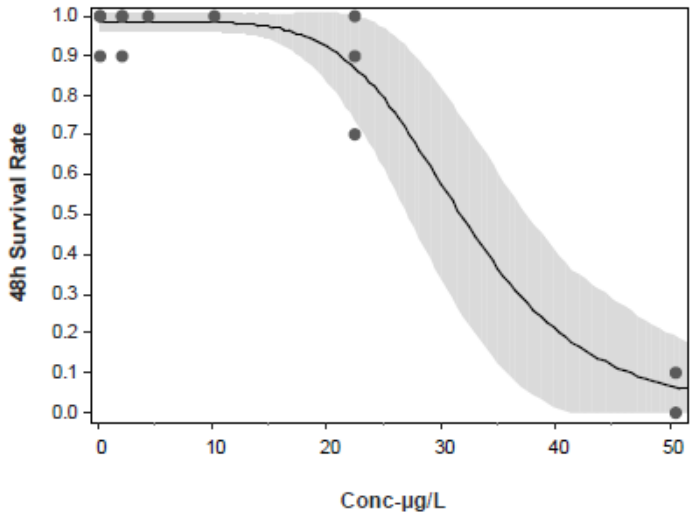


Figure 3-9: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Clutha River natural water sample. 3P Log-Logistic fitted.

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^{2+} after 48 hours when compared to the negative control. However, exposure to 48 mg/L Cu^{2+} 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^{2+}). The acute toxicity test for *Daphnia* resulted in an EC_{10} of 23 µg/L Cu^{2+}, with a upper 95% confidence limit of 30 µg/L Cu^{2+} and an EC_{50} of 41 µg/L Cu^{2+}, with a 95% confidence interval of 33-51 µg/L Cu^{2+} (Figure 3-10, Table 3-13 and Appendix J).

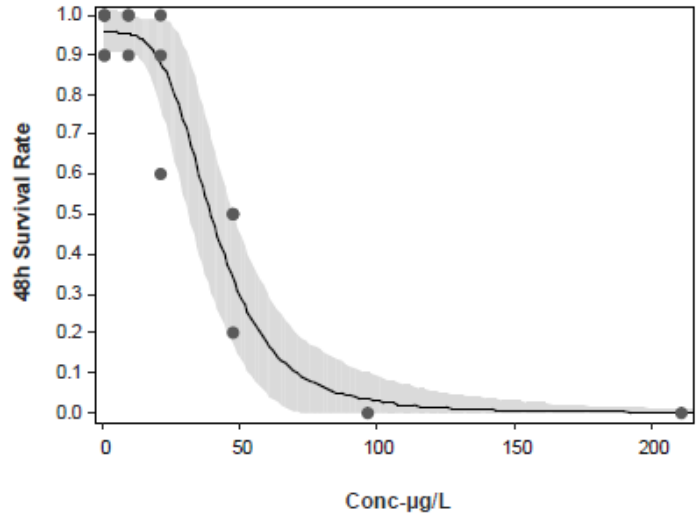


Figure 3-10: Survival of *D. thomsoni* (compared to control) versus measured copper concentrations for Waihou River natural water sample. 3P Log-Logistic fitted.

Table 3-13: Toxicity statistics as statistically derived by CETIS™ (µg/L copper) for *D. thomsoni* 48-hour survival in each natural water tested. Values in parentheses are the EC_{50} value 95% confidence intervals.

Natural water	EC_{10}[a] (95% CL) µg/L Cu^{2+}	EC_{50}[a] (95% CL) µg/L Cu^{2+}	NOEC[b] µg/L Cu^{2+}	LOEC[c] µg/L Cu^{2+}	TEC[d] µg/L Cu^{2+}
Mahurangi	89 (65-150)[f]	211 (159-255)	51.0	156	89.2
Hoteo	180 (178-183)[f]	272 (261-284)	151	470	266
Okutua[e]	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

[a] EC_x: The statistically determined test Concentration causing a X% Effect on the endpoint after the specified exposure period. [b] NOEC: The highest tested Concentration causing No Observed Effect relative to the controls. [c] LOEC: The Lowest tested Concentration causing an Observed Effect relative to the controls. [d] TEC: Threshold Effect Concentration, the geometric mean of NOEC and LOEC. [e] As pH adjusted for testing. [f] EC_{15} value as EC_{10} 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 µg/L and higher, with EC_{50} values ranging from 343 to 826 µg/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 µg/L and above, with EC_{50} values ranging from 32 to 272 µg/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.

Natural water	pH	Hardness mg/L $CaCO_3$	DOC mg/L	Zinc		Copper	
				EC_{10}[a] (95% CL) µg/L Zn^{2+}	EC_{50}[a] (95% CL) µg/L Zn^{2+}	EC_{10}[a] (95% CL) µg/L Cu^{2+}	EC_{50}[a] (95% CL) µg/L Cu^{2+}
Mahurangi	7.58	73.5	2.18	511 (459-551)	826 (782-873)	89 (65-150)[c]	211 (159-255)
Hoteo	7.49	58.1	3.73	374 (279-442)	751 (661-853)	180 (178-183)[c]	272 (261-284)
Okutua	6.03[b]	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)

[a] EC_x: The statistically determined test Concentration causing a X% Effect on the endpoint after the specified exposure period. [b] As pH adjusted for testing. [c] EC_{15} value as EC_{10} 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 a short period relative to the organism's life span. Refer to Warne et al. (Warne 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 guideline value (DGV)	A guideline value recommended for generic application to all Australian and New Zealand fresh or marine waterbodies in the absence of a more specific guideline value (for example site-specific) in the Australian and New Zealand 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_{50}	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 in a toxicity test that has no statistically significant adverse effect on the 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 members of other groups and that form a reproductively isolated group that will not normally breed with members of another group. (Chemical species are 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

7 References

ANZECC (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand. Canberra.

ANZG (2018) Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian state and territory governments, Canberra ACT, Australia. Available at www.waterquality.gov.au/anz-guidelines

Burns, C.W., Duggan, I.C., Banks, J.C., Hogg, I.D. (2017) A new, subalpine species of *Daphnia* (Cladocera, Anomopoda) in the *D. carinata* species complex, in the South Island, New Zealand. *Hydrobiologia,* 798: 151-169.

Gadd, J., Albert, A., Mohsin, M. Thompson, K. Bell, S. (2022) Bioavailability of zinc to an alga and a native daphnid in New Zealand natural waters, *NIWA Client Report* 2021356AK Prepared for Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia. January 2022: 52

NIWA (2022) Cladocera Culture Maintenance - *Daphnia magna* and *Daphnia thomsoni*, Ecotoxicology Standard Operating Procedure 11.0. Version 6.0. April 2022. Prepared for Internal Use Only, National Institute of Water and Atmospheric Research Ltd. (NIWA) Ecotoxicology Laboratory, Hamilton, New Zealand.

NIWA (2022b) Cladocera Acute Toxicity Test, Ecotoxicology Standard Operating Procedure 10.0. Version 3.0. March 2022. Prepared for Internal Use Only, National Institute of Water and Atmospheric Research Ltd. (NIWA) Ecotoxicology Laboratory, Hamilton, New Zealand.

OECD (2004) OECD/OCDE 202: *Daphnia* sp., Acute Immobilisation Test. OECD Guideline for testing of chemicals. April 2004. Organization for Economic Co-operation and Development: 12.

OECD (2019). Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals. OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 23 (second edition), OECD Publishing, Paris.

Ouwerkerk, K. (2017) The effect of dissolved organic carbon (DOC) on the acute toxicity of copper and zinc for different freshwater species. Internship report AEW70424 for study undertaken at NIWA, Hamilton Ecotoxicology Laboratory. Aquatic Ecology and Water Quality Management Group, Department of Environmental Sciences, Wageningen University, Wageningen, The Netherlands: 33

Tidepool (2001-2022) CETIS -Comprehensive Environmental Toxicity Information System. CETIS Users Guide v2.1.3 Tidepool Scientific Software, MacKinleyville, Ca.: 305.

Warne, M.S., Batley, G.E., van Dam, R.A., Chapman, J.C., Fox, D.R., Hickey, C.W., Stauber, J.L. (2018) Revised method for deriving Australian and New Zealand water quality guideline values for toxicants - update of 2015 version. Prepared for the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian state and territory governments. August 2015 - updated October 2018. Canberra.

Appendix A Hill Labs results - Natural water characterisation

R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand

0508 HILL LAB (44 555 22)
+64 7 858 2000
mail@hill-labs.co.nz
www.hill-labs.co.nz

Certificate of Analysis

Client:	NIWA Corporate	**Lab No:**	3569077	SUPv1
Contact:	K Thompson C/- NIWA Corporate PO Box 11115 Hillcrest Hamilton 3251	**Date Received:** **Date Reported:** **Quote No:** **Order No:** **Client Reference:** **Submitted By:**	03-May-2024 09-May-2024 130803 U333268 Requisition 138984 K Thompson	

Sample Type: Aqueous

Sample Name:		Clutha 13-Feb-2024	Waihou 30-Apr-2024	Okutua 07-Feb-2024	Redwoods 07-Feb-2024
Lab Number:		3569077.1	3569077.2	3569077.3	3569077.4
Total Hardness	g/m³ as $CaCO_3$	33.7 ± 2.0	15.66 ± 0.73	2.74 ± 0.17	73.5 ± 3.4
Dissolved Calcium	g/m³	12.27 ± 0.77	3.25 ± 0.21	0.483 ± 0.045	16.1 ± 1.1
Dissolved Magnesium	g/m³	0.737 ± 0.052	1.83 ± 0.13	0.372 ± 0.029	8.09 ± 0.55
Dissolved Non-Purgeable Organic Carbon (DNPOC)	g/m³	0.38 ± 0.14	< 0.3 ± 0.14	11.9 ± 2.4	2.18 ± 0.46

Sample Name:		Hoteo 07-Feb-2024
Lab Number:		3569077.5
Total Hardness	g/m³ as $CaCO_3$	58.1 ± 2.7
Dissolved Calcium	g/m³	13.87 ± 0.87
Dissolved Magnesium	g/m³	5.70 ± 0.39
Dissolved Non-Purgeable Organic Carbon (DNPOC)	g/m³	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 : Online Edition.	1.0 g/m³ as $CaCO_3$	1-5
Filtration for dissolved metals analysis	Sample filtration through 0.45µm membrane filter and preservation with nitric acid. APHA 3030 B : Online Edition.	-	1-5
Dissolved Calcium	Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.	0.05 g/m³	1-5
Dissolved Magnesium	Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.	0.02 g/m³	1-5
Dissolved Non-Purgeable Organic Carbon (DNPOC)	Acidification, purging to remove inorganic C, super-critical persulphate oxidation at 375°C, IR detection. APHA 5310 C (modified) : Online Edition.	0.3 g/m³	1-5

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.

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

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.

Ara Heron BSc (Tech)
Client Services Manager - Environmental

Appendix B Hill Labs results – Zinc

R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand

0508 HILL LAB (44 555 22)
+64 7 858 2000
mail@hill-labs.co.nz
www.hill-labs.co.nz

Certificate of Analysis

Client:	NIWA Corporate	**Lab No:**	3578828	SUPv1
Contact:	K Thompson	**Date Received:**	13-May-2024	
	C/- NIWA Corporate	**Date Reported:**	16-May-2024	
	PO Box 11115	**Quote No:**	130803	
	Hillcrest	**Order No:**	U333504	
	Hamilton 3251	**Client Reference:**		
		Submitted By:	K Thompson	

Sample Type: Aqueous

	Sample Name:	Clutha 100ug/L T0 09-May-2024 1:00 pm	Clutha 220ug/L T0 09-May-2024 1:00 pm	Clutha 484ug/L T0 09-May-2024 1:00 pm	Clutha 1056ug/L T0 09-May-2024 1:00 pm
	Lab Number:	3578828.1	3578828.2	3578828.3	3578828.4
Dissolved Zinc	g/m³	0.106 ± 0.010	0.233 ± 0.022	0.517 ± 0.049	1.12 ± 0.11

	Sample Name:	Clutha 2343ug/L T0 09-May-2024 1:00 pm	Clutha 100ug/L T48 09-May-2024 1:00 pm	Clutha 220ug/L T48 09-May-2024 1:00 pm	Clutha 484ug/L T48 09-May-2024 1:00 pm
	Lab Number:	3578828.5	3578828.6	3578828.7	3578828.8
Dissolved Zinc	g/m³	2.47 ± 0.23	0.132 ± 0.013	0.219 ± 0.021	0.487 ± 0.046

	Sample Name:	Clutha 1056ug/L T48 09-May-2024 1:00 pm	Clutha 2343ug/L T48 09-May-2024 1:00 pm	Waihou 100ug/L T0 09-May-2024 1:00 pm	Waihou 220ug/L T0 09-May-2024 1:00 pm
	Lab Number:	3578828.9	3578828.10	3578828.11	3578828.12
Dissolved Zinc	g/m³	1.043 ± 0.097	2.31 ± 0.22	0.109 ± 0.011	0.236 ± 0.022

	Sample Name:	Waihou 484ug/L T0 09-May-2024 1:00 pm	Waihou 1056ug/L T0 09-May-2024 1:00 pm	Waihou 2343ug/L T0 09-May-2024 1:00 pm	Waihou 100ug/L T48 09-May-2024 1:00 pm
	Lab Number:	3578828.13	3578828.14	3578828.15	3578828.16
Dissolved Zinc	g/m³	0.518 ± 0.049	1.13 ± 0.11	2.46 ± 0.23	0.123 ± 0.012

	Sample Name:	Waihou 220ug/L T48 09-May-2024 1:00 pm	Waihou 484ug/L T48 09-May-2024 1:00 pm	Waihou 1056ug/L T48 09-May-2024 1:00 pm	Waihou 2343ug/L T48 09-May-2024 1:00 pm
	Lab Number:	3578828.17	3578828.18	3578828.19	3578828.20
Dissolved Zinc	g/m³	0.223 ± 0.021	0.482 ± 0.045	1.09 ± 0.11	2.36 ± 0.22

	Sample Name:	Redwoods 100ug/L T0 10-May-2024 1:00 pm	Redwoods 220ug/L T0 10-May-2024 1:00 pm	Redwoods 484ug/L T0 10-May-2024 1:00 pm	Redwoods 1056ug/L T0 10-May-2024 1:00 pm
	Lab Number:	3578828.21	3578828.22	3578828.23	3578828.24
Dissolved Zinc	g/m³	0.1032 ± 0.0097	0.232 ± 0.022	0.491 ± 0.046	1.08 ± 0.11

	Sample Name:	Redwoods 2343ug/L T0 10-May-2024 1:00 pm	Redwoods 100ug/L T48 10-May-2024 1:00 pm	Redwoods 220ug/L T48 10-May-2024 1:00 pm	Redwoods 484ug/L T48 10-May-2024 1:00 pm
	Lab Number:	3578828.25	3578828.26	3578828.27	3578828.28
Dissolved Zinc	g/m³	2.38 ± 0.23	0.457 ± 0.043	0.254 ± 0.024	0.487 ± 0.046

	Sample Name:	Redwoods 1056ug/L T48 10-May-2024 1:00 pm	Redwoods 2343ug/L T48 10-May-2024 1:00 pm	Hoteo 100ug/L T0 10-May-2024 1:00 pm	Hoteo 220ug/L T0 10-May-2024 1:00 pm
	Lab Number:	3578828.29	3578828.30	3578828.31	3578828.32
Dissolved Zinc	g/m³	1.034 ± 0.097	2.30 ± 0.22	0.1047 ± 0.0098	0.229 ± 0.022

	Sample Name:	Hoteo 484ug/L T0 10-May-2024 1:00 pm	Hoteo 1056ug/L T0 10-May-2024 1:00 pm	Hoteo 2343ug/L T0 10-May-2024 1:00 pm	Hoteo 100ug/L T48 10-May-2024 1:00 pm
	Lab Number:	3578828.33	3578828.34	3578828.35	3578828.36
Dissolved Zinc	g/m³	0.519 ± 0.049	1.08 ± 0.11	2.44 ± 0.23	0.513 ± 0.048

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.

Sample Type: Aqueous					
Sample Name:		Hoteo 220ug/L T48 10-May-2024 1:00 pm	Hoteo 484ug/L T48 10-May-2024 1:00 pm	Hoteo 1056ug/L T48 10-May-2024 1:00 pm	Hoteo 2343ug/L T48 10-May-2024 1:00 pm
Lab Number:		3578828.37	3578828.38	3578828.39	3578828.40
Dissolved Zinc	g/m^3	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 indicated, analyses were performed at Hill Labs, 28 Duke Street, Frankton, Hamilton 3204.

Sample Type: Aqueous			
Test	**Method Description**	**Default Detection Limit**	**Sample No**
Filtration for dissolved metals analysis	Sample filtration through 0.45µm membrane filter and 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^3	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.

Ara Heron BSc (Tech)
Client Services Manager - Environmental

Appendix C Hill Labs results - Copper

R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand

0508 HILL LAB (44 555 22)
+64 7 858 2000
mail@hill-labs.co.nz
www.hill-labs.co.nz

Certificate of Analysis

Client:	NIWA Corporate	**Lab No:**	3575453	SUPv1
Contact:	K Thompson	**Date Received:**	09-May-2024	
	C/- NIWA Corporate	**Date Reported:**	14-May-2024	
	PO Box 11115	**Quote No:**	130803	
	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/m³	0.00092 ± 0.00035	0.00068 ± 0.00034	0.0113 ± 0.0011	0.00818 ± 0.00082
Dissolved Zinc	g/m³	0.00311 ± 0.00073	0.00338 ± 0.00074	-	-

	Sample Name:	Waihou Cu 23.4 T0	Waihou Cu 23.4 T48	Waihou Cu 51.5 T0	Waihou Cu 51.5 T48
	Lab Number:	3575453.5	3575453.6	3575453.7	3575453.8
Dissolved Copper	g/m³	0.0234 ± 0.0022	0.0191 ± 0.0018	0.0533 ± 0.0049	0.0431 ± 0.0040

	Sample Name:	Waihou Cu 113 T0	Waihou Cu 113 T48	Waihou Cu 249 T0	Waihou Cu 249 T48
	Lab Number:	3575453.9	3575453.10	3575453.11	3575453.12
Dissolved Copper	g/m³	0.1016 ± 0.0093	0.0918 ± 0.0084	0.229 ± 0.021	0.191 ± 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
Dissolved Copper	g/m³	< 0.0005 ± 0.00034	< 0.0005 ± 0.00034	0.0249 ± 0.0023	0.0211 ± 0.0020
Dissolved Zinc	g/m³	0.00330 ± 0.00073	0.00314 ± 0.00073	-	-

	Sample Name:	Redwoods Cu 51.5 T0	Redwoods Cu 51.5 T48	Redwoods Cu 154.5 T0	Redwoods Cu 154.5 T48
	Lab Number:	3575453.17	3575453.18	3575453.19	3575453.20
Dissolved Copper	g/m³	0.0525 ± 0.0048	0.0501 ± 0.0046	0.156 ± 0.015	0.155 ± 0.015

	Sample Name:	Redwoods Cu 463.5 T0	Redwoods Cu 463.5 T48	Redwoods Cu 1390.5 T0	Redwoods Cu 1390.5 T48
	Lab Number:	3575453.21	3575453.22	3575453.23	3575453.24
Dissolved Copper	g/m³	0.466 ± 0.043	0.429 ± 0.040	1.44 ± 0.14	1.20 ± 0.11

	Sample Name:	Hoteo Control T0	Hoteo Control T48	Hoteo Cu 23.4 T0	Hoteo Cu 23.4 T48
	Lab Number:	3575453.25	3575453.26	3575453.27	3575453.28
Dissolved Copper	g/m³	0.00105 ± 0.00035	0.00098 ± 0.00035	0.0240 ± 0.0023	0.0251 ± 0.0024
Dissolved Zinc	g/m³	0.00418 ± 0.00077	0.00453 ± 0.00079	-	-

	Sample Name:	Hoteo Cu 51.5 T0	Hoteo Cu 51.5 T48	Hoteo Cu 154.5 T0	Hoteo Cu 154.5 T48
	Lab Number:	3575453.29	3575453.30	3575453.31	3575453.32
Dissolved Copper	g/m³	0.0529 ± 0.0049	0.0527 ± 0.0049	0.149 ± 0.014	0.153 ± 0.014

	Sample Name:	Hoteo Cu 463.5 T0	Hoteo Cu 463.5 T48	Hoteo Cu 1390.5 T0	Hoteo Cu 1390.5 T48
	Lab Number:	3575453.33	3575453.34	3575453.35	3575453.36
Dissolved Copper	g/m³	0.465 ± 0.043	0.471 ± 0.043	1.42 ± 0.13	1.22 ± 0.12

	Sample Name:	Clutha Control T0	Clutha Control T48	Clutha Cu 2.2 T0	Clutha Cu 2.2 T48
	Lab Number:	3575453.37	3575453.38	3575453.39	3575453.40
Dissolved Copper	g/m³	< 0.0005 ± 0.00034	< 0.0005 ± 0.00034	0.00235 ± 0.00040	0.00203 ± 0.00038
Dissolved Zinc	g/m³	0.00271 ± 0.00071	0.00299 ± 0.00072	-	-

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.

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 indicated, 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/m³	1-48
Dissolved Zinc	Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.	0.0010 g/m³	1-2, 13-14, 25-26, 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.

This certificate of analysis must not be reproduced, except in full, without the written consent of the signatory.

Ara Heron BSc (Tech)
Client Services Manager - Environmental

Appendix D Hill Labs results – Okutua, zinc and copper

R J Hill Laboratories Limited
28 Duke Street Frankton 3204
Private Bag 3205
Hamilton 3240 New Zealand

0508 HILL LAB (44 555 22)
+64 7 858 2000
mail@hill-labs.co.nz
www.hill-labs.co.nz

Certificate of Analysis

Client:	NIWA Corporate	**Lab No:**	3599299	SUPv2
Contact:	K Thompson	**Date Received:**	05-Jun-2024	
	C/- NIWA Corporate	**Date Reported:**	21-Jun-2024	**(Amended)**
	PO Box 11115	**Quote No:**	130803	
	Hillcrest	**Order No:**	U334528	
	Hamilton 3251	**Client Reference:**	Okutua	
		Submitted By:	K Thompson	

Sample Type: 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	3599299.3	3599299.4
Dissolved Copper	g/m³	-	0.00126 ± 0.00035	-	-
Total Copper	g/m³	0.00130 ± 0.00038	-	0.0143 ± 0.0015	0.0137 ± 0.0015
Dissolved Zinc	g/m³	-	0.00441 ± 0.00078	-	-
Total Zinc	g/m³	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	3599299.7	3599299.8
Dissolved Copper	g/m³	-	0.0283 ± 0.0026	0.0601 ± 0.0055	0.0578 ± 0.0053
Total Copper	g/m³	0.0284 ± 0.0029	-	-	-

	Sample Name:	Okutua Cu 113 To	Okutua Cu 113 Tend	Okutua Cu 249 To	Okutua Cu 249 Tend
	Lab Number:	3599299.9	3599299.10	3599299.11	3599299.12
Dissolved Copper	g/m³	-	-	-	0.284 ± 0.026
Total Copper	g/m³	0.136 ± 0.014	0.136 ± 0.014	0.283 ± 0.029	-

	Sample Name:	Okutua Zn 100 To	Okutua Zn 100 Tend	Okutua Zn 220 To	Okutua Zn 220 Tend
	Lab Number:	3599299.13	3599299.14	3599299.15	3599299.16
Dissolved Zinc	g/m³	-	0.108 ± 0.011	-	0.220 ± 0.021
Total Zinc	g/m³	0.1099 ± 0.0089	-	0.252 ± 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	3599299.19	3599299.20
Dissolved Zinc	g/m³	0.495 ± 0.047	0.468 ± 0.044	1.07 ± 0.10	1.09 ± 0.11

	Sample Name:	Okutua Zn 2343 To	Okutua Zn 2343 Tend
	Lab Number:	3599299.21	3599299.22
Total Copper	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 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.

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/m³	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/m³	1, 3-5, 9-11, 21-22
Dissolved Zinc	Filtered sample, ICP-MS, trace level. APHA 3125 B : Online Edition.	0.0010 g/m³	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/m³	1, 13, 15, 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.

Ara Heron BSc (Tech)
Client Services Manager - Environmental

Appendix E Toxicity tests raw data

Zinc

Acute *D. thomsoni* 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 T0 & T48
	2	10	7	4	
	3	10	6	2	
Mahurangi 2343	1	10	5	0	Sent to hill labs for analysis T0 & T48
	2	10	4	0	
	3	10	4	0	
Mahurangi 5154	1	10	0	0	Not sent to Hill labs
	2	10	0	0	
	3	10	0	0	
Mahurangi 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**
Hoteo Control 0	1	10	10	10	Sent to hills with coppers
Hoteo Control 0	2	10	10	10	
Hoteo Control 0	3	10	10	10	
Hoteo Control 0	4	10	10	10	
Hoteo Control 0	5	10	10	10	
Hoteo 100	1	10	10	10	Sent to hill labs for analysis T0 & T48
	2	10	10	10	
	3	10	10	10	
Hoteo 220	1	10	10	10	Sent to hill labs for analysis T0 & T48
	2	10	10	10	
	3	10	10	10	
Hoteo 484	1	10	9	6	Sent to hill labs for analysis T0 & T48
	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	4	Sent to hill labs for analysis T0 & T48
	2	10	10	3	
	3	10	9	3	
Okutua 1065	1	10	0	0	Sent to hill labs for analysis T0 & T48
	2	10	0	0	
	3	10	0	0	
Okutua 2343	1	10	0	0	Sent to hill labs for analysis T0 & T48
	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	
Natural water Nominal Zinc µg/L	**Replicate**	**# *Daphnia* exposed**	**24 h Survival**	**48 h Survival**	**Comment**
Clutha Control 0	1	10	10	10	Sent to hill labs with coppers
Clutha Control 0	2	10	10	10	
Clutha Control 0	3	10	10	10	
Clutha Control 0	4	10	10	9	
Clutha Control 0	5	10	10	10	
Clutha 45	1	10	10	10	Not sent to Hill labs
	2	10	10	10	
	3	10	10	10	
Clutha 100	1	10	10	10	Sent to hill labs for analysis T0 & T48
	2	10	10	10	
	3	10	10	10	
Clutha 220	1	10	10	9	Sent to hill labs for analysis T0 & T48
	2	10	10	9	
	3	10	10	8	
Clutha 484	1	10	9	6	Sent to hill labs for analysis T0 & T48
	2	10	9	6	
	3	10	8	4	
Clutha 1056	1	10	5	0	Sent to hill labs for analysis T0 & T48
	2	10	8	1	
	3	10	8	3	
Clutha 2343	1	10	0	0	Sent to hill labs for analysis T0 & T48
	2	10	0	0	
	3	10	1	0	
Clutha 5154	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
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 Copper µg/L	**Replicate**	**# *Daphnia* exposed**	**24 h Survival**	**48 h Survival**	**Comment**
Hoteo Control 0	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Hoteo Control 0	2	10	10	10	
Hoteo Control 0	3	10	10	10	
Hoteo Control 0	4	10	10	10	
Hoteo Control 0	5	10	10	10	
Hoteo 1.0	1	10	10	10	Not sent to Hill labs
Hoteo 1.0	2	10	10	10	
Hoteo 1.0	3	10	10	10	
Hoteo 2.2	1	10	10	10	Not sent to Hill labs
Hoteo 2.2	2	10	10	10	
Hoteo 2.2	3	10	10	10	
Hoteo 4.8	1	10	10	10	Not sent to Hill labs
Hoteo 4.8	2	10	10	10	
Hoteo 4.8	3	10	10	10	
Hoteo 10.6	1	10	10	10	Not sent to Hill labs
Hoteo 10.6	2	10	10	10	
Hoteo 10.6	3	10	10	10	
Hoteo 23.4	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Hoteo 23.4	2	10	10	10	
Hoteo 23.4	3	10	10	10	
Hoteo 51.5	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Hoteo 51.5	2	10	10	10	
Hoteo 51.5	3	10	10	10	
Hoteo 154.5	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Hoteo 154.5	2	10	10	10	
Hoteo 154.5	3	10	10	10	
Hoteo 463.5	1	10	0	0	Sent to Hill labs for analysis T0 and T48
Hoteo 463.5	2	10	1	0	
Hoteo 463.5	3	10	2	1	
Hoteo 1390.5	1	10	0	0	Sent to Hill labs for analysis T0 and T48
Hoteo 1390.5	2	10	0	0	
Hoteo 1390.5	3	10	0	0	

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	4	
Okutua 113	3	10	0	0	
Okutua 249	1	10	0	0	
Okutua 249	2	10	0	0	
Okutua 249	3	10	0	0	
Natural water Nominal Copper µg/L	**Replicate**	**# *Daphnia* exposed**	**24 h Survival**	**48 h Survival**	**Comment**
Clutha Control 0	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Clutha Control 0	2	10	10	10	
Clutha Control 0	3	10	10	10	
Clutha Control 0	4	10	10	9	
Clutha Control 0	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	
Clutha 2.2	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Clutha 2.2	2	10	10	9	
Clutha 2.2	3	10	10	10	
Clutha 4.8	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Clutha 4.8	2	10	10	10	
Clutha 4.8	3	10	10	10	
Clutha 10.6	1	10	10	10	Sent to Hill labs for analysis T0 and T48
Clutha 10.6	2	10	10	10	
Clutha 10.6	3	10	10	10	
Clutha 23.4	1	10	8	7	Sent to Hill labs for analysis T0 and T48
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 T0 and T48
Clutha 51.5	2	10	1	1	
Clutha 51.5	3	10	0	0	

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 T0 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 T0 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 T0 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 T0 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 T0 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 1 of 2)
Test Code/ID: 24.003.1 Zn / 15-8888-6853

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	17-8062-8370	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:24	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	224919757782DA5F34CA239A904E497A	**Editor ID:**	
Batch ID:	03-1254-1573	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	08 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Redwoods
Ending Date:	10 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	09-2771-2013	**Code:**	24.003.1 Zn	**Project:**	Special Studies
Sample Date:	07 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	07 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	240	490	342.9	---	0.06421	6.42%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		103	6	0	2.56	0.09742	CDF	1.0000	Non-Significant Effect
		240	6	0	2.56	0.09742	CDF	1.0000	Non-Significant Effect
		490*	6	4.283	2.56	0.09742	CDF	0.0021	Significant Effect
		1055*	6	22.99	2.56	0.09742	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.80484	0.451209	4	166.2	<1.0E-05	Significant Effect
Error	0.0325818	0.0027152	12			
Total	1.83742		16			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
	Levene Equality of Variance Test	19.76	5.412	3.3E-05	Unequal Variances
	Mod Levene Equality of Variance Test	1.458	7.847	0.3105	Equal Variances
Distribution	Anderson-Darling A2 Test	4.306	3.878	<1.0E-05	Non-Normal Distribution
	D'Agostino Skewness Test	3.014	2.576	0.0026	Non-Normal Distribution
	Kolmogorov-Smirnov D Test	0.4412	0.2405	<1.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.5509	0.848	<1.0E-05	Non-Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
3.2	D	5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
103		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
240		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
490		3	0.9000	0.8996	0.9004	0.9000	0.9000	0.9000	0.0000	0.00%	10.00%
1055		3	0.2667	0.0000	0.5535	0.2000	0.2000	0.4000	0.0667	43.30%	73.33%
2350		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
3.2	D	5	1.4120	1.4120	1.4120	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
103		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
240		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
490		3	1.2490	1.2490	1.2490	1.2490	1.2490	1.2490	0.0000	0.00%	11.54%
1055		3	0.5373	0.2203	0.8544	0.4636	0.4636	0.6847	0.0737	23.75%	61.95%
2350		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.76%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	17-8062-8370	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:24	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	224919757782DA5F34CA239A904E497A	**Editor ID:**	

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		

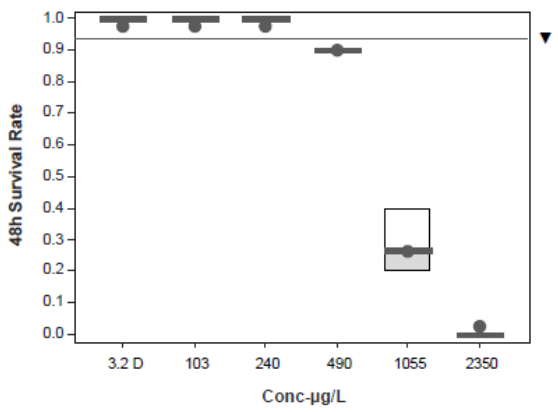
Angular (Corrected) Transformed Detail

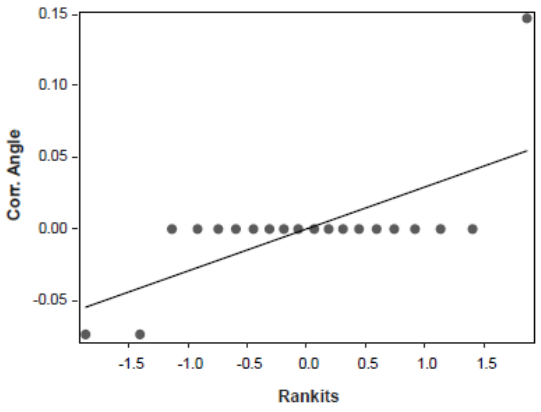
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
3.2	D	1.4120	1.4120	1.4120	1.4120	1.4120
103		1.4120	1.4120	1.4120		
240		1.4120	1.4120	1.4120		
490		1.2490	1.2490	1.2490		
1055		0.4636	0.6847	0.4636		
2350		0.1588	0.1588	0.1588		

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		

Graphics





Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	15-8302-1928	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:24	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	224919757782DA5F34CA239A904E497A	**Editor ID:**	
Batch ID:	03-1254-1573	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	08 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Redwoods
Ending Date:	10 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	09-2771-2013	**Code:**	24.003.1 Zn	**Project:**	Special Studies
Sample Date:	07 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	07 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
4	-7.72	22.94	24.43	0.9753	0.00%	1	Yes	1.702	0.2123	Non-Significant Lack-of-Fit

Point Estimates

Level	µg/L	95% LCL	95% UCL
LC5	433.6	377.4	473.3
LC10	510.6	459.2	551
LC15	565	516.6	605.7
LC20	609.8	563.4	650.6
LC25	649.4	604.5	690.5
LC40	755.7	712.5	799.2
LC50	825.9	781.6	872.6

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	1	1.518E-05	1	1	65870	<1.0E-05	Significant Parameter
γ	4.57	0.2874	3.963	5.176	15.9	<1.0E-05	Significant Parameter
δ	825.9	21.46	780.6	871.1	38.49	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	500400000	166800000	3	1.446E+09	<1.0E-05	Significant Effect
Lack of Fit	0.5239	0.1746	3	1.702	0.2123	Non-Significant Lack-of-Fit
Pure Error	1.437	0.1026	14			
Residual	1.961	0.1153	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	2.194	27.59	1.0000	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	1.961	27.59	1.0000	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	1.371	3.687	0.3286	Equal Variances
Distribution	Anderson-Darling A2 Test	1.661	2.492	<1.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.7373	0.9044	0.0001	Non-Normal Distribution
Overdispersion	Tarone C(α) Overdispersion Test	1.291	1.645	0.0984	Non-Significant Overdispersion

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	15-8302-1928	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:24	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	224919757782DA5F34CA239A904E497A	**Editor ID:**	

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	Code	Count	Mean	Median	Min	Max	Std Err	Std Dev	CV%	%Effect	ΣA/ΣB
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		

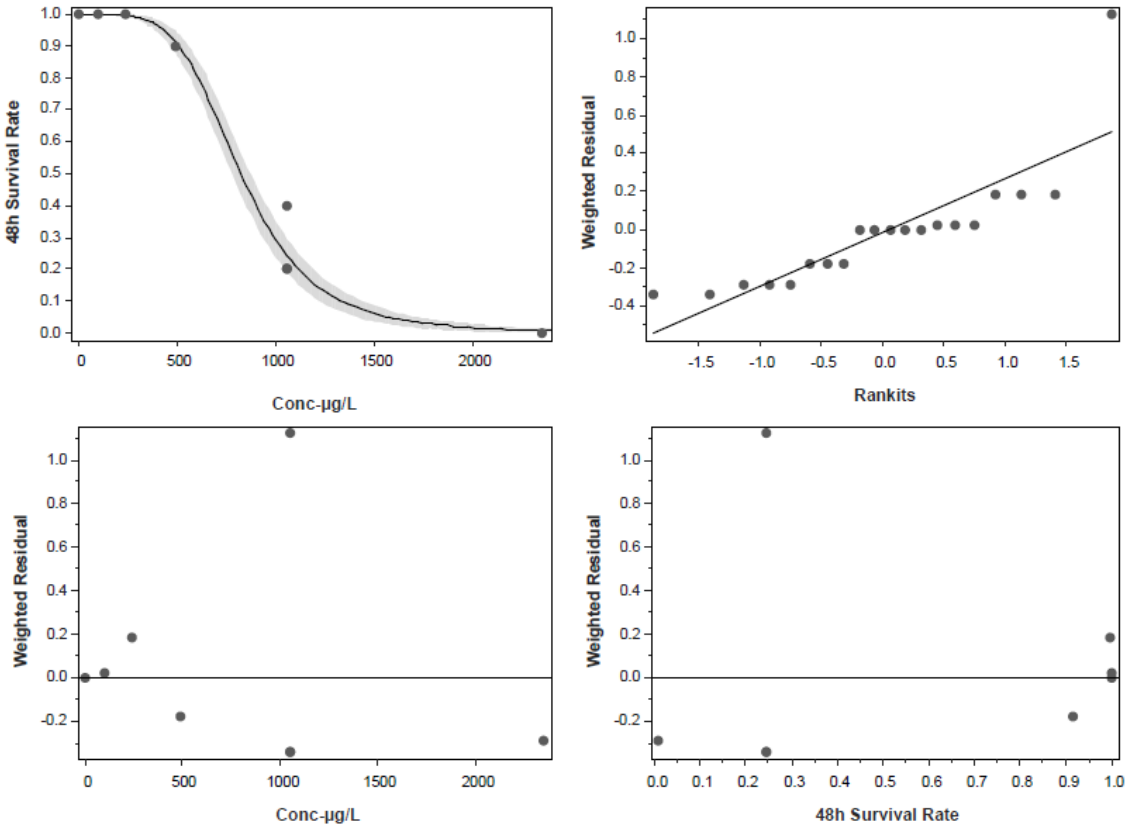
48h Survival Rate Binomials

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		

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	15-8302-1928	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:24	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	224919757782DA5F34CA239A904E497A	**Editor ID:**	

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Copper

CETIS Analytical Report

Report Date: 27 May-24 14:09 (p 1 of 2)
Test Code/ID: 24.003.1 Cu / 11-3123-4564

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	06-4463-1136	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:08	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	5D241F0A16458046CC0427C2488552BC	**Editor ID:**	
Batch ID:	15-9420-8257	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	04 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Redwoods
Ending Date:	06 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	04-1166-2908	**Code:**	24.003.1 Cu	**Project:**	Special Studies
Sample Date:	03 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	03 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	51	156	89.2	---	0.06018	6.02%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		23	6	0	2.466	0.08906	CDF	1.0000	Non-Significant Effect
		51	6	0	2.466	0.08906	CDF	1.0000	Non-Significant Effect
		156*	6	11.55	2.466	0.08906	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.410318	0.136773	3	55.93	<1.0E-05	Significant Effect
Error	0.0244562	0.0024456	10			
Total	0.434774		13			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
	Levene Equality of Variance Test	5.781	6.552	0.0148	Equal Variances
	Mod Levene Equality of Variance Test	656.6	9.78	<1.0E-05	Unequal Variances
Distribution	Anderson-Darling A2 Test	3.824	3.878	<1.0E-05	Non-Normal Distribution
	D'Agostino Skewness Test	0.2587	2.576	0.7958	Normal Distribution
	Kolmogorov-Smirnov D Test	0.4286	0.262	<1.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.5624	0.8239	1.9E-05	Non-Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.25	D	5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
23		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
51		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
156		3	0.7000	0.4516	0.9484	0.7000	0.6000	0.8000	0.0577	14.29%	30.00%
450		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
1320		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.25	D	5	1.4120	1.4120	1.4120	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
23		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
51		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
156		3	0.9948	0.7201	1.2690	0.9912	0.8861	1.1070	0.0638	11.12%	29.55%
450		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.76%
1320		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.76%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	06-4463-1136	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:08	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	5D241F0A16458046CC0427C2488552BC	**Editor ID:**	

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	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		

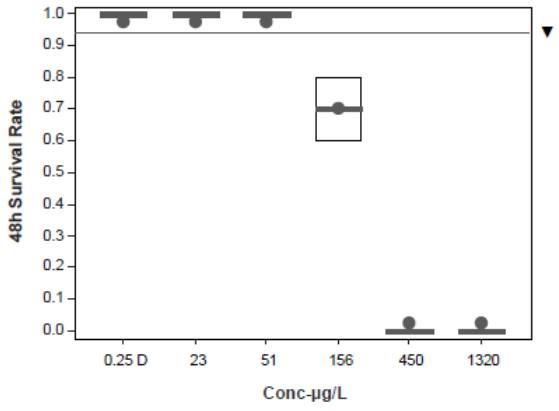
Angular (Corrected) Transformed Detail

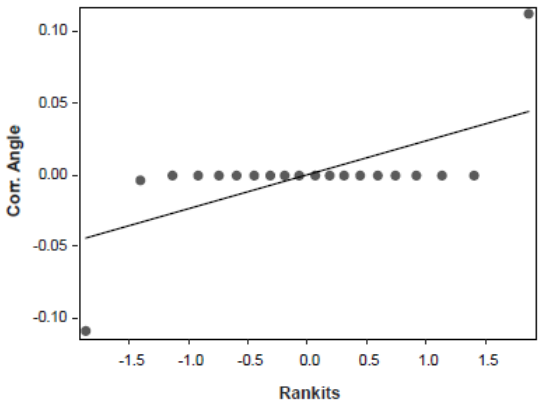
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0.25	D	1.4120	1.4120	1.4120	1.4120	1.4120
23		1.4120	1.4120	1.4120		
51		1.4120	1.4120	1.4120		
156		1.1070	0.8861	0.9912		
450		0.1588	0.1588	0.1588		
1320		0.1588	0.1588	0.1588		

48h 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	10/10	10/10
23		10/10	10/10	10/10		
51		10/10	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		

Graphics





Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

CETIS Analytical Report

Report Date: 27 May-24 14:09 (p 1 of 2)
Test Code/ID: 24.003.1 Cu / 11-3123-4564

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	01-0618-7910	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:09	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	5D241F0A16458046CC0427C2488552BC	**Editor ID:**	
Batch ID:	15-9420-8257	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	04 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Redwoods
Ending Date:	06 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	04-1166-2908	**Code:**	24.003.1 Cu	**Project:**	Special Studies
Sample Date:	03 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	03 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	850200	200	Yes	Two-Point Interpolation

Point Estimates

Level	µg/L	95% LCL	95% UCL
LC15	89.35	64.88	149.7
LC20	107.6	69.26	209.2
LC25	129.6	73.2	207.5
LC40	181.5	127.9	227.4
LC50	211.2	158.6	255.3

48h Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0.25	D	5	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	50/50	1.0000	0.00%
23		3	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	30/30	1.0000	0.00%
51		3	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	30/30	1.0000	0.00%
156		3	0.7000	0.7000	0.6000	0.8000	14.29%	30.00%	21/30	0.7000	30.00%
450		3	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/30	0.0000	100.00%
1320		3	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/30	0.0000	100.00%

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	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		

48h Survival Rate Binomials

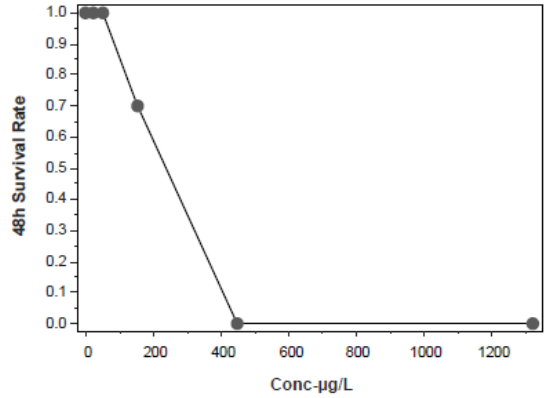
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0.25	D	10/10	10/10	10/10	10/10	10/10
23		10/10	10/10	10/10		
51		10/10	10/10	10/10		
156		8/10	6/10	7/10		
450		0/10	0/10	0/10		
1320		0/10	0/10	0/10		

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	01-0618-7910	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:09	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	5D241F0A16458046CC0427C2488552BC	**Editor ID:**	

Graphics



Appendix G CETIS statistical analyses – Hoteo

Zinc

CETIS Analytical Report

Report Date: 27 May-24 14:26 (p 1 of 2)
Test Code/ID: 24.003.2 Zn / 18-3585-2031

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	05-6314-4525	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:26	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	8CB123CEE5801E9A4A603D8DCEB372A	**Editor ID:**	
Batch ID:	20-4051-4804	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	08 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Hoteo
Ending Date:	10 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	04-6256-9350	**Code:**	24.003.2 Zn	**Project:**	Special Studies
Sample Date:	07 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	07 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	235	505	344.5	---	0.09253	9.25%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		105	6	0	2.56	0.1503	CDF	1.0000	Non-Significant Effect
		235	6	0	2.56	0.1503	CDF	1.0000	Non-Significant Effect
		505*	6	7.106	2.56	0.1503	CDF	2.5E-05	Significant Effect
		1055*	6	13.67	2.56	0.1503	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.66646	0.416616	4	64.46	<1.0E-05	Significant Effect
Error	0.0775609	0.0064634	12			
Total	1.74402		16			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
	Levene Equality of Variance Test	5.924	5.412	0.0072	Unequal Variances
	Mod Levene Equality of Variance Test	22.1	7.847	0.0005	Unequal Variances
Distribution	Anderson-Darling A2 Test	2.777	3.878	<1.0E-05	Non-Normal Distribution
	D'Agostino Skewness Test	0.9691	2.576	0.3325	Normal Distribution
	Kolmogorov-Smirnov D Test	0.3824	0.2405	<1.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.7463	0.848	0.0004	Non-Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
4.35	D	5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
105		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
235		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
505		3	0.7000	0.4516	0.9484	0.7000	0.6000	0.8000	0.0577	14.29%	30.00%
1055		3	0.3333	0.0000	0.7128	0.3000	0.2000	0.5000	0.0882	45.83%	66.67%
2400		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
4.35	D	5	1.4120	1.4120	1.4120	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
105		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
235		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
505		3	0.9948	0.7201	1.2690	0.9912	0.8861	1.1070	0.0638	11.12%	29.55%
1055		3	0.6096	0.2048	1.0140	0.5796	0.4636	0.7854	0.0941	26.73%	56.83%
2400		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.76%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	05-6314-4525	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:26	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	8CB123CEE5801E9A4A603D8DCEB372A	**Editor ID:**	

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		
505		0.6000	0.7000	0.8000		
1055		0.2000	0.3000	0.5000		
2400		0.0000	0.0000	0.0000		

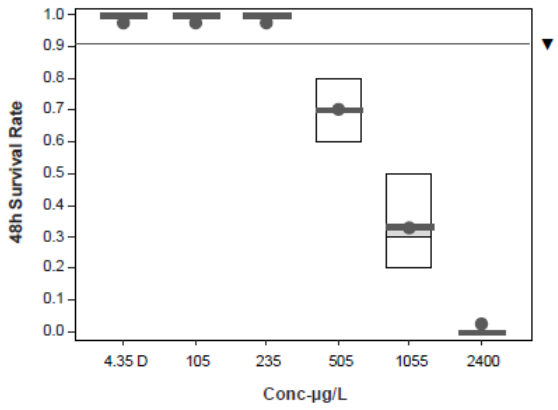
Angular (Corrected) Transformed Detail

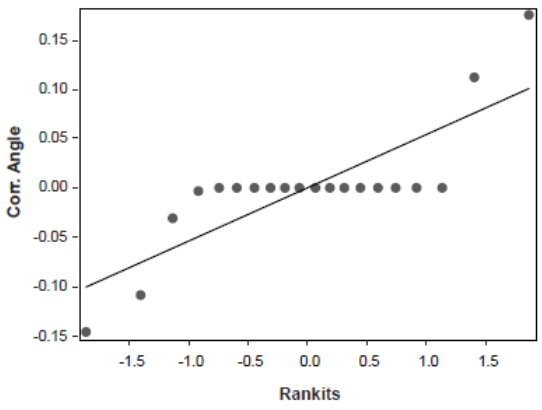
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
4.35	D	1.4120	1.4120	1.4120	1.4120	1.4120
105		1.4120	1.4120	1.4120		
235		1.4120	1.4120	1.4120		
505		0.8861	0.9912	1.1070		
1055		0.4636	0.5796	0.7854		
2400		0.1588	0.1588	0.1588		

48h Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
4.35	D	10/10	10/10	10/10	10/10	10/10
105		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





Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

CETIS Analytical Report

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	05-7411-6460	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:26	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	8CB123CEE5801E9A4A603D8DCEB372A	**Editor ID:**	
Batch ID:	20-4051-4804	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	08 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Hoteo
Ending Date:	10 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	04-6256-9350	**Code:**	24.003.2 Zn	**Project:**	Special Studies
Sample Date:	07 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	07 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
8	-12.01	31.51	33	0.9001	0.63%	0.9987	Yes	4.816	0.0166	Significant Lack-of-Fit

Point Estimates

Level	μg/L	95% LCL	95% UCL
LC5	295.4	188.7	357.1
LC10	374.3	278.7	441.9
LC15	433.4	343.4	504.6
LC20	483.9	397.5	558
LC25	530.1	445.9	606.9
LC40	660.3	576.1	748.4
LC50	750.7	661.1	852.5

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	0.9987	0.002998	0.9924	1.005	333.1	<1.0E-05	Significant Parameter
γ	3.157	0.3554	2.407	3.907	8.883	<1.0E-05	Significant Parameter
δ	750.7	44.77	656.3	845.2	16.77	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	50060	16690	3	38650	<1.0E-05	Significant Effect
Lack of Fit	3.728	1.243	3	4.816	0.0166	Significant Lack-of-Fit
Pure Error	3.612	0.258	14			
Residual	7.34	0.4318	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	8.369	27.59	0.9580	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	7.34	27.59	0.9787	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	15.46	3.687	0.0006	Unequal Variances
Distribution	Anderson-Darling A2 Test	1.153	2.492	0.0052	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.886	0.9044	0.0227	Non-Normal Distribution
Overdispersion	Tarone C(α) Overdispersion Test	0.8812	1.645	0.1891	Non-Significant Overdispersion

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	05-7411-6460	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:26	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	8CB123CEE5801E9A4A603D8DCEB372A	**Editor ID:**	

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**ΣA/ΣB**
4.35	D	5	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%	50/50
105		3	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%	30/30
235		3	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%	30/30
505		3	0.7000	0.7000	0.6000	0.8000	0.0577	0.1000	14.29%	30.00%	21/30
1055		3	0.3333	0.3000	0.2000	0.5000	0.0882	0.1528	45.83%	66.67%	10/30
2400		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**
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		
505		0.6000	0.7000	0.8000		
1055		0.2000	0.3000	0.5000		
2400		0.0000	0.0000	0.0000		

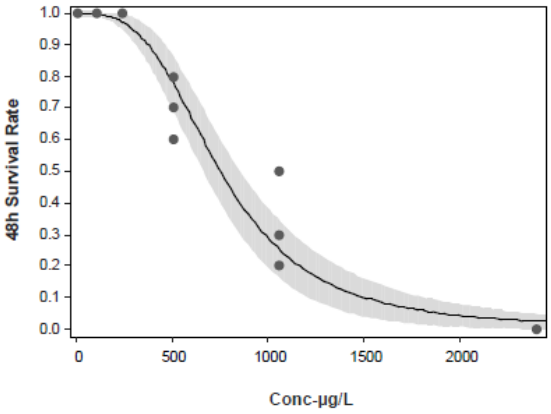
48h Survival Rate Binomials

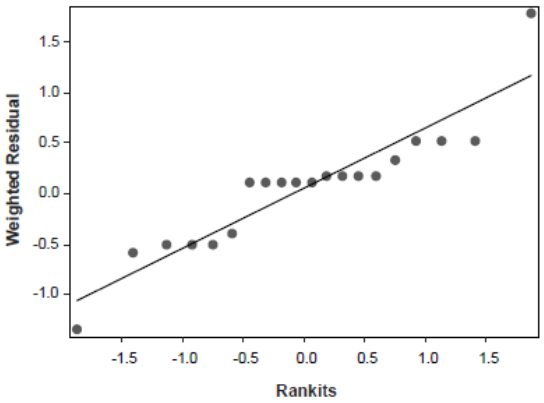
Conc-µg/L	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**
4.35	D	10/10	10/10	10/10	10/10	10/10
105		10/10	10/10	10/10		
235		10/10	10/10	10/10		
505		6/10	7/10	8/10		
1055		2/10	3/10	5/10		
2400		0/10	0/10	0/10		

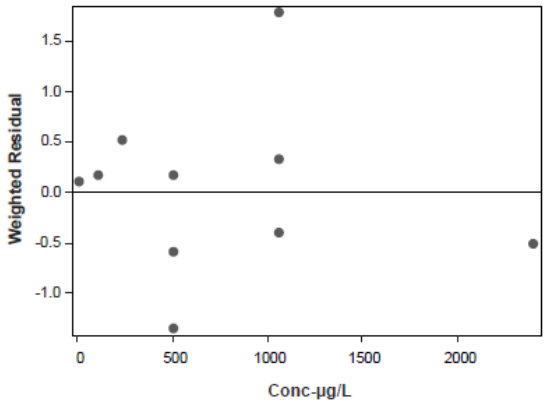
Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

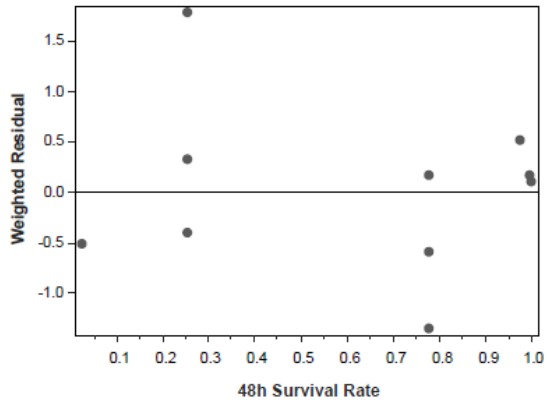
Analysis ID:	05-7411-6460	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:26	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	8CB123CEE5801E9A4A603D8DCEB372A	**Editor ID:**	

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]









Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Copper

CETIS Analytical Report

Report Date: 27 May-24 14:15 (p 1 of 2)
Test Code/ID: 24.003.2 Cu / 11-1449-0779

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	02-8623-2987	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:14	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	7540DE25745EA65EB54EBC125F9B5243	**Editor ID:**	

Batch ID:	19-5192-3864	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team	
Start Date:	04 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Hoteo	
Ending Date:	06 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable	
Test Length:	48h	**Taxon:**		**Source:**	Field Collected	**Age:**

Sample ID:	03-6699-5858	**Code:**	24.003.2 Cu	**Project:**	Special Studies
Sample Date:	03 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	03 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	151	470	266.4	---	0.05224	5.22%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		24.5	6	0	2.56	0.07182	CDF	1.0000	Non-Significant Effect
		53	6	0	2.56	0.07182	CDF	1.0000	Non-Significant Effect
		151	6	0	2.56	0.07182	CDF	1.0000	Non-Significant Effect
		470*	6	42.74	2.56	0.07182	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.5512	0.8878	4	601.7	<1.0E-05	Significant Effect
Error	0.0177062	0.0014755	12			
Total	3.56891		16			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
	Levene Equality of Variance Test	19.76	5.412	3.3E-05	Unequal Variances
	Mod Levene Equality of Variance Test	1.458	7.847	0.3105	Equal Variances
Distribution	Anderson-Darling A2 Test	4.306	3.878	<1.0E-05	Non-Normal Distribution
	D'Agostino Skewness Test	3.014	2.576	0.0026	Non-Normal Distribution
	Kolmogorov-Smirnov D Test	0.4412	0.2405	<1.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.5509	0.848	<1.0E-05	Non-Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.1	D	5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
24.5		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
53		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
151		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
470		3	0.0333	0.0000	0.1768	0.0000	0.0000	0.1000	0.0333	173.21%	96.67%
1320		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.1	D	5	1.4120	1.4120	1.4120	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
24.5		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
53		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
151		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	0.00%
470		3	0.2131	-0.0206	0.4468	0.1588	0.1588	0.3218	0.0543	44.15%	84.91%
1320		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.76%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	02-8623-2987	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:14	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	7540DE25745EA65EB54EBC125F9B5243	**Editor ID:**	

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		

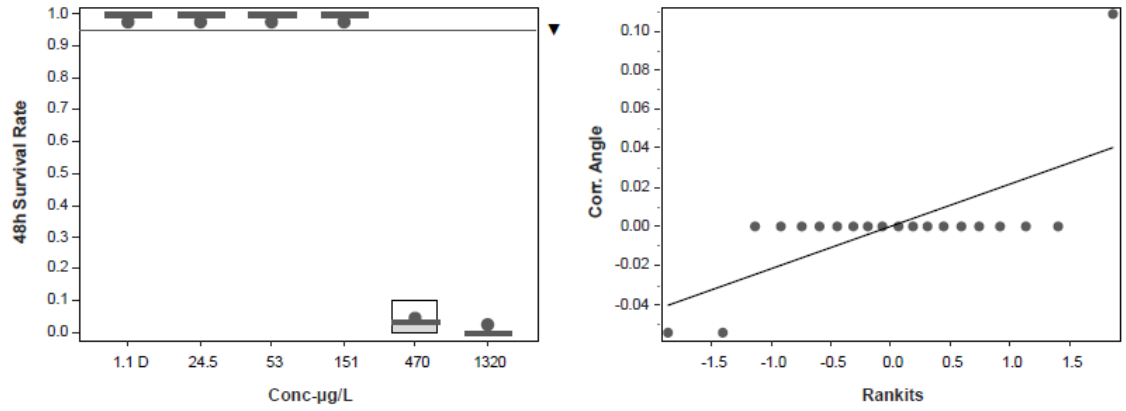
Angular (Corrected) Transformed Detail

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		
470		0.1588	0.1588	0.3218		
1320		0.1588	0.1588	0.1588		

48h Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1.1	D	10/10	10/10	10/10	10/10	10/10
24.5		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



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

CETIS Analytical Report

Report Date: 27 May-24 14:15 (p 1 of 2)
Test Code/ID: 24.003.2 Cu / 11-1449-0779

Daphnia thomsoni 48-h Acute Survival Test — NIWA Ecotoxicology

Analysis ID:	01-8576-5485	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:14	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	7540DE25745EA65EB54EBC125F9B5243	**Editor ID:**	
Batch ID:	19-5192-3864	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	04 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Hoteo
Ending Date:	06 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	03-6699-5858	**Code:**	24.003.2 Cu	**Project:**	Special Studies
Sample Date:	03 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	03 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1141069	200	Yes	Two-Point Interpolation

Point Estimates

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
LC50	271.8	260.8	283.9

48h Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
1.1	D	5	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	50/50	1.0000	0.00%
24.5		3	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	30/30	1.0000	0.00%
53		3	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	30/30	1.0000	0.00%
151		3	1.0000	1.0000	1.0000	1.0000	0.00%	0.00%	30/30	1.0000	0.00%
470		3	0.0333	0.0000	0.0000	0.1000	173.21%	96.67%	1/30	0.0333	96.67%
1320		3	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/30	0.0000	100.00%

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		

48h Survival Rate Binomials

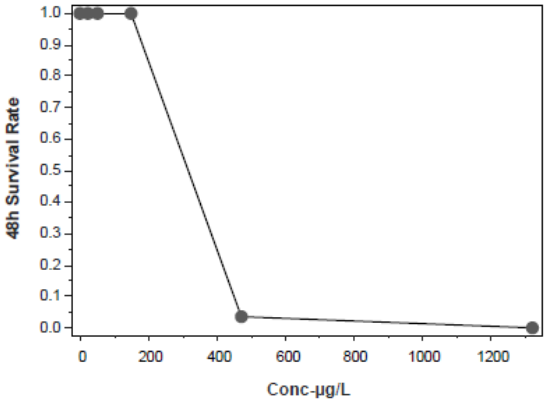
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1.1	D	10/10	10/10	10/10	10/10	10/10
24.5		10/10	10/10	10/10		
53		10/10	10/10	10/10		
151		10/10	10/10	10/10		
470		0/10	0/10	1/10		
1320		0/10	0/10	0/10		

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	01-8576-5485	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:14	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	7540DE25745EA65EB54EBC125F9B5243	**Editor ID:**	

Graphics



Appendix H CETIS statistical analyses – Okutua (pH adjusted)

Zinc

CETIS Analytical Report

Report Date: 26 Jun-24 12:01 (p 1 of 3)
Test Code/ID: 24.003.3 Zn / 12-5207-5074

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	13-5720-1591	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 12:00	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:	26 Jun-24 11:56	**MD5 Hash:**	764B00A97DDBA7F034CD818B89CF20D9	**Editor ID:**	008-408-407-6
Batch ID:	21-1207-3831	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	29 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Okutua
Ending Date:	31 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	07-2399-9627	**Code:**	24.003.3 Zn	**Project:**	Special Studies
Sample Date:	28 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	28 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	109	235	160	---	0.1552	15.84%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		109	6	0.7077	2.466	0.2405	CDF	0.7430	Non-Significant Effect
		235*	6	3.817	2.466	0.2405	CDF	0.0051	Significant Effect
		485*	6	7.84	2.466	0.2405	CDF	2.1E-05	Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend Test	0.395	0.05	0.3950	Non-Significant Control Trend

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.24958	0.416528	3	23.35	7.8E-05	Significant Effect
Error	0.178402	0.0178402	10			
Total	1.42799		13			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.308	11.34	0.2301	Equal Variances
	Levene Equality of Variance Test	4.538	6.552	0.0296	Equal Variances
	Mod Levene Equality of Variance Test	0.5359	9.78	0.6746	Equal Variances
Distribution	Anderson-Darling A2 Test	0.4094	3.878	0.3493	Normal Distribution
	D'Agostino Skewness Test	0.2473	2.576	0.8047	Normal Distribution
	Kolmogorov-Smirnov D Test	0.181	0.262	0.2526	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9571	0.8239	0.6749	Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
4.15	D	5	0.9800	0.9245	1.0000	1.0000	0.9000	1.0000	0.0200	4.56%	0.00%
109		3	0.9333	0.6465	1.0000	1.0000	0.8000	1.0000	0.0667	12.37%	4.76%
235		3	0.7000	0.2697	1.0000	0.6000	0.6000	0.9000	0.1000	24.74%	28.57%
485		3	0.3333	0.1899	0.4768	0.3000	0.3000	0.4000	0.0333	17.32%	65.99%
1080		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
2300		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (008-408-407-6)

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	13-5720-1591	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 12:00	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:	26 Jun-24 11:56	**MD5 Hash:**	764B00A97DDBA7F034CD818B89CF20D9	**Editor ID:**	008-408-407-6

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
4.15	D	5	1.3790	1.2890	1.4700	1.4120	1.2490	1.4120	0.0326	5.28%	0.00%
109		3	1.3100	0.8731	1.7480	1.4120	1.1070	1.4120	0.1016	13.43%	5.00%
235		3	1.0070	0.4865	1.5280	0.8861	0.8861	1.2490	0.1210	20.81%	26.99%
485		3	0.6147	0.4640	0.7654	0.5796	0.5796	0.6847	0.0350	9.87%	55.44%
1080		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.49%
2300		3	0.1588	0.1588	0.1588	0.1588	0.1588	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		

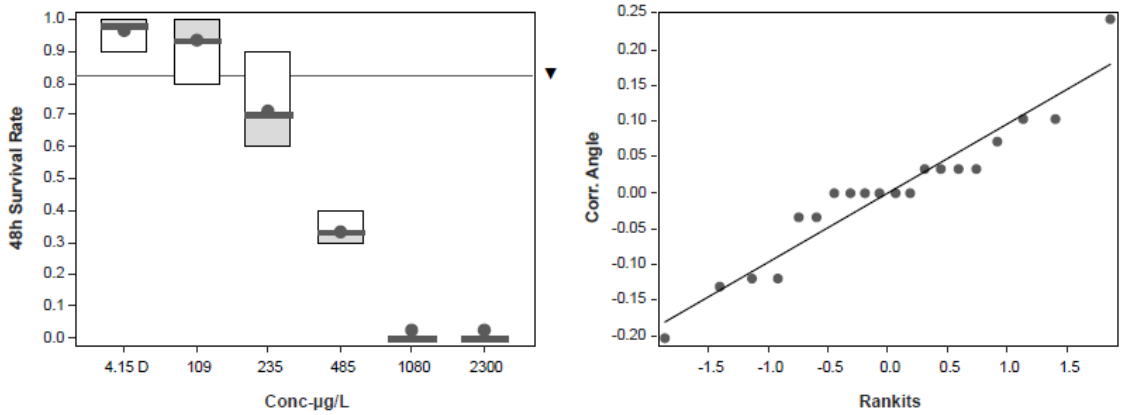
Angular (Corrected) Transformed Detail

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		0.1588	0.1588	0.1588		

48h Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
4.15	D	10/10	10/10	10/10	10/10	9/10
109		10/10	8/10	10/10		
235		6/10	6/10	9/10		
485		4/10	3/10	3/10		
1080		0/10	0/10	0/10		
2300		0/10	0/10	0/10		

Graphics



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (008-408-407-6)

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	15-2921-5324	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 12:00	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	26 Jun-24 11:56	**MD5 Hash:**	764B00A97DDBA7F034CD818B89CF20D9	**Editor ID:**	008-408-407-6
Batch ID:	21-1207-3831	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	29 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Okutua
Ending Date:	31 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	07-2399-9627	**Code:**	24.003.3 Zn	**Project:**	Special Studies
Sample Date:	28 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	28 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
7	-17.28	42.07	43.55	0.8442	3.99%	0.9766	Yes	0.8779	0.4761	Non-Significant Lack-of-Fit

Point Estimates

Level	µg/L	95% LCL	95% UCL
LC5	125.4	---	167.2
LC10	161.8	77.84	207.5
LC15	189.5	118	238.8
LC20	213.4	147.4	265.7
LC25	235.5	172.7	290.5
LC40	298.3	239.2	362.4
LC50	342.6	281.9	416.3

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	0.9766	0.01846	0.9376	1.016	52.89	<1.0E-05	Significant Parameter
γ	2.93	0.4877	1.901	3.959	6.008	1.4E-05	Significant Parameter
δ	342.6	31.78	275.5	409.6	10.78	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	2679	892.9	3	1072	<1.0E-05	Significant Effect
Lack of Fit	2.243	0.7476	3	0.8779	0.4761	Non-Significant Lack-of-Fit
Pure Error	11.92	0.8516	14			
Residual	14.17	0.8333	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	14.8	27.59	0.6102	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	14.17	27.59	0.6554	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	0.5301	3.687	0.7489	Equal Variances
Distribution	Anderson-Darling A2 Test	0.484	2.492	0.2325	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9468	0.9044	0.3214	Normal Distribution
Control Trend	Mann-Kendall Trend Test	0.395	0.05	0.3950	Non-Significant Control Trend
Overdispersion	Tarone C(α) Overdispersion Test	1.162	1.645	0.1226	Non-Significant Overdispersion

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	15-2921-5324	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 12:00	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	26 Jun-24 11:56	**MD5 Hash:**	764B00A97DDBA7F034CD818B89CF20D9	**Editor ID:**	008-408-407-6

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	Code	Count	Mean	Median	Min	Max	Std Err	Std Dev	CV%	%Effect	ΣA/ΣB
4.15	D	5	0.9800	1.0000	0.9000	1.0000	0.0200	0.0447	4.56%	0.00%	49/50
109		3	0.9333	1.0000	0.8000	1.0000	0.0667	0.1155	12.37%	4.76%	28/30
235		3	0.7000	0.6000	0.6000	0.9000	0.1000	0.1732	24.74%	28.57%	21/30
485		3	0.3333	0.3000	0.3000	0.4000	0.0333	0.0577	17.32%	65.99%	10/30
1080		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/30
2300		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
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		

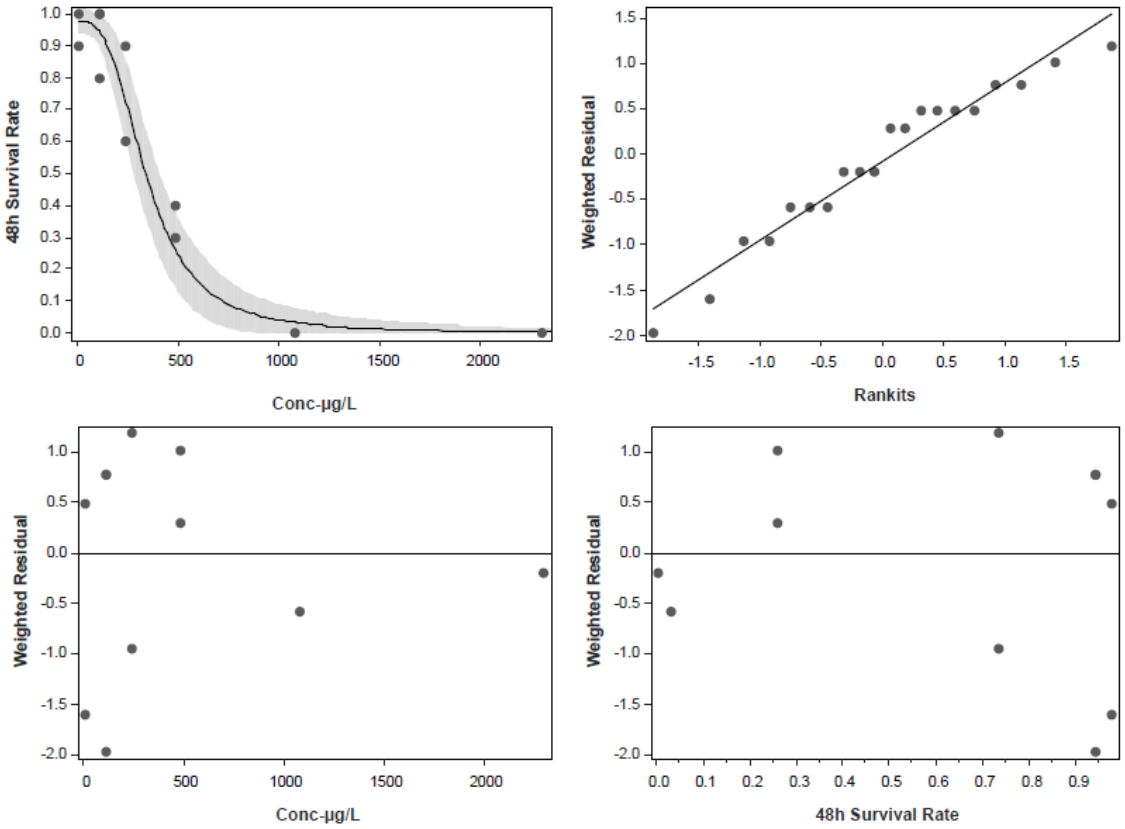
48h Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
4.15	D	10/10	10/10	10/10	10/10	9/10
109		10/10	8/10	10/10		
235		6/10	6/10	9/10		
485		4/10	3/10	3/10		
1080		0/10	0/10	0/10		
2300		0/10	0/10	0/10		

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	15-2921-5324	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 12:00	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	26 Jun-24 11:56	**MD5 Hash:**	764B00A97DDBA7F034CD818B89CF20D9	**Editor ID:**	008-408-407-6

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (008-408-407-6)

Copper

CETIS Analytical Report

Report Date: 26 Jun-24 11:48 (p 1 of 2)
Test Code/ID: 24.003.3 Cu / 16-2293-8617

Daphnia thomsoni 48-h Acute Survival Test — NIWA Ecotoxicology

Analysis ID:	08-9689-2974	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 11:47	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:	26 Jun-24 11:46	**MD5 Hash:**	733EADCD93E99B00DEDE9048F1D8118	**Editor ID:**	008-408-407-6
Batch ID:	01-3332-2169	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	29 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Okutua
Ending Date:	31 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	02-9373-9603	**Code:**	24.003.3 Cu	**Project:**	Special Studies
Sample Date:	28 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	28 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	59	136	89.58	---	0.1873	19.12%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		59	6	0.1781	2.306	0.2814	CDF	0.8631	Non-Significant Effect
		136*	6	8.566	2.306	0.2814	CDF	2.7E-05	Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Control Trend	Mann-Kendall Trend Test	0.395	0.05	0.3950	Non-Significant Control Trend

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.34796	1.17398	2	42.05	5.7E-05	Significant Effect
Error	0.223362	0.0279202	8			
Total	2.57132		10			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.512	9.21	0.0635	Equal Variances
	Levene Equality of Variance Test	8.878	8.649	0.0093	Unequal Variances
	Mod Levene Equality of Variance Test	0.9841	13.27	0.4361	Equal Variances
Distribution	Anderson-Darling A2 Test	0.906	3.878	0.0209	Normal Distribution
	D'Agostino Skewness Test	1.632	2.576	0.1026	Normal Distribution
	Kolmogorov-Smirnov D Test	0.2672	0.2906	0.0272	Normal Distribution
	Shapiro-Wilk W Normality Test	0.838	0.7725	0.0298	Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.3	D	5	0.9800	0.9245	1.0000	1.0000	0.9000	1.0000	0.0200	4.56%	0.00%
59		3	0.9667	0.8232	1.0000	1.0000	0.9000	1.0000	0.0333	5.97%	1.36%
136		3	0.1333	0.0000	0.7070	0.0000	0.0000	0.4000	0.1333	173.21%	86.39%
280		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
1.3	D	5	1.3790	1.2890	1.4700	1.4120	1.2490	1.4120	0.0326	5.28%	0.00%
59		3	1.3580	1.1240	1.5910	1.4120	1.2490	1.4120	0.0543	6.93%	1.58%
136		3	0.3341	-0.4202	1.0880	0.1588	0.1588	0.6847	0.1753	90.89%	75.78%
280		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.49%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (008-408-407-6)

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	08-9689-2974	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 11:47	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:	26 Jun-24 11:46	**MD5 Hash:**	733EADCD93E99B00DEDE9048F1D8118	**Editor ID:**	008-408-407-6

48h Survival Rate Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1.3	D	1.0000	1.0000	1.0000	1.0000	0.9000
59		1.0000	1.0000	0.9000		
136		0.0000	0.4000	0.0000		
280		0.0000	0.0000	0.0000		

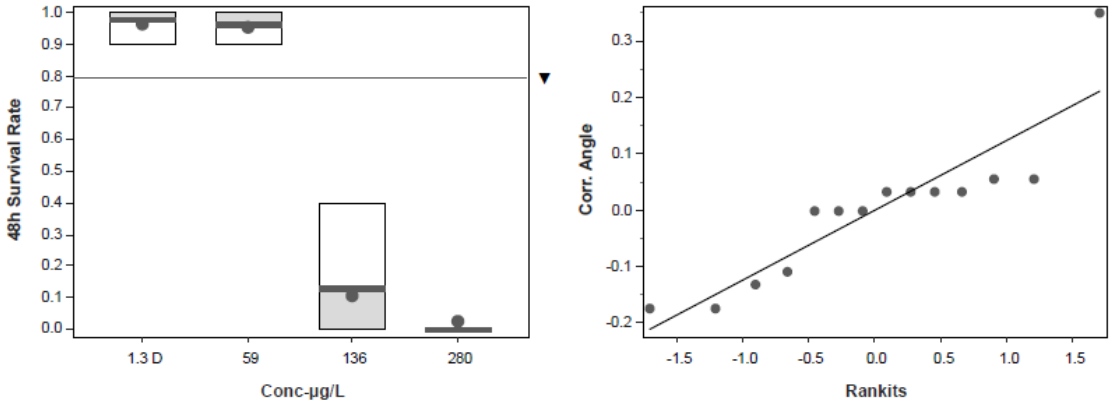
Angular (Corrected) Transformed Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1.3	D	1.4120	1.4120	1.4120	1.4120	1.2490
59		1.4120	1.4120	1.2490		
136		0.1588	0.6847	0.1588		
280		0.1588	0.1588	0.1588		

48h Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1.3	D	10/10	10/10	10/10	10/10	9/10
59		10/10	10/10	9/10		
136		0/10	4/10	0/10		
280		0/10	0/10	0/10		

Graphics



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (008-408-407-6)

CETIS Analytical Report

Report Date: 26 Jun-24 11:49 (p 1 of 3)
Test Code/ID: 24.003.3 Cu / 16-2293-8617

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	16-0302-2229	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 11:48	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	26 Jun-24 11:46	**MD5 Hash:**	2F6FF92B610D6BE0CFE8F75A74FEF8CB	**Editor ID:**	008-408-407-6
Batch ID:	01-3332-2169	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	29 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Okutua
Ending Date:	31 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	02-9373-9603	**Code:**	24.003.3 Cu	**Project:**	Special Studies
Sample Date:	28 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	28 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
3	-11.95	31.4	32.89	0.8992	2.19%	0.9908	Yes	0.2843	0.8359	Non-Significant Lack-of-Fit

Point Estimates

Level	μg/L	95% LCL	95% UCL
LC5	66.66	---	83.06
LC10	74.44	---	92.61
LC15	79.71	---	99.46
LC20	83.92	---	105.2
LC25	87.56	---	110.5
LC40	97.01	51.26	125.4
LC50	103	78.09	135.8

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	0.9908	0.0103	0.969	1.012	96.21	<1.0E-05	Significant Parameter
γ	6.766	2.09	2.358	11.18	3.238	0.0048	Significant Parameter
δ	103	10.65	80.52	125.5	9.667	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	12680	4228	3	3338	<1.0E-05	Significant Effect
Lack of Fit	1.236	0.4122	3	0.2843	0.8359	Non-Significant Lack-of-Fit
Pure Error	20.3	1.45	14			
Residual	21.53	1.267	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	17.34	27.59	0.4316	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	21.53	27.59	0.2034	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	0.5486	3.687	0.7365	Equal Variances
Distribution	Anderson-Darling A2 Test	2.284	2.492	<1.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.7972	0.9044	0.0008	Non-Normal Distribution
Control Trend	Mann-Kendall Trend Test	0.395	0.05	0.3950	Non-Significant Control Trend
Overdispersion	Tarone C(α) Overdispersion Test	2.681	1.645	0.0037	Significant Overdispersion

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (008-408-407-6)

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	16-0302-2229	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 11:48	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	26 Jun-24 11:46	**MD5 Hash:**	2F6FF92B610D6BE0CFE8F75A74FEF8CB	**Editor ID:**	008-408-407-6

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	Code	Count	Mean	Median	Min	Max	Std Err	Std Dev	CV%	%Effect	ΣA/ΣB
1.3	D	5	0.9800	1.0000	0.9000	1.0000	0.0200	0.0447	4.56%	0.00%	49/50
14		3	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.04%	30/30
28		3	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.04%	30/30
59		3	0.9667	1.0000	0.9000	1.0000	0.0333	0.0577	5.97%	1.36%	29/30
136		3	0.1333	0.0000	0.0000	0.4000	0.1333	0.2309	173.21%	86.39%	4/30
280		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
1.3	D	1.0000	1.0000	1.0000	1.0000	0.9000
14		1.0000	1.0000	1.0000		
28		1.0000	1.0000	1.0000		
59		1.0000	1.0000	0.9000		
136		0.0000	0.4000	0.0000		
280		0.0000	0.0000	0.0000		

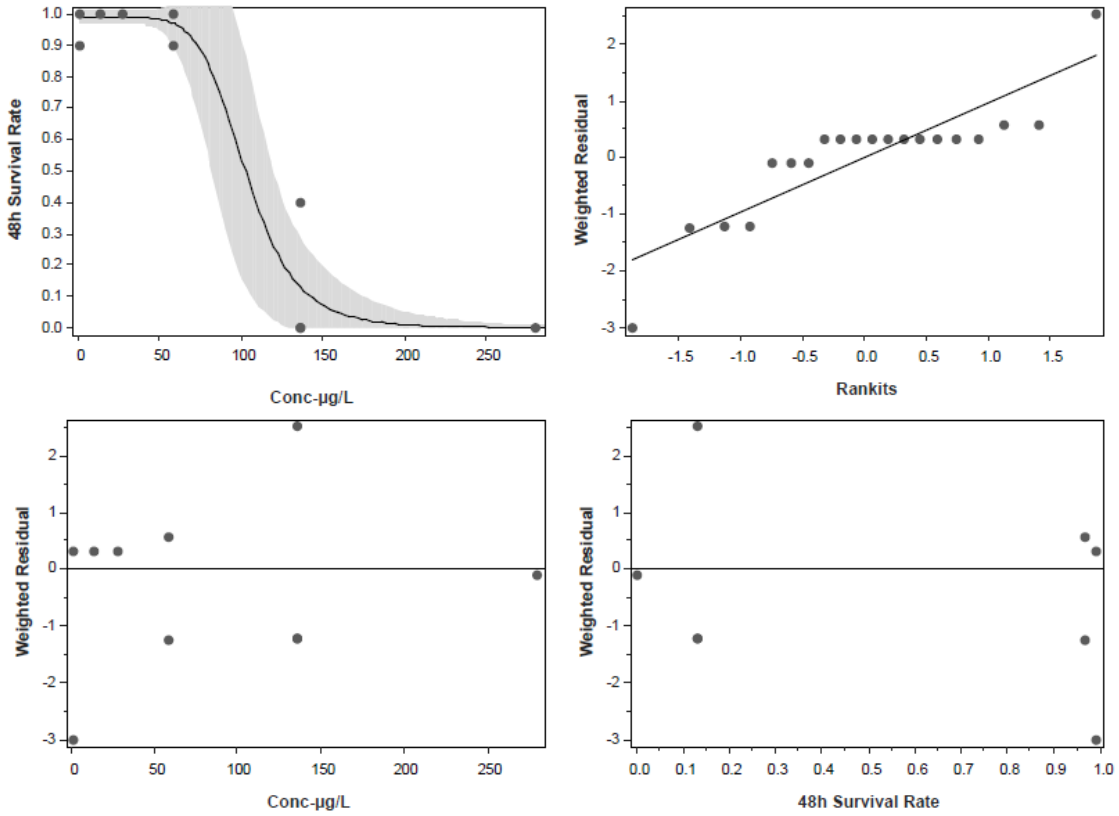
48h Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1.3	D	10/10	10/10	10/10	10/10	9/10
14		10/10	10/10	10/10		
28		10/10	10/10	10/10		
59		10/10	10/10	9/10		
136		0/10	4/10	0/10		
280		0/10	0/10	0/10		

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	16-0302-2229	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	26 Jun-24 11:48	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:	26 Jun-24 11:46	**MD5 Hash:**	2F6FF92B610D6BE0CFE8F75A74FEF8CB	**Editor ID:**	008-408-407-6

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (008-408-407-6)

Appendix I CETIS statistical analyses – Clutha

Zinc

CETIS Analytical Report

Report Date: 24 May-24 13:43 (p 1 of 2)
Test Code/ID: 24.003.4 Zn / 02-6719-5256

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	05-4485-8179	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	24 May-24 13:41	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	A16B6493E9BF68A2A853143B3283FDFA	**Editor ID:**	
Batch ID:	13-2989-6792	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	07 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Clutha river
Ending Date:	09 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	16-8019-3246	**Code:**	24.003.4 Zn	**Project:**	Special Studies
Sample Date:	06 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	06 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	225	505	337.1	---	0.1328	13.55%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		119	6	-0.3969	2.56	0.2103	CDF	1.0000	Non-Significant Effect
		225	6	2.163	2.56	0.2103	CDF	0.1028	Non-Significant Effect
		505*	6	6.824	2.56	0.2103	CDF	3.7E-05	Significant Effect
		1080*	6	12.49	2.56	0.2103	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.61111	0.652778	4	51.62	<1.0E-05	Significant Effect
Error	0.151764	0.012647	12			
Total	2.76287		16			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
	Levene Equality of Variance Test	3.283	5.412	0.0490	Equal Variances
	Mod Levene Equality of Variance Test	1.723	7.847	0.2485	Equal Variances
Distribution	Anderson-Darling A2 Test	0.8802	3.878	0.0242	Normal Distribution
	D'Agostino Skewness Test	0.05141	2.576	0.9590	Normal Distribution
	Kolmogorov-Smirnov D Test	0.2059	0.2405	0.0538	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9142	0.848	0.1178	Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
2.9	D	5	0.9800	0.9245	1.0000	1.0000	0.9000	1.0000	0.0200	4.56%	0.00%
119		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.04%
225		3	0.8667	0.7232	1.0000	0.9000	0.8000	0.9000	0.0333	6.66%	11.56%
505		3	0.5333	0.2465	0.8202	0.6000	0.4000	0.6000	0.0667	21.65%	45.58%
1080		3	0.1333	0.0000	0.5128	0.1000	0.0000	0.3000	0.0882	114.56%	86.39%
2400		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
2.9	D	5	1.3790	1.2890	1.4700	1.4120	1.2490	1.4120	0.0326	5.28%	0.00%
119		3	1.4120	1.4110	1.4130	1.4120	1.4120	1.4120	0.0000	0.00%	-2.36%
225		3	1.2020	0.9982	1.4050	1.2490	1.1070	1.2490	0.0473	6.82%	12.88%
505		3	0.8190	0.5302	1.1080	0.8861	0.6847	0.8861	0.0671	14.20%	40.63%
1080		3	0.3534	-0.1738	0.8805	0.3218	0.1588	0.5796	0.1225	60.05%	74.38%
2400		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.49%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	05-4485-8179	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	24 May-24 13:41	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	A16B6493E9BF68A2A853143B3283FDFA	**Editor ID:**	

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		

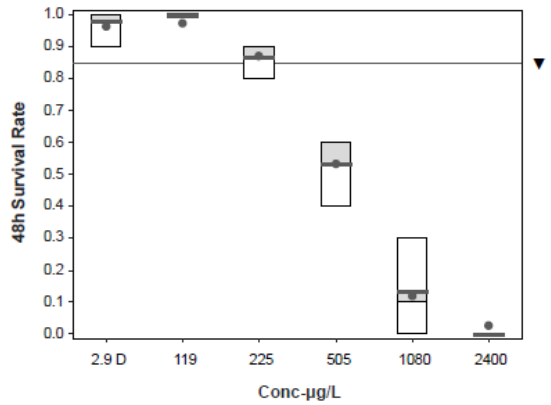
Angular (Corrected) Transformed Detail

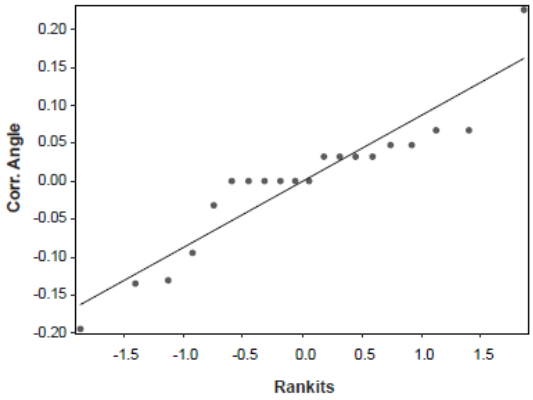
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
2.9	D	1.4120	1.4120	1.4120	1.2490	1.4120
119		1.4120	1.4120	1.4120		
225		1.2490	1.2490	1.1070		
505		0.8861	0.8861	0.6847		
1080		0.1588	0.3218	0.5796		
2400		0.1588	0.1588	0.1588		

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





Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	16-3085-5703	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	24 May-24 13:41	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	A16B6493E9BF68A2A853143B3283FDFA	**Editor ID:**	
Batch ID:	13-2989-6792	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	07 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Clutha river
Ending Date:	09 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	16-8019-3246	**Code:**	24.003.4 Zn	**Project:**	Special Studies
Sample Date:	06 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	06 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
10	-16.95	41.4	42.89	0.8282	3.01%	0.9855	Yes	0.7813	0.5238	Non-Significant Lack-of-Fit

Point Estimates

Level	μg/L	95% LCL	95% UCL
LC5	186.5	---	246.8
LC10	242.7	124.4	311.2
LC15	285.7	180.5	360.3
LC20	323	224.3	402.6
LC25	357.4	262.8	441.6
LC40	456.3	365.5	555.4
LC50	526.4	432.1	641.3

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	0.9855	0.01405	0.9559	1.015	70.13	<1.0E-05	Significant Parameter
γ	2.838	0.4606	1.866	3.81	6.161	1.0E-05	Significant Parameter
δ	526.4	49.1	422.8	630	10.72	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	4741	1580	3	1903	<1.0E-05	Significant Effect
Lack of Fit	2.024	0.6748	3	0.7813	0.5238	Non-Significant Lack-of-Fit
Pure Error	12.09	0.8636	14			
Residual	14.11	0.8303	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	12.98	27.59	0.7374	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	14.11	27.59	0.6590	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	0.6852	3.687	0.6481	Equal Variances
Distribution	Anderson-Darling A2 Test	0.898	2.492	0.0219	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.9137	0.9044	0.0752	Normal Distribution
Overdispersion	Tarone C(α) Overdispersion Test	1.043	1.645	0.1485	Non-Significant Overdispersion

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	16-3085-5703	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	24 May-24 13:41	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	A16B6493E9BF68A2A853143B3283FDFA	**Editor ID:**	

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	Code	Count	Mean	Median	Min	Max	Std Err	Std Dev	CV%	%Effect	ΣA/ΣB
2.9	D	5	0.9800	1.0000	0.9000	1.0000	0.0200	0.0447	4.56%	0.00%	49/50
119		3	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.04%	30/30
225		3	0.8667	0.9000	0.8000	0.9000	0.0333	0.0577	6.66%	11.56%	26/30
505		3	0.5333	0.6000	0.4000	0.6000	0.0667	0.1155	21.65%	45.58%	16/30
1080		3	0.1333	0.1000	0.0000	0.3000	0.0882	0.1528	114.56%	86.39%	4/30
2400		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
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		

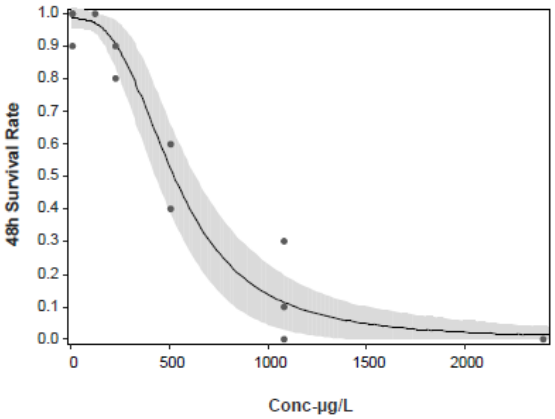
48h Survival Rate Binomials

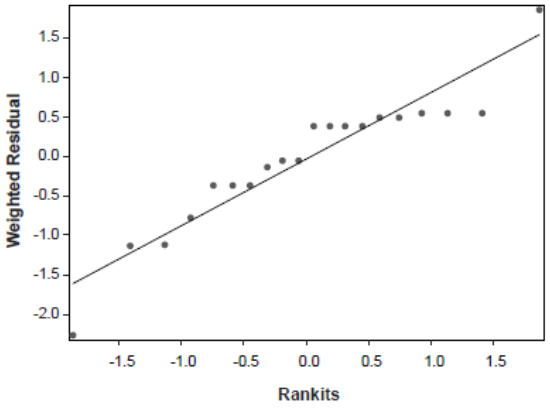
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
2.9	D	10/10	10/10	10/10	9/10	10/10
119		10/10	10/10	10/10		
225		9/10	9/10	8/10		
505		6/10	6/10	4/10		
1080		0/10	1/10	3/10		
2400		0/10	0/10	0/10		

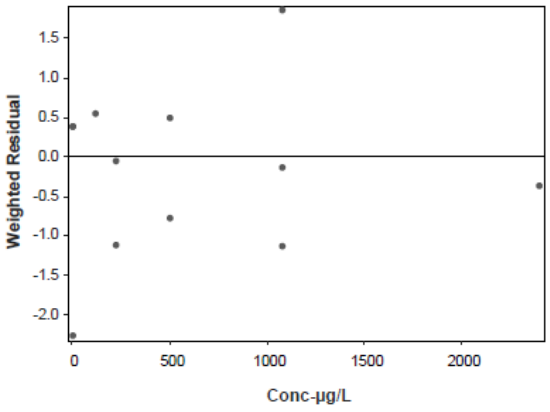
Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

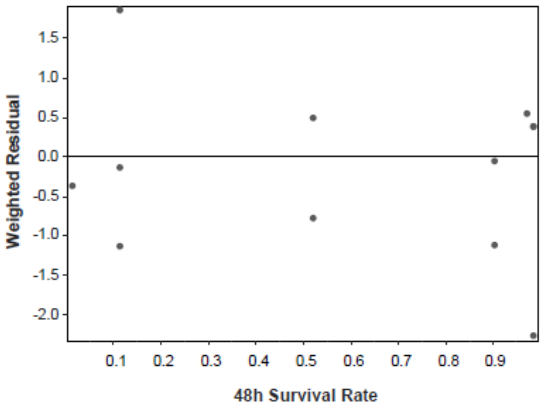
Analysis ID:	16-3085-5703	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	24 May-24 13:41	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	A16B6493E9BF68A2A853143B3283FDFA	**Editor ID:**	

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]









Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Copper

CETIS Analytical Report

Report Date: 27 May-24 13:55 (p 1 of 3)
Test Code/ID: 24.003.4 Cu / 06-7607-7132

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	14-2862-3054	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:55	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	68560BC405F85A2458FD72874CB5B6A9	**Editor ID:**	
Batch ID:	17-3956-0696	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	02 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Clutha river
Ending Date:	04 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	10-6877-4232	**Code:**	24.003.4 Cu	**Project:**	Special Studies
Sample Date:	01 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	01 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	22.5	50.5	33.71	---	0.1229	12.54%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		2.2	6	0.2906	2.624	0.1962	CDF	1.0000	Non-Significant Effect
		4.4	6	-0.436	2.624	0.1962	CDF	1.0000	Non-Significant Effect
		10.3	6	-0.436	2.624	0.1962	CDF	1.0000	Non-Significant Effect
		22.5	6	2.167	2.624	0.1962	CDF	0.1199	Non-Significant Effect
		50.5*	6	14.87	2.624	0.1962	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.11472	0.622944	5	59.44	<1.0E-05	Significant Effect
Error	0.146723	0.0104802	14			
Total	3.26144		19			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test				Indeterminate
	Levene Equality of Variance Test	3.979	4.695	0.0187	Equal Variances
	Mod Levene Equality of Variance Test	1.966	6.632	0.1886	Equal Variances
Distribution	Anderson-Darling A2 Test	1.608	3.878	1.2E-05	Non-Normal Distribution
	D'Agostino Kurtosis Test	1.78	2.576	0.0751	Normal Distribution
	D'Agostino Skewness Test	1.407	2.576	0.1594	Normal Distribution
	D'Agostino-Pearson K2 Omnibus Test	5.148	9.21	0.0762	Normal Distribution
	Kolmogorov-Smirnov D Test	0.3	0.2235	5.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.86	0.866	0.0079	Non-Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.25	D	5	0.9800	0.9245	1.0000	1.0000	0.9000	1.0000	0.0200	4.56%	0.00%
2.2		3	0.9667	0.8232	1.0000	1.0000	0.9000	1.0000	0.0333	5.97%	1.36%
4.4		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.04%
10.3		3	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.04%
22.5		3	0.8667	0.4872	1.0000	0.9000	0.7000	1.0000	0.0882	17.63%	11.56%
50.5		3	0.0667	0.0000	0.2101	0.1000	0.0000	0.1000	0.0333	86.60%	93.20%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	14-2862-3054	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:55	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	68560BC405F85A2458FD72874CB5B6A9	**Editor ID:**	

Angular (Corrected) Transformed Summary

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%
22.5		3	1.2170	0.6903	1.7450	1.2490	0.9912	1.4120	0.1225	17.43%	11.75%
50.5		3	0.2674	0.0337	0.5012	0.3218	0.1588	0.3218	0.0543	35.18%	80.61%

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		

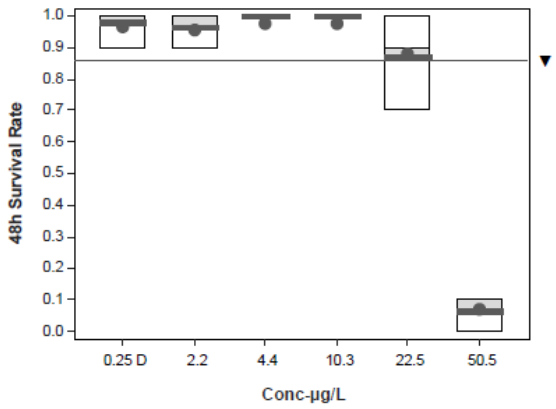
Angular (Corrected) Transformed Detail

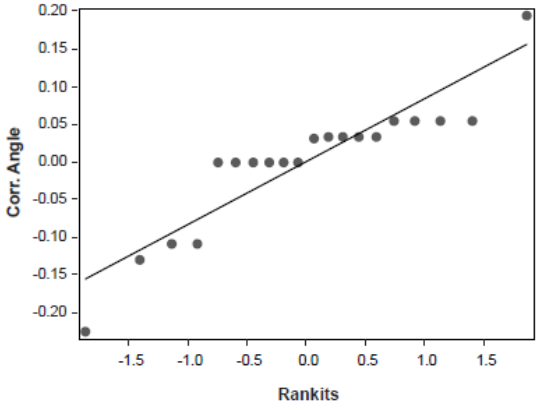
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0.25	D	1.4120	1.4120	1.4120	1.2490	1.4120
2.2		1.4120	1.2490	1.4120		
4.4		1.4120	1.4120	1.4120		
10.3		1.4120	1.4120	1.4120		
22.5		0.9912	1.4120	1.2490		
50.5		0.3218	0.3218	0.1588		

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





Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

CETIS Analytical Report

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	15-7079-1881	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:55	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	68560BC405F85A2458FD72874CB5B6A9	**Editor ID:**	

Batch ID:	17-3956-0696	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	02 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Clutha river
Ending Date:	04 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**

Sample ID:	10-6877-4232	**Code:**	24.003.4 Cu	**Project:**	Special Studies
Sample Date:	01 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	01 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
5	-13.36	34.21	35.7	0.8309	2.21%	0.9856	Yes	0.5387	0.6635	Non-Significant Lack-of-Fit

Point Estimates

Level	µg/L	95% LCL	95% UCL
LC5	19.16	---	22.77
LC10	21.81	---	25.62
LC15	23.63	---	27.65
LC20	25.1	16.13	29.34
LC25	26.38	19	30.87
LC40	29.75	23.91	35.29
LC50	31.92	26.38	38.62

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	0.9856	0.01032	0.9639	1.007	95.47	<1.0E-05	Significant Parameter
γ	5.769	1.197	3.244	8.294	4.82	0.0002	Significant Parameter
δ	31.92	2.638	26.35	37.48	12.1	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	9621	3207	3	3137	<1.0E-05	Significant Effect
Lack of Fit	1.799	0.5995	3	0.5387	0.6635	Non-Significant Lack-of-Fit
Pure Error	15.58	1.113	14			
Residual	17.38	1.022	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	14.59	27.59	0.6251	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	17.38	27.59	0.4290	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	0.5962	3.687	0.7051	Equal Variances
Distribution	Anderson-Darling A2 Test	4.186	2.492	<1.0E-05	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.6397	0.9044	<1.0E-05	Non-Normal Distribution
Overdispersion	Tarone C(α) Overdispersion Test	0.8299	1.645	0.2033	Non-Significant Overdispersion

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	15-7079-1881	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:55	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	68560BC405F85A2458FD72874CB5B6A9	**Editor ID:**	

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	Code	Count	Mean	Median	Min	Max	Std Err	Std Dev	CV%	%Effect	ΣA/ΣB
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		

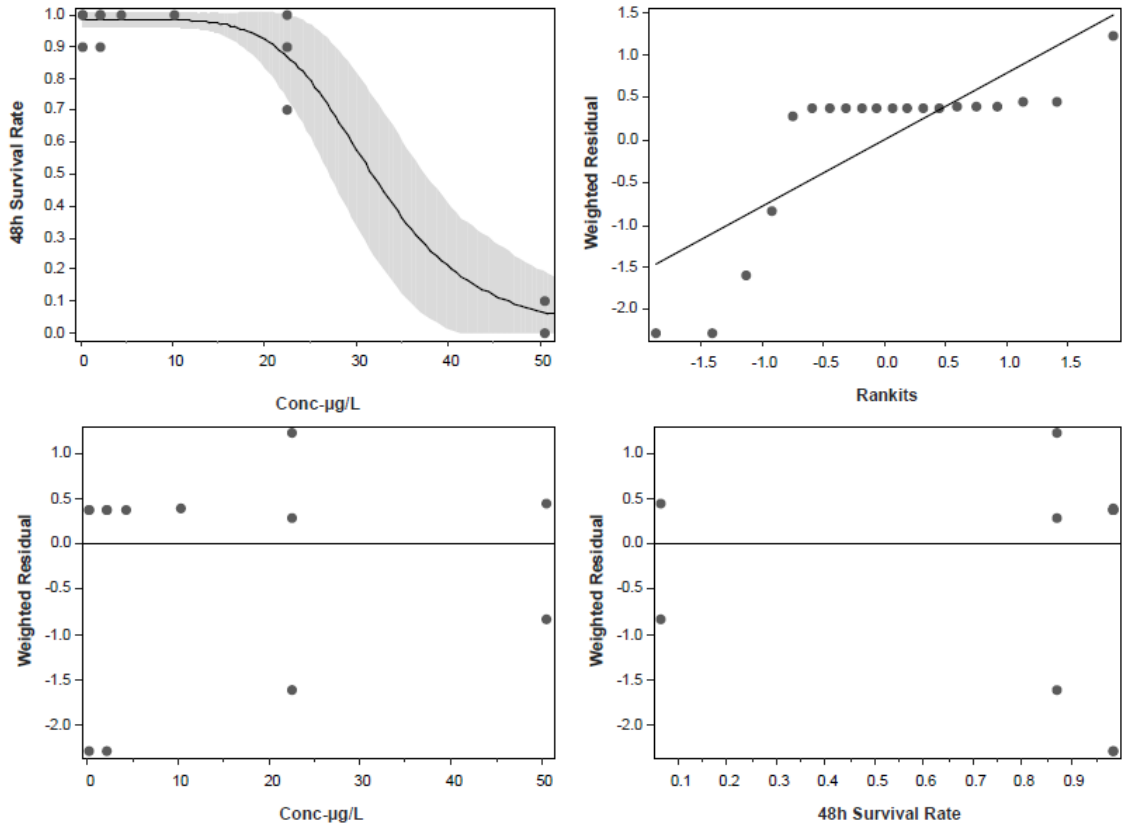
48h Survival Rate Binomials

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		

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	15-7079-1881	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:55	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	68560BC405F85A2458FD72874CB5B6A9	**Editor ID:**	

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Appendix J CETIS statistical analyses – Waihou

Zinc

CETIS Analytical Report

Report Date: 27 May-24 13:35 (p 1 of 2)
Test Code/ID: 24.003.5 Zn / 16-7680-7646

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	20-4181-6750	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:34	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	1A253C1D48BDA445832ED5DA447D9A7B	**Editor ID:**	

Batch ID:	11-2219-0889	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	07 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Waihou
Ending Date:	09 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**

Sample ID:	06-1101-1726	**Code:**	24.003.5 Zn	**Project:**	Special Studies
Sample Date:	06 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	06 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	116	230	163.3	---	0.1242	12.93%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		116	6	0.5547	2.466	0.1932	CDF	0.8869	Non-Significant Effect
		230*	6	3.443	2.466	0.1932	CDF	0.0095	Significant Effect
		500*	6	8.47	2.466	0.1932	CDF	1.1E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.931589	0.31053	3	26.98	4.1E-05	Significant Effect
Error	0.115087	0.0115087	10			
Total	1.04668		13			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	0.7195	11.34	0.8686	Equal Variances
	Levene Equality of Variance Test	0.8939	6.552	0.4776	Equal Variances
	Mod Levene Equality of Variance Test	0.09641	9.78	0.9592	Equal Variances
Distribution	Anderson-Darling A2 Test	0.8152	3.878	0.0349	Normal Distribution
	D'Agostino Skewness Test	0.6961	2.576	0.4864	Normal Distribution
	Kolmogorov-Smirnov D Test	0.2182	0.262	0.0696	Normal Distribution
	Shapiro-Wilk W Normality Test	0.8807	0.8239	0.0595	Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
3.25	D	5	0.9600	0.8920	1.0000	1.0000	0.9000	1.0000	0.0245	5.71%	0.00%
116		3	0.9333	0.7899	1.0000	0.9000	0.9000	1.0000	0.0333	6.19%	2.78%
230		3	0.7667	0.4798	1.0000	0.7000	0.7000	0.9000	0.0667	15.06%	20.14%
500		3	0.4000	0.1516	0.6484	0.4000	0.3000	0.5000	0.0577	25.00%	58.33%
1110		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
2450		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
3.25	D	5	1.3470	1.2360	1.4580	1.4120	1.2490	1.4120	0.0399	6.63%	0.00%
116		3	1.3030	1.0700	1.5370	1.2490	1.2490	1.4120	0.0543	7.22%	3.23%
230		3	1.0770	0.7073	1.4470	0.9912	0.9912	1.2490	0.0860	13.82%	20.03%
500		3	0.6833	0.4277	0.9388	0.6847	0.5796	0.7854	0.0594	15.06%	49.27%
1110		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.21%
2450		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.21%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	20-4181-6750	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:34	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	1A253C1D48BDA445832ED5DA447D9A7B	**Editor ID:**	

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		

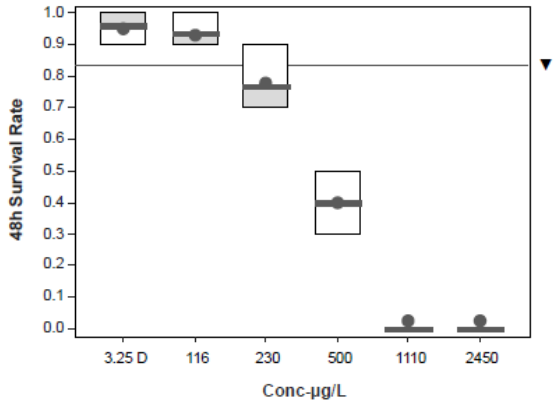
Angular (Corrected) Transformed Detail

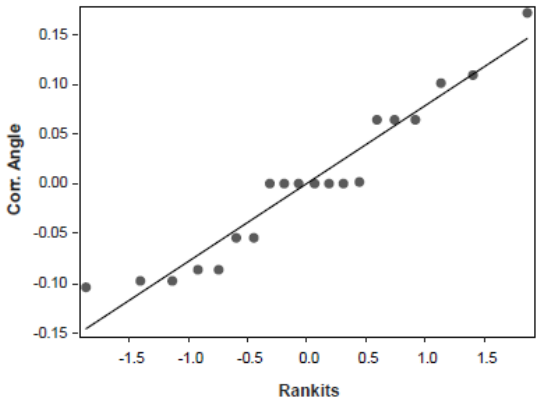
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
3.25	D	1.4120	1.2490	1.4120	1.2490	1.4120
116		1.2490	1.4120	1.2490		
230		1.2490	0.9912	0.9912		
500		0.7854	0.6847	0.5796		
1110		0.1588	0.1588	0.1588		
2450		0.1588	0.1588	0.1588		

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		

Graphics





Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

CETIS Analytical Report

Report Date: 27 May-24 13:36 (p 1 of 3)
Test Code/ID: 24.003.5 Zn / 16-7680-7646

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	02-3974-0228	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:35	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	1A253C1D48BDA445832ED5DA447D9A7B	**Editor ID:**	

Batch ID:	11-2219-0889	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	07 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Waihou
Ending Date:	09 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**

Sample ID:	06-1101-1726	**Code:**	24.003.5 Zn	**Project:**	Special Studies
Sample Date:	06 May-24	**Material:**	Zinc sulfate	**Source:**	Solution made by NIWA
Receipt Date:	06 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
9	-17.38	42.26	43.75	0.8533	4.30%	0.9529	Yes	1.949	0.1682	Non-Significant Lack-of-Fit

Point Estimates

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
LC50	403.7	344	473.8

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	0.9529	0.01943	0.9119	0.9939	49.05	<1.0E-05	Significant Parameter
γ	3.22	0.484	2.199	4.241	6.653	<1.0E-05	Significant Parameter
δ	403.7	31.4	337.5	470	12.86	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1601	533.7	3	983.6	<1.0E-05	Significant Effect
Lack of Fit	2.717	0.9058	3	1.949	0.1682	Non-Significant Lack-of-Fit
Pure Error	6.507	0.4648	14			
Residual	9.224	0.5426	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	11.78	27.59	0.8134	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	9.224	27.59	0.9330	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	0.5622	3.687	0.7275	Equal Variances
Distribution	Anderson-Darling A2 Test	0.9658	2.492	0.0150	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.9004	0.9044	0.0420	Non-Normal Distribution
Overdispersion	Tarone C(α) Overdispersion Test	0.9324	1.645	0.1756	Non-Significant Overdispersion

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	02-3974-0228	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:35	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	1A253C1D48BDA445832ED5DA447D9A7B	**Editor ID:**	

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	Code	Count	Mean	Median	Min	Max	Std Err	Std Dev	CV%	%Effect	ΣA/ΣB
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		

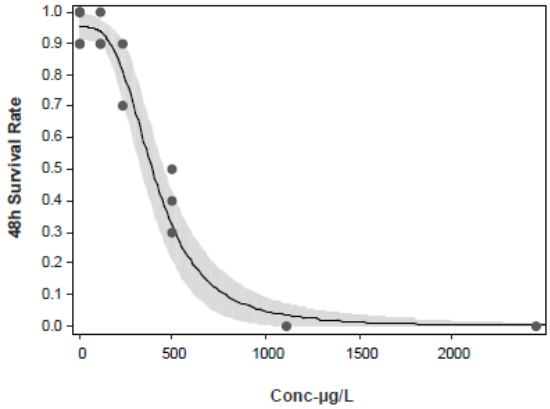
48h Survival Rate Binomials

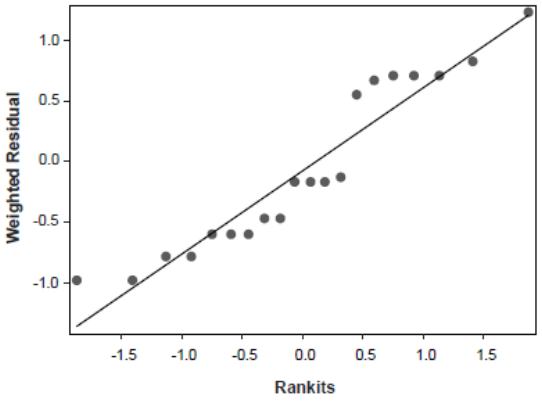
Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
3.25	D	10/10	9/10	10/10	9/10	10/10
116		9/10	10/10	9/10		
230		9/10	7/10	7/10		
500		5/10	4/10	3/10		
1110		0/10	0/10	0/10		
2450		0/10	0/10	0/10		

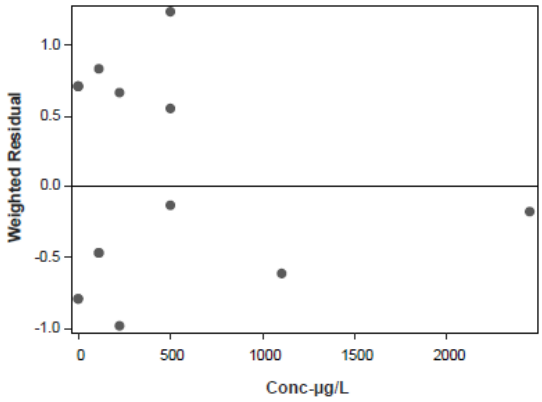
Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

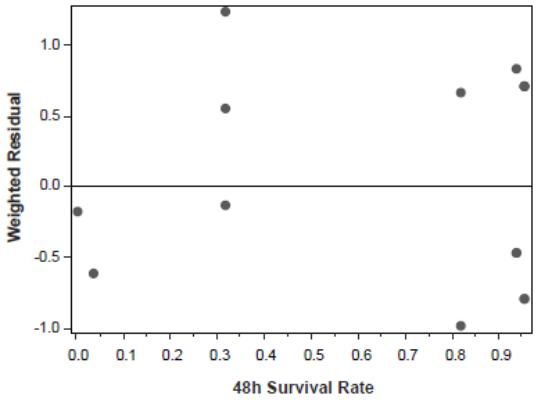
Analysis ID:	02-3974-0228	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 13:35	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	1A253C1D48BDA445832ED5DA447D9A7B	**Editor ID:**	

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]









Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Copper

CETIS Analytical Report

Report Date: 27 May-24 14:02 (p 1 of 2)
Test Code/ID: 24.003.5 Cu / 15-1536-4540

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	18-8837-2322	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:01	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	0B07C01911269DEBFC612C1A53753B59	**Editor ID:**	
Batch ID:	20-8394-3851	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	04 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Waihou
Ending Date:	06 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	02-5559-2325	**Code:**	24.003.5 Cu	**Project:**	Special Studies
Sample Date:	03 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	03 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	21.1	48	31.82	---	0.2037	21.22%

Bonferroni Adj t Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		9.75	6	-0.09164	2.466	0.2924	CDF	1.0000	Non-Significant Effect
		21.1	6	1.387	2.466	0.2924	CDF	0.2934	Non-Significant Effect
		48*	6	5.64	2.466	0.2924	CDF	0.0003	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.988236	0.329412	3	12.5	0.0010	Significant Effect
Error	0.263566	0.0263566	10			
Total	1.2518		13			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.722	11.34	0.2930	Equal Variances
	Levene Equality of Variance Test	3.001	6.552	0.0817	Equal Variances
	Mod Levene Equality of Variance Test	0.9186	9.78	0.4865	Equal Variances
Distribution	Anderson-Darling A2 Test	0.9171	3.878	0.0197	Normal Distribution
	D'Agostino Skewness Test	1.231	2.576	0.2184	Normal Distribution
	Kolmogorov-Smirnov D Test	0.2914	0.262	0.0021	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.8995	0.8239	0.1110	Normal Distribution

48h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.8	D	5	0.9600	0.8920	1.0000	1.0000	0.9000	1.0000	0.0245	5.71%	0.00%
9.75		3	0.9667	0.8232	1.0000	1.0000	0.9000	1.0000	0.0333	5.97%	-0.69%
21.1		3	0.8333	0.3162	1.0000	0.9000	0.6000	1.0000	0.1202	24.98%	13.19%
48		3	0.4000	0.0000	0.8303	0.5000	0.2000	0.5000	0.1000	43.30%	58.33%
97		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
211		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0.8	D	5	1.3470	1.2360	1.4580	1.4120	1.2490	1.4120	0.0399	6.63%	0.00%
9.75		3	1.3580	1.1240	1.5910	1.4120	1.2490	1.4120	0.0543	6.93%	-0.81%
21.1		3	1.1820	0.5136	1.8510	1.2490	0.8861	1.4120	0.1554	22.77%	12.21%
48		3	0.6781	0.2167	1.1400	0.7854	0.4636	0.7854	0.1073	27.39%	49.65%
97		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.21%
211		3	0.1588	0.1588	0.1588	0.1588	0.1588	0.1588	0.0000	0.00%	88.21%

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

Analysis ID:	18-8837-2322	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:01	**Analysis:**	Parametric-Multiple Comparison	**Status Level:**	1
Edit Date:		**MD5 Hash:**	0B07C01911269DEBFC612C1A53753B59	**Editor ID:**	

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		0.0000	0.0000	0.0000		
211		0.0000	0.0000	0.0000		

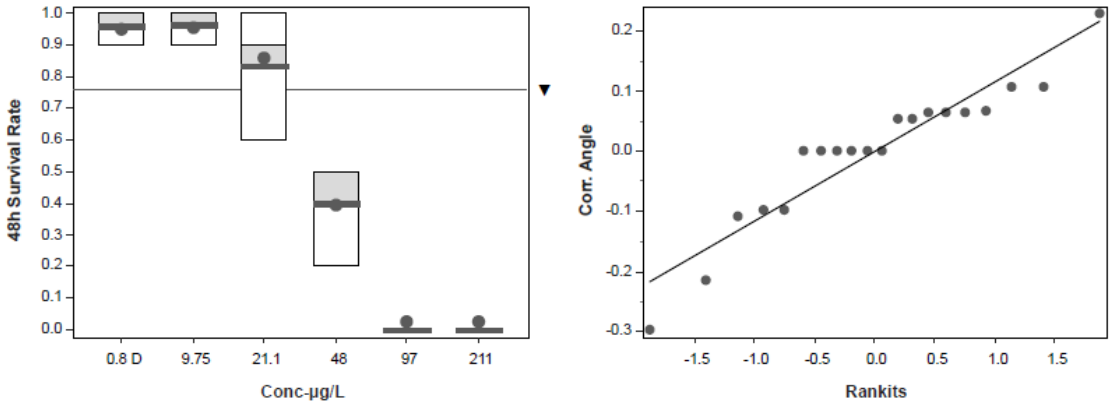
Angular (Corrected) Transformed Detail

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		0.1588	0.1588	0.1588		
211		0.1588	0.1588	0.1588		

48h Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0.8	D	10/10	9/10	10/10	9/10	10/10
9.75		10/10	9/10	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



Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

CETIS Analytical Report

Report Date: 27 May-24 14:04 (p 1 of 3)
Test Code/ID: 24.003.5 Cu / 15-1536-4540

Daphnia thomsoni 48-h Acute Survival Test — **NIWA Ecotoxicology**

Analysis ID:	15-5899-0665	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:02	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	0B07C01911269DEBFC612C1A53753B59	**Editor ID:**	
Batch ID:	20-8394-3851	**Test Type:**	Survival (48h)	**Analyst:**	Ecotox Team
Start Date:	04 May-24	**Protocol:**	NIWA SOP 10 (2022)	**Diluent:**	Waihou
Ending Date:	06 May-24	**Species:**	Daphnia thomsoni (Water flea)	**Brine:**	Not Applicable
Test Length:	48h	**Taxon:**		**Source:**	Field Collected **Age:**
Sample ID:	02-5559-2325	**Code:**	24.003.5 Cu	**Project:**	Special Studies
Sample Date:	03 May-24	**Material:**	Copper	**Source:**	Solution made by NIWA
Receipt Date:	03 May-24	**CAS (PC):**		**Station:**	Lab Solution
Sample Age:	24h	**Client:**	Hydrotoxy Research		

Non-Linear Regression Options

Model Name and Function	Weighting Function	PTBS Function	X Trans	Y Trans
3P Log-Logistic: μ=α/[1+[x/δ]^γ]	Binomial [ω=n/[p·q]]	Off [μ*=μ]	None	None

Regression Summary

Iters	LL	AICc	BIC	Adj R2	PMSD	Thresh	Optimize	F Stat	P-Value	Decision(α:5%)
10	-18.87	45.23	46.72	0.8551	5.32%	0.9576	Yes	0.7865	0.5212	Non-Significant Lack-of-Fit

Point Estimates

Level	µg/L	95% LCL	95% UCL
LC5	18.78	---	24.99
LC10	22.83	---	29.64
LC15	25.78	---	33.09
LC20	28.24	13.19	35.97
LC25	30.45	18.71	38.55
LC40	36.51	27.81	45.61
LC50	40.6	32.61	50.55

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
α	0.9576	0.02412	0.9067	1.008	39.69	<1.0E-05	Significant Parameter
γ	3.818	0.8905	1.939	5.697	4.287	0.0005	Significant Parameter
δ	40.6	4.128	31.89	49.31	9.836	<1.0E-05	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1989	662.9	3	615.5	<1.0E-05	Significant Effect
Lack of Fit	2.641	0.8803	3	0.7865	0.5212	Non-Significant Lack-of-Fit
Pure Error	15.67	1.119	14			
Residual	18.31	1.077	17			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Model Fit	Likelihood Ratio GOF Test	19.38	27.59	0.3073	Non-Significant Heterogeneity
	Pearson Chi-Sq GOF Test	18.31	27.59	0.3695	Non-Significant Heterogeneity
Variance	Mod Levene Equality of Variance	1.144	3.687	0.4114	Equal Variances
Distribution	Anderson-Darling A2 Test	0.7253	2.492	0.0584	Normal Distribution
	Shapiro-Wilk W Normality Test	0.893	0.9044	0.0305	Non-Normal Distribution
Overdispersion	Tarone C(α) Overdispersion Test	1.394	1.645	0.0816	Non-Significant Overdispersion

Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Daphnia thomsoni 48-h Acute Survival Test **NIWA Ecotoxicology**

Analysis ID:	15-5899-0665	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:02	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	0B07C01911269DEBFC612C1A53753B59	**Editor ID:**	

48h Survival Rate Summary

			Calculated Variate(A/B)								
Conc-µg/L	**Code**	**Count**	**Mean**	**Median**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**ΣA/ΣB**
0.8	D	5	0.9600	1.0000	0.9000	1.0000	0.0245	0.0548	5.71%	0.00%	48/50
9.75		3	0.9667	1.0000	0.9000	1.0000	0.0333	0.0577	5.97%	-0.69%	29/30
21.1		3	0.8333	0.9000	0.6000	1.0000	0.1202	0.2082	24.98%	13.19%	25/30
48		3	0.4000	0.5000	0.2000	0.5000	0.1000	0.1732	43.30%	58.33%	12/30
97		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%	0/30
211		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**
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		0.0000	0.0000	0.0000		
211		0.0000	0.0000	0.0000		

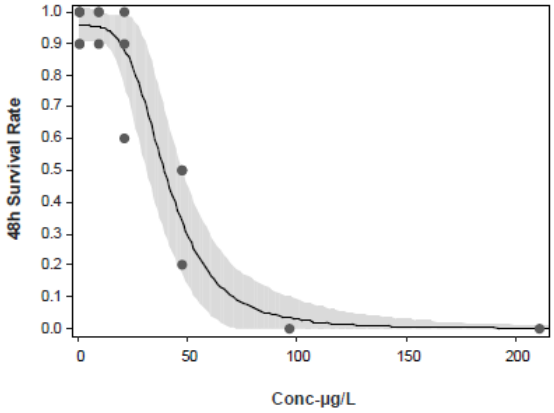
48h Survival Rate Binomials

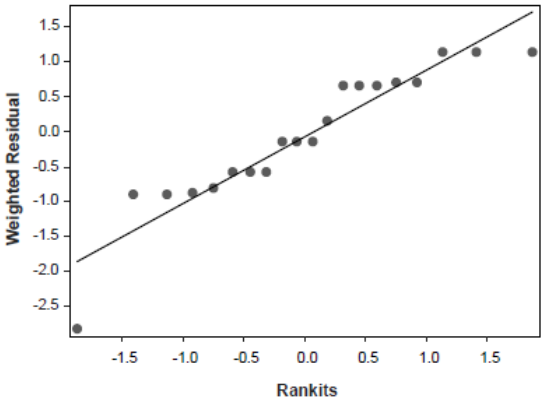
Conc-µg/L	**Code**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**
0.8	D	10/10	9/10	10/10	9/10	10/10
9.75		10/10	9/10	10/10		
21.1		10/10	9/10	6/10		
48		5/10	2/10	5/10		
97		0/10	0/10	0/10		
211		0/10	0/10	0/10		

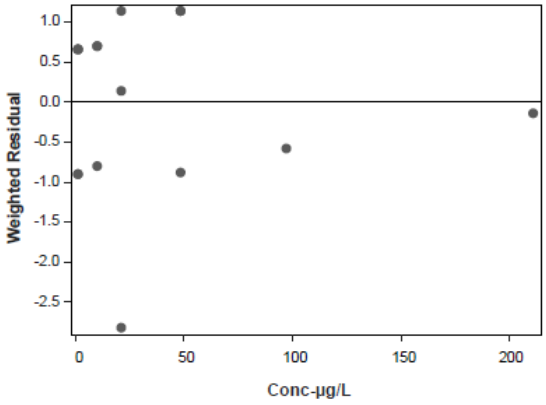
Daphnia thomsoni 48-h Acute Survival Test NIWA Ecotoxicology

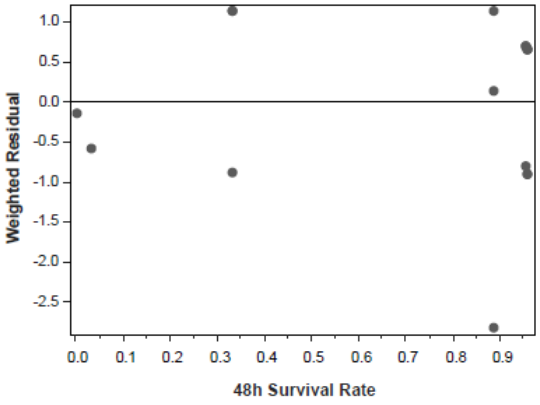
Analysis ID:	15-5899-0665	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.4
Analyzed:	27 May-24 14:02	**Analysis:**	Nonlinear Regression (NLR)	**Status Level:**	1
Edit Date:		**MD5 Hash:**	0B07C01911269DEBFC612C1A53753B59	**Editor ID:**	

Graphics Model: 3P Log-Logistic: μ=α/[1+[x/δ]^γ] Distribution: Binomial [ω=n/[p·q]]









Convergent Rounding (4 sf) CETIS™ v2.1.4.5 (009-951-268-0) Analyst:_____ QA:_____

Appendix K Physico-chemical data for zinc tests

Table K-1: Summary of physico-chemical measures from acute *D. thomsoni* zinc toxicity testing with Mahurangi Stream 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 concentration (µg/L Zn^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{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 concentration (µg/L Zn^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{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 pH adjusted Okutua Creek 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 (µg/L Zn^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{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 concentration (µg/L Zn^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}
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 with Clutha 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 (µg/L Zn^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}
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 with Waihou 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 (µg/L Zn^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{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 with Mahurangi Stream 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 (µg/L Cu^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{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 with Hoteo 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 (µg/L Cu^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{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 pH adjusted Okutua Creek 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 (µg/L Cu^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}
0 Control	6.0	6.5	8.4	8.8	32.0	34.6	19	20

Nominal concentration (µg/L Cu^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}
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 with Clutha 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 (µg/L Cu^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}
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 with Waihou 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 (µg/L Cu^{2+})	pH		Dissolved Oxygen (mg/L)		Conductivity (µS/cm)		Temperature (°C)	
	T_0	T_{48}	T_0	T_{48}	T_0	T_{48}	T_0	T_{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 zinc toxicity testing data. Shaded values are nominal concentrations. na = not available.

Notes incl. source	Duration	Temp. °C	pH mean (min.-max.)	Hardness mg/L $CaCO_3$	DOC mg/L	EC_{50}[a] (95% CL or ± 2 SD) µg/L Zn^{2+}
Fish - Smelt - *Retropinna retropinna*						
Juvenile wet weight 1.8 g; Head-tail base length 57 mm. NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not.	96 h	15	7.4-7.7	≈ 40	<0.5	5,601 n=1
Fish - Common bully - *Gobiomorphus cotidianus*						
NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not.	96 h	15	7.4-7.7	≈ 40	<0.5	3,060 (684-5,435) n=12
Juvenile wet weight 0.45 g; Head-tail base length 32 mm. Ouwerkerk (2017)	96 h	16	7.24 (6.93-7.54)	14.2	0.3[a]	1,868 (1,581-2,207) n=1
Juvenile wet weight 0.75 g; Head-tail base length 37.5 mm. Ouwerkerk (2017)	96 h	16	7.05 (6.65-7.38)	14.2	1.3[a]	3,888 (3,655-4,136) n=1
Juvenile wet weight 0.63 g; Head-tail base length 37 mm. Ouwerkerk (2017)	96 h	16	7.21 (6.87-7.58)	14.2	4.3[a]	2,171 (1,654-2,848) n=1
Juvenile wet weight 0.45 g; Head-tail base length 32 mm. Ouwerkerk (2017)	10 d	16	7.24 (6.93-7.54)	14.2	0.3[a]	382 (284-514) n=1
Juvenile wet weight 0.75 g; Head-tail base length 37.5 mm. Ouwerkerk (2017)	10 d	16	7.05 (6.65-7.38)	14.2	1.3[a]	244 (121-493) n=1
Juvenile wet weight 0.63 g; Head-tail base length 37 mm. Ouwerkerk (2017)	10 d	16	7.21 (6.87-7.58)	14.2	4.3[a]	166 (94-293) n=1
Fish - Inanga - *Galaxias maculatus*						
Juvenile wet weight 0.3 g, length 40 mm. NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not.	96 h	15	7.4-7.7	≈ 40	<0.5	3,493 (3,073-3,969 n=1
Invertebrate - Snail - *Potamopyrgus antipodarum*						
NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not. Survival.	96 h	20	7.4-7.7	≈ 40	<0.5	1,561 (1,345-1,812) n=1
NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not. Morbidity (mobility).	96 h	20	7.4-7.7	≈ 40	<0.5	936 (796-1,101) n=1

Notes incl. source	Duration	Temp. °C	pH mean (min.-max.)	Hardness mg/L $CaCO_3$	DOC mg/L	EC_{50}[a] (95% CL or ± 2 SD) µg/L Zn^{2+}
Ouwerkerk (2017). Survival.	96 h	19	7.06 (6.94-7.18)	14.2	0.3[a]	532 (474-597) n=1
Ouwerkerk (2017). Survival.	96 h	20	7.02 (6.86-7.10)	14.2	1.3[a]	819 (694-968) n=1
Ouwerkerk (2017). Survival.	96 h	21	7.03 (6.86-7.13)	14.2	4.3[a]	1,004 (896-1,125) n=1
Ouwerkerk (2017). Survival.	96 h	20	6.99 (6.94-7.06)	14.2	9.3[a]	1,372 (1,222-1,541) n=1
Ouwerkerk (2017). Morbidity (mobility).	96 h	19	7.06 (6.94-7.18)	14.2	0.3[a]	446 (403-492) n=1
Ouwerkerk (2017). Morbidity (mobility).	96 h	20	7.02 (6.86-7.10)	14.2	1.3[a]	459 (401-525) n=1
Ouwerkerk (2017). Morbidity (mobility).	96 h	21	7.03 (6.86-7.13)	14.2	4.3[a]	723 (655-771) n=1
Ouwerkerk (2017). Morbidity (mobility).	96 h	20	6.99 (6.94-7.06)	14.2	9.3[a]	993 (905-1089) n=1
Invertebrate - Amphipod - *Paracalliope fluviatilis*						
NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not.	48 h	20	7.4	48	na	887 (564-1,210) n=5
Ouwerkerk (2017). Morbidity (mobility)	48 h	20	7.32 (7.21-7.37)	14.2	0.3[a]	482 (403-577) n=1
Ouwerkerk (2017). Morbidity (mobility)	48 h	20	7.21 (7.16-7.25)	14.2	1.3[a]	293 (242-353) n=1
Ouwerkerk (2017). Morbidity (mobility)	48 h	20	7.37 (7.21-7.48)	14.2	4.3[a]	579 (500-672) n=1
Ouwerkerk (2017). Morbidity (mobility)	48 h	20	7.43 (7.13-7.58)	14.2	9.3[a]	823 (671-950) n=1
Invertebrate – Water flea – *Daphnia thomsoni*						
NIWA study FWWQ2009	48 h	20	7.5 (7.2-7.7)	14.0	0.42[b]	270 (209-348) n=1
NIWA study FWWQ2009	48 h	20	7.4 (7.1-7.6)	14.0	1.76[b]	240 (202-303) n=1
NIWA study FWWQ2009	48 h	20	7.4 (7.0-7.8)	14.0	3.66[b]	856 (na-na) n=1
NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not.	48 h	20	7.8	48	na	998 (812-1,226) n=1
Bivalve – Freshwater mussel juvenile - *Echyridella menziesii*						
NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not.	48 h	20	7.4-7.7	≈ 40	<0.5	354 (212-518) n=1
NIWA reference toxicant data base (unpublished). Zinc stock concentration verified, test dilutions not.	96 h	20	7.4-7.7	≈ 40	<0.5	261 (172-341) n=1

[a] Humic acid reference carbon source. [b] Manuka leaf extract carbon source.

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

Notes incl. source	Duration	Temp. °C	pH mean (min.-max.)	Hardness mg/L $CaCO_3$	DOC mg/L	EC_{50}[a] (95% CL) µg/L Cu^{2+}
Fish - Common bully - *Gobiomorphus cotidianus*						
Juvenile wet weight 0.45 g; Head-tail base length 32 mm. Ouwerkerk (2017)	96 h	16	7.29 (7.05-7.80)	14.2	0.3[a]	476 (289-565) n=1
Juvenile wet weight 0.75 g; Head-tail base length 37.5 mm. Ouwerkerk (2017)	96 h	16	7.22 (6.84-7.52)	14.2	1.3[a]	601 (477-757) n=1
Juvenile wet weight 0.63 g; Head-tail base length 37 mm. Ouwerkerk (2017)	96 h	16	7.18 (6.87-7.58)	14.2	4.3[a]	1,789 (1,000-3,200) n=1
Juvenile wet weight 0.45 g; Head-tail base length 32 mm. Ouwerkerk (2017)	10 d	16	7.29 (7.05-7.80)	14.2	0.3[a]	125 (93-163) n=1
Juvenile wet weight 0.75 g; Head-tail base length 37.5 mm. Ouwerkerk (2017)	10 d	16	7.22 (6.84-7.52)	14.2	1.3[a]	534 (496-574) n=1
Juvenile wet weight 0.63 g; Head-tail base length 37 mm. Ouwerkerk (2017)	10 d	16	7.18 (6.87-7.58)	14.2	4.3[a]	1,006 (797-1,270) n=1
Invertebrate - Snail - *Potamopyrgus antipodarum*						
Ouwerkerk (2017). Survival	96 h	20	6.86 (6.65-7.1)	14.2	0.3[a]	17 (14-20) n=1
Ouwerkerk (2017). Survival	96 h	20	7.06 (7.0-7.14)	14.2	1.3[a]	36 (31-41) n=1
Ouwerkerk (2017). Survival	96 h	20	7.06 (7.03-7.16)	14.2	4.3[a]	77 (67-89) n=1
Ouwerkerk (2017). Survival	96 h	20	6.89 (6.78-7.06)	14.2	9.3[a]	110 (102-120) n=1
Ouwerkerk (2017). Morbidity (mobility)	96 h	20	6.86 (6.65-7.1)	14.2	0.3[a]	14 (13-15) n=1
Ouwerkerk (2017). Morbidity (mobility)	96 h	20	7.06 (7.0-7.14)	14.2	1.3[a]	34 (30-38) n=1
Ouwerkerk (2017). Morbidity (mobility)	96 h	20	7.06 (7.03-7.16)	14.2	4.3[a]	52 (47-58) n=1
Ouwerkerk (2017). Morbidity (mobility)	96 h	20	6.89 (6.78-7.06)	14.2	9.3[a]	91 (83-100) n=1
Invertebrate - Amphipod - *Paracalliope fluviatilis*						
Ouwerkerk (2017). Morbidity (mobility)	48 h	21	7.37 (7.31-7.44)	14.2	0.3[a]	70 (58-84) n=1
Ouwerkerk (2017). Morbidity (mobility)	48 h	20	7.29 (7.18-7.35)	14.2	1.3[a]	92 (80-108) n=1
Ouwerkerk (2017). Morbidity (mobility)	48 h	20	7.48 (7.35-53)	14.2	4.3[a]	263 (184-308) n=1
Ouwerkerk (2017). Morbidity (mobility)	48 h	20	7.53 (7.47-7.59)	14.2	9.3[a]	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[b]	28 (na-na) n=1
NIWA study FWWQ2009	48 h	20	7.7 (7.5-7.8)	14.0	1.70[b]	215 (164-246) n=1
NIWA study FWWQ2009	48 h	20	7.7 (7.5-7.8)	14.0	2.52[b]	597 (558-614) n=1

[a] 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.[1] 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[2] 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.[3] 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 µg/L.

---

[1] 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).
[2] Stauber et al., 2022.
[3] 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 µg/L (at pH 8.5, hardness 90 mg/L as $CaCO_3$ and DOC 0.5 mg/L) to >1000 µg/L in natural waters with high DOC (20-25 mg/L) and lower pH.

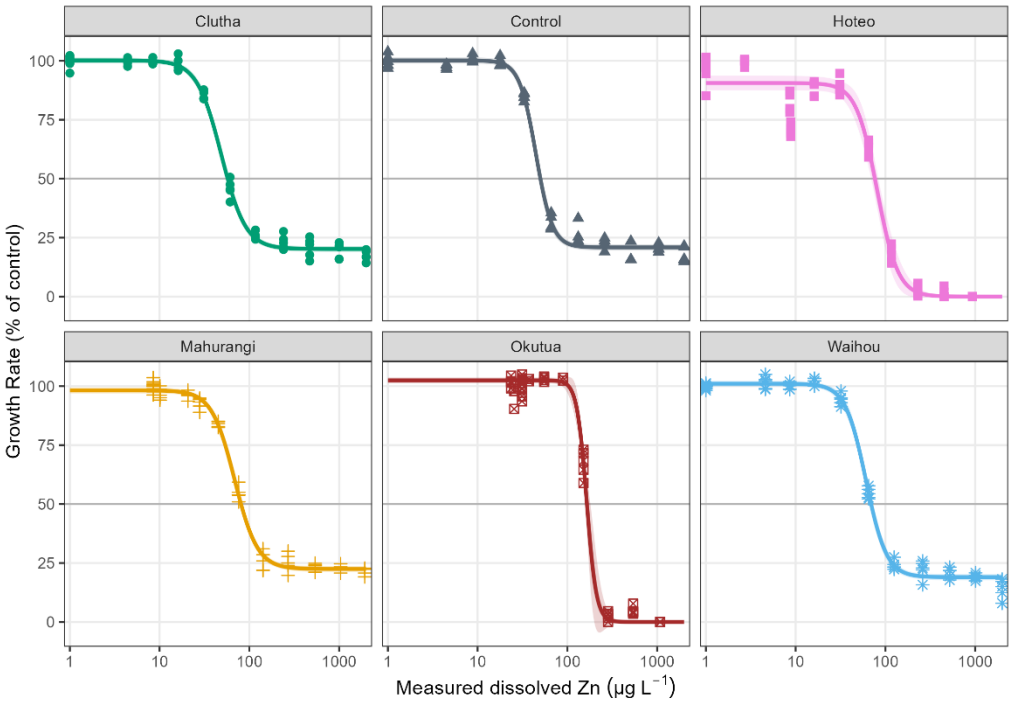


Figure D.1: Concentration-response curves for *R. subcapitata* after exposure to **zinc** for 24 hours. Note that for most of the waters tested growth rate did not reduce to zero after 24 hours of exposure. EC50 calculated as 50% of control growth rate.

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 (95% confidence interval, µg Zn/L)	Model	pH	Hardness (mg/L as $CaCO_3$)	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

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.

Test	pH	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

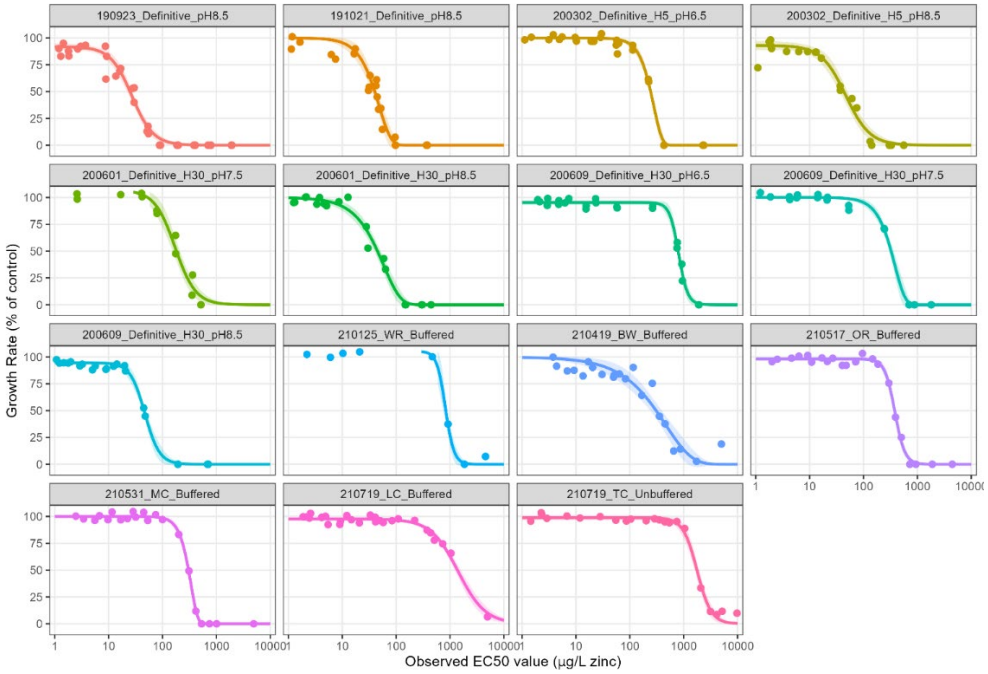


Figure D.2: Concentration-response curves for *Chlorella* sp. after exposure to **zinc** for 24 hours. This includes tests in synthetic waters at various pH and hardness values and in natural waters that varied by pH, hardness and DOC.

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.1: 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 [4]	Canadian copper GVs[5]	pH, DOC, multiple cations/anions
Zinc		
***R. subcapitata* chronic MLR** [6]	Chronic zinc DGVs for Australia & NZ [8]	pH, DOC
***Chlorella* sp. chronic MLR** [7]	Chronic zinc DGVs for Australia & NZ [8]	pH, DOC, hardness
***R. subcapitata* BLM** [6]	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 $CaCO_3$/L.[9] 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,[10] 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.

[4] 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.

[5] ECCC, 2021; B.C. Ministry of Environment and Climate Change Strategy, 2019.

[6] DeForest et al., 2023.

[7] Price et al., 2023.

[8] ANZG, 2024.

[9] Markich, King, and Wilson, 2006.

[10] 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. data points)
Cerotophyllum demersum	96-h biomass	pH 7.0 Hardness 35-335 mg $CaCO_3$/L DOC 0.1 mg/L*	Copper (3)
Chlorella sp.	24-h growth rate inhibition	pH 6.1-8.3 Hardness 3-355 mg $CaCO_3$/L DOC 0.69-20 mg/L	Zinc (15)
R. subcapitata	24-h growth rate inhibition	pH 7.2-8.2 Hardness 9-110 mg $CaCO_3$/L 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 under-predicts 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.

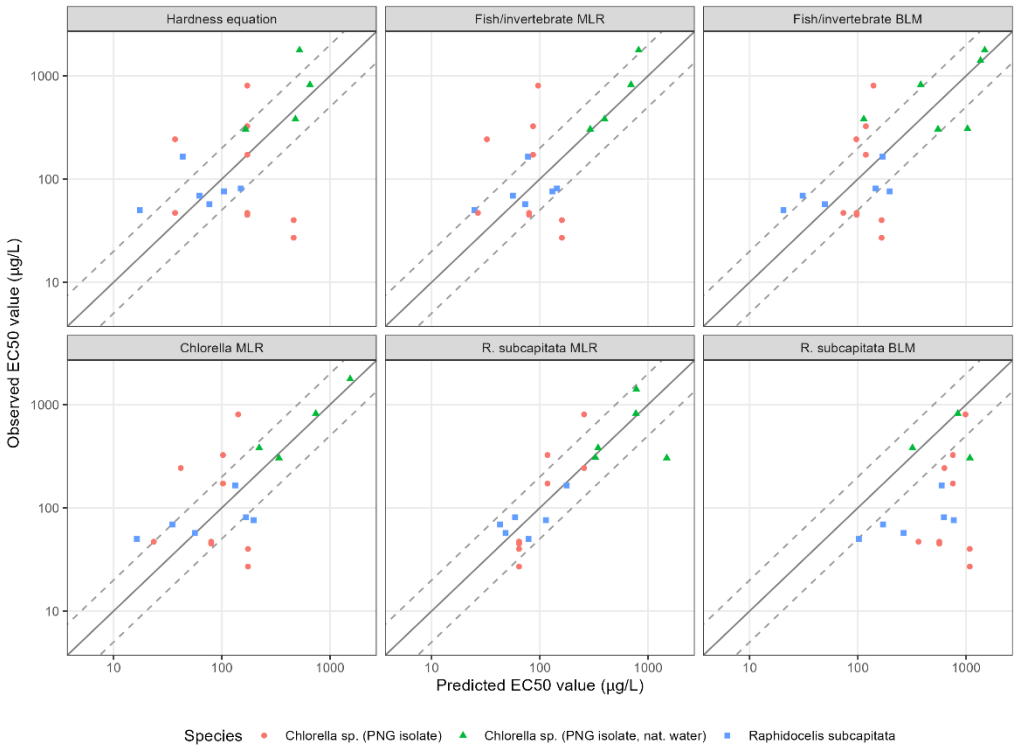


Figure E.1: Observed EC50 values for acute **zinc** toxicity to algal species, compared to EC50 values predicted with various models. Solid line is line of perfect agreement between observed and predicted EC50 values. Dashed lines indicate a factor of ±2 difference. The closest agreement is shown using 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^2	$RF_{x,2.0}$ †	Residual scores ‡	Overall score (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: † Proportion of predictions within a factor of two. ‡ 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))|)[11] 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.

[11] Besser et al., 2021..

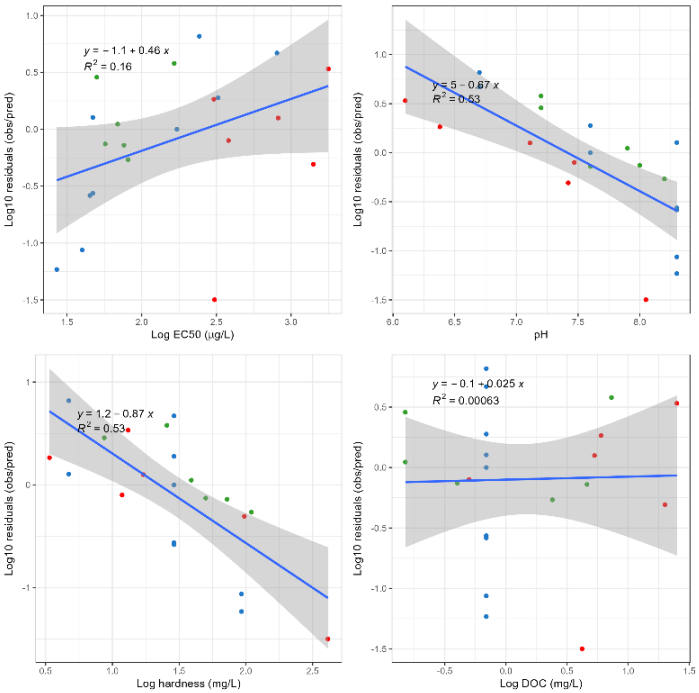


Figure E.2: Model evaluation plots for **zinc** plant/algae data with hardness regression. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.

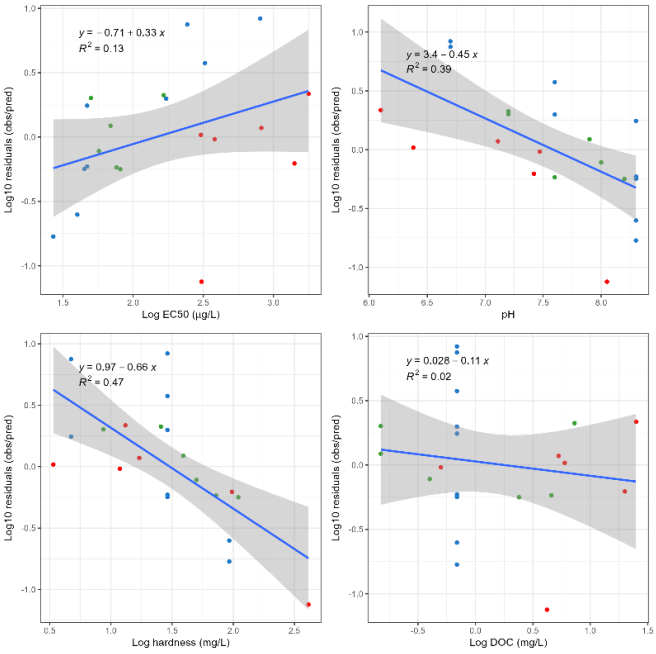


Figure E.3: Model evaluation plots for **zinc** plant/algae data with pooled fish/invertebrate MLR. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.

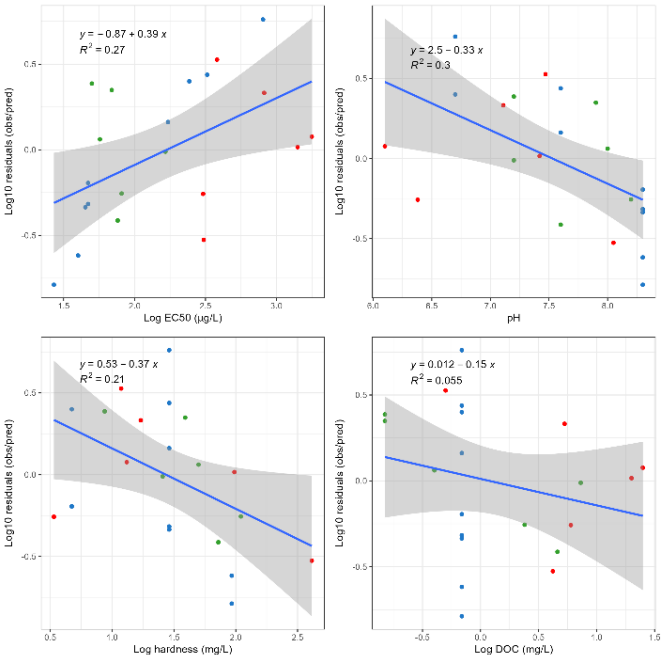


Figure E.4: Model evaluation plots for **zinc** plant/algae data with pooled fish/invertebrate BLM. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.

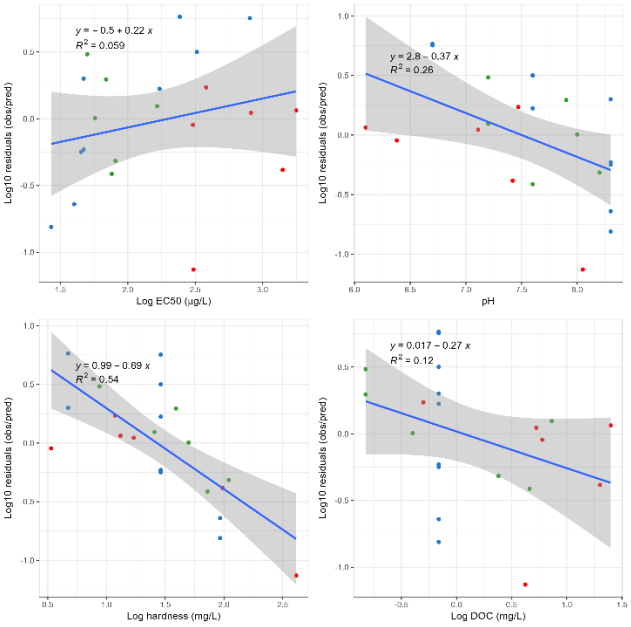


Figure E.5: Model evaluation plots for **zinc** plant/algae data with *Chlorella* sp. MLR. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.

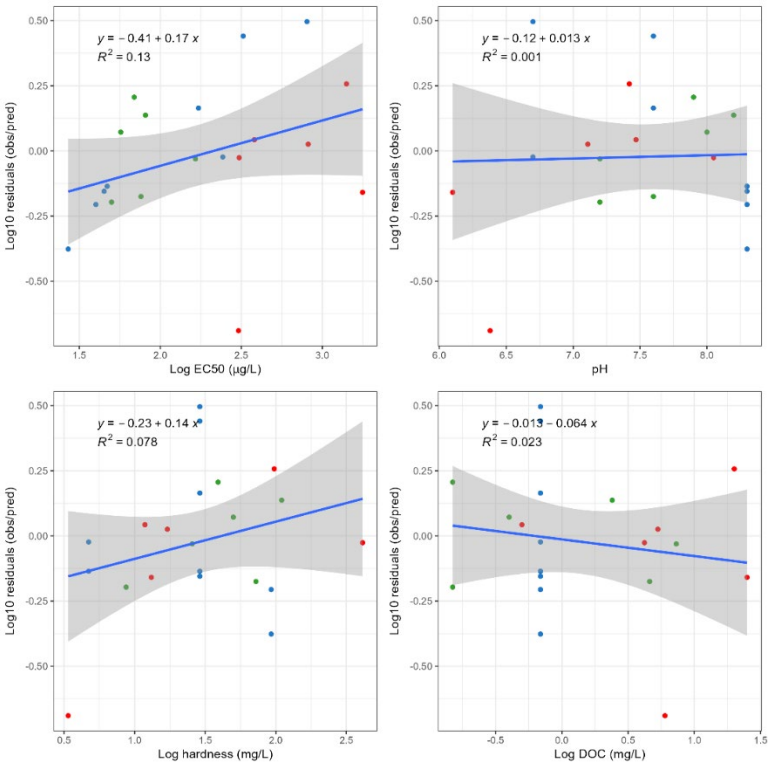


Figure E.6: Model evaluation plots for **zinc** plant/algae data with *R. subcapitata*. MLR. Green points are *R. subcapitata*, blue points are *Chlorella* sp. in synthetic waters, red points are *Chlorella* sp. in natural waters.

Appendix Table E.4: Residual scores for the plant/algal models.

Model	Slopes (p-values) of observed/predicted EC50 versus:			
	Observed EC50	pH	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	0.39 (0.02)	-0.33 (0.01)	-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)
***R. subcapitata* 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

Appendix Table F.1: Cross-species model performance metrics for **copper**.

Model	Correlation coefficient (pred.vs obs.)	RF $_{x,2.0}$ †	Slopes (p-values) of observed/predicted EC50 versus: ‡				
			Log (obs. EC50)*	pH	Log (hardness)	Log (DOC)	Log (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/ invertebrate 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/ invertebrate 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)

Notes: † Proportion of predictions within a factor of two. ‡ 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))|)[12] 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.

[12] Besser et al., 2021..

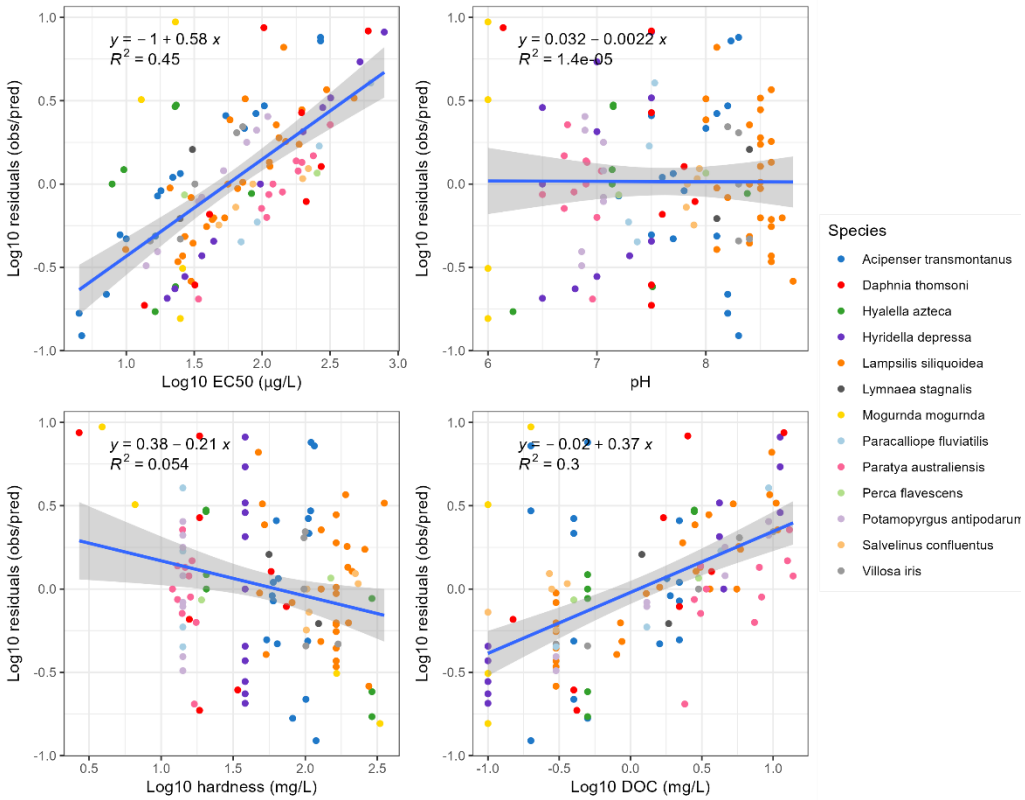


Figure F.1: Model evaluation plots for **copper** cross-validation of hardness regression model.

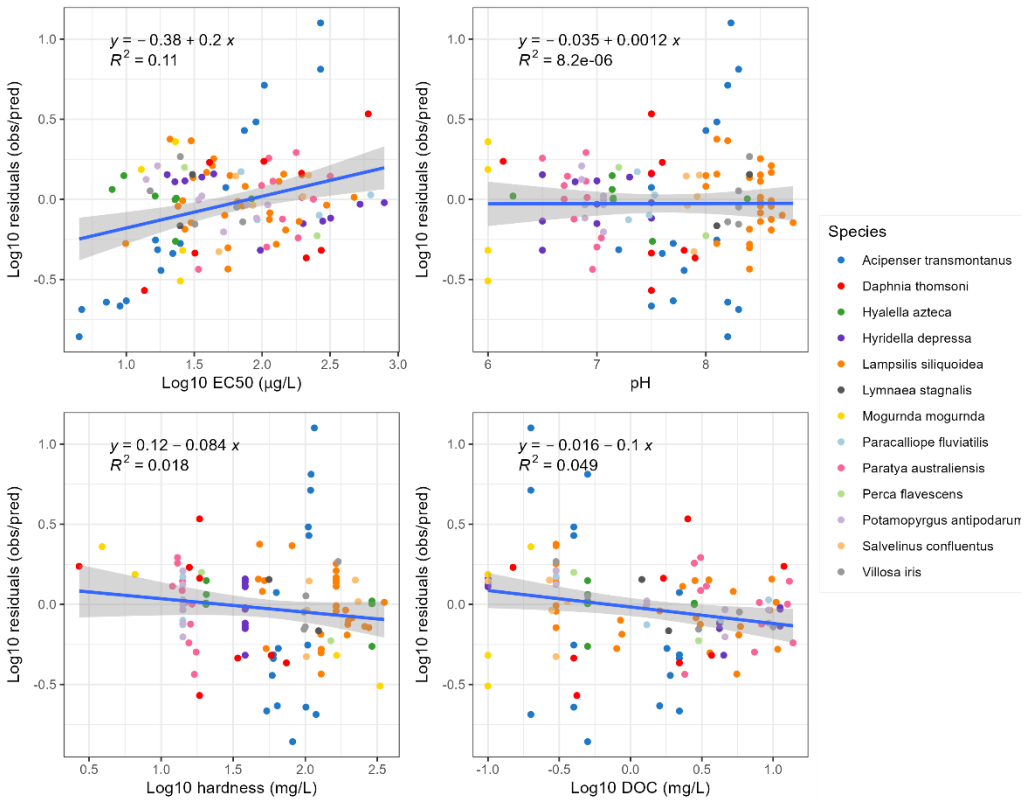


Figure F.2: Model evaluation plots for **copper** cross-validation of pooled fish/invertebrate MLR model.

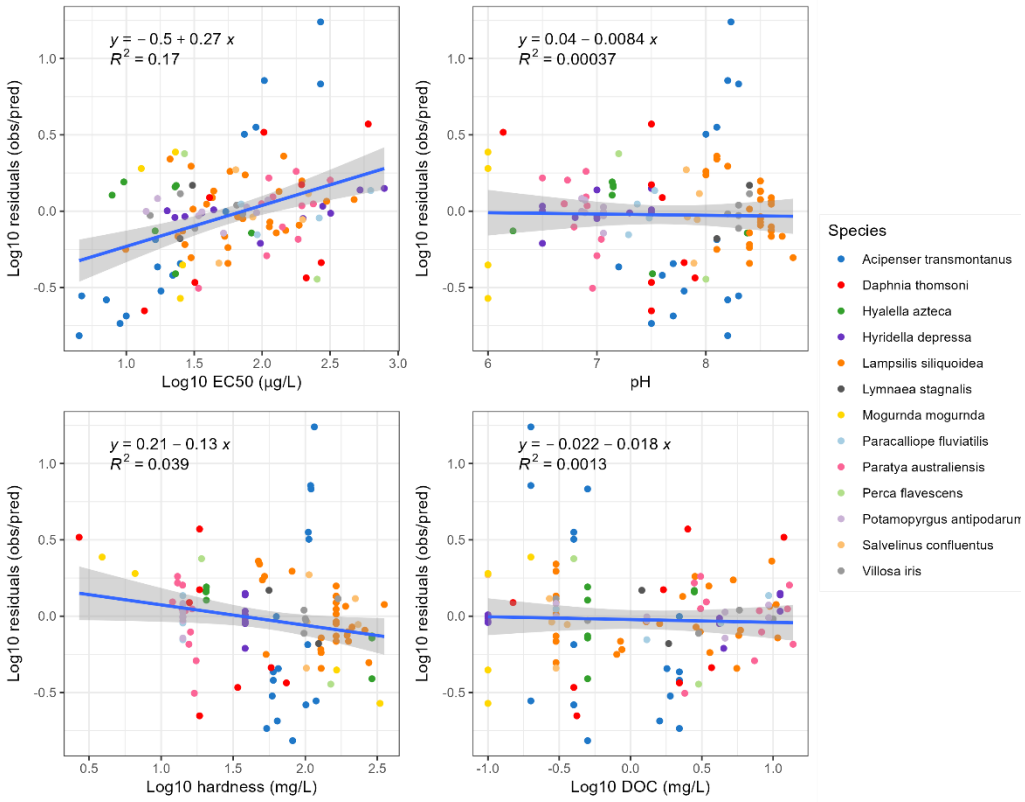


Figure F.3: Model evaluation plots for **copper** cross-validation of trophic-level MLR models. .

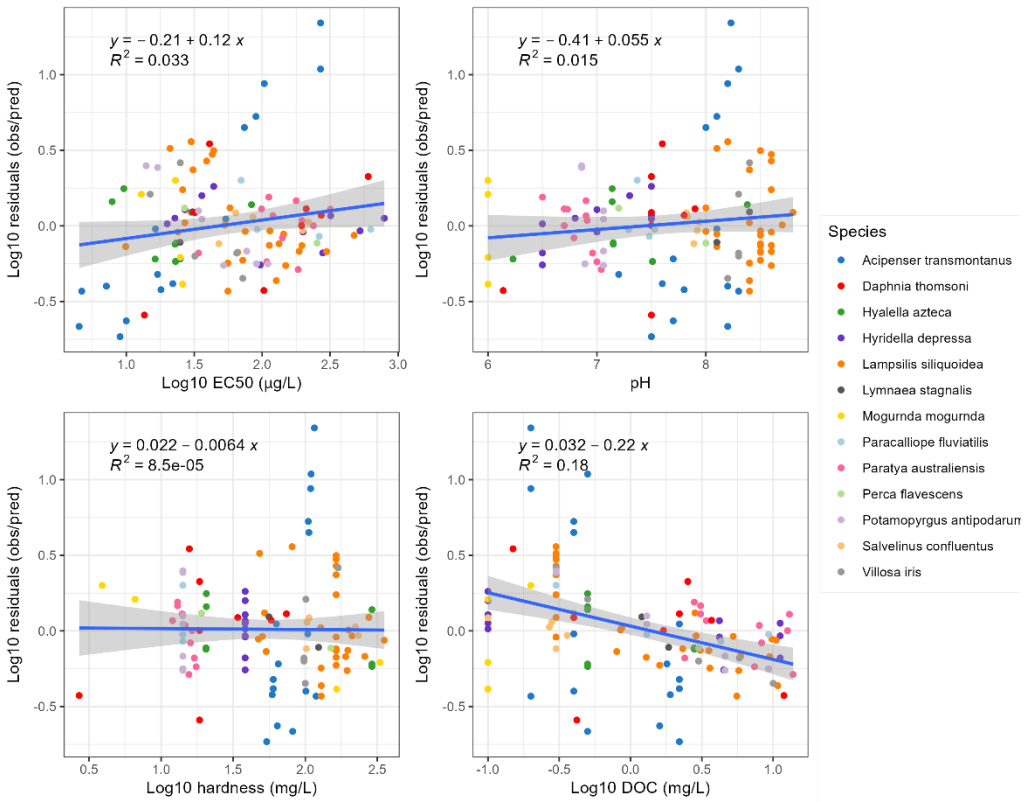


Figure F.4: Model evaluation plots for **copper** cross-validation of BLM. .

F.2 Zinc cross-species validation plots

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

Model	Correlation coefficient (pred.vs obs.)	RF $_{x,2.0}$ †	Slopes (p-values) of observed/predicted EC50 versus: ‡				
			Log (obs. EC50)*	pH	Log (hardness)	Log (DOC)	Log (Alkalinity)
Hardness 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/ invertebrate 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/ invertebrate 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)

Notes: † Proportion of predictions within a factor of two. ‡ 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))|)[13] 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.

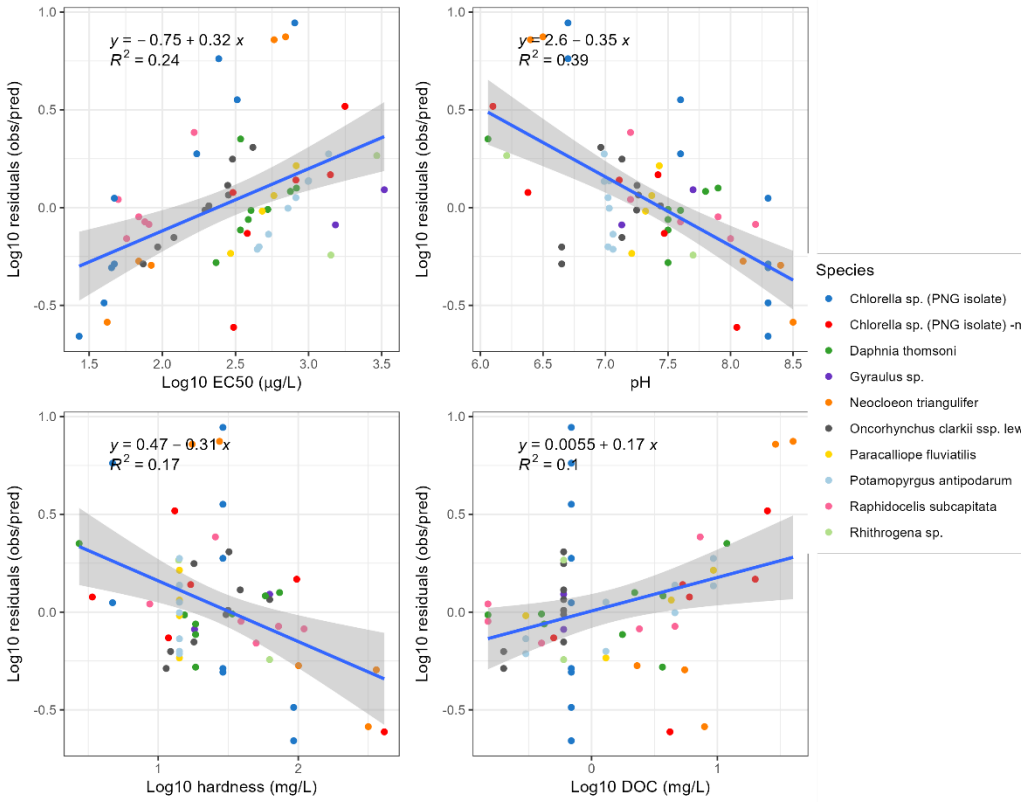


Figure F.5: Model evaluation plots for **zinc** cross-validation of hardness regression model.

[13] Besser et al., 2021..

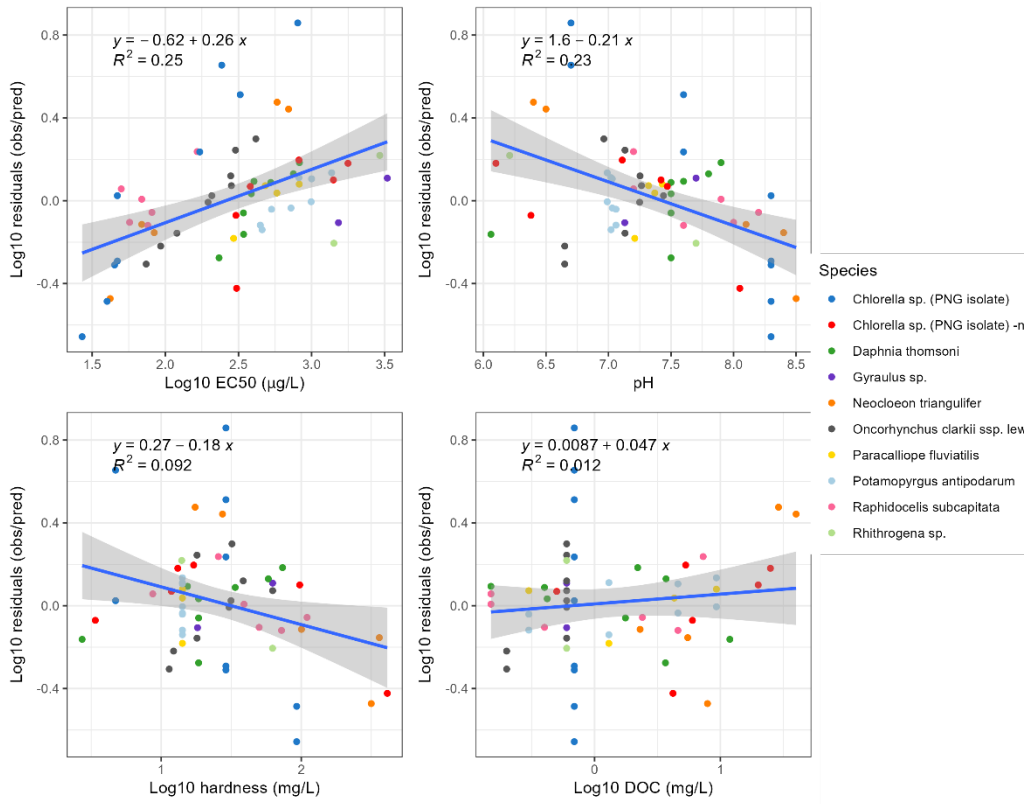


Figure F.6: Model evaluation plots for **zinc** cross-validation of pooled fish/invertebrate MLR.

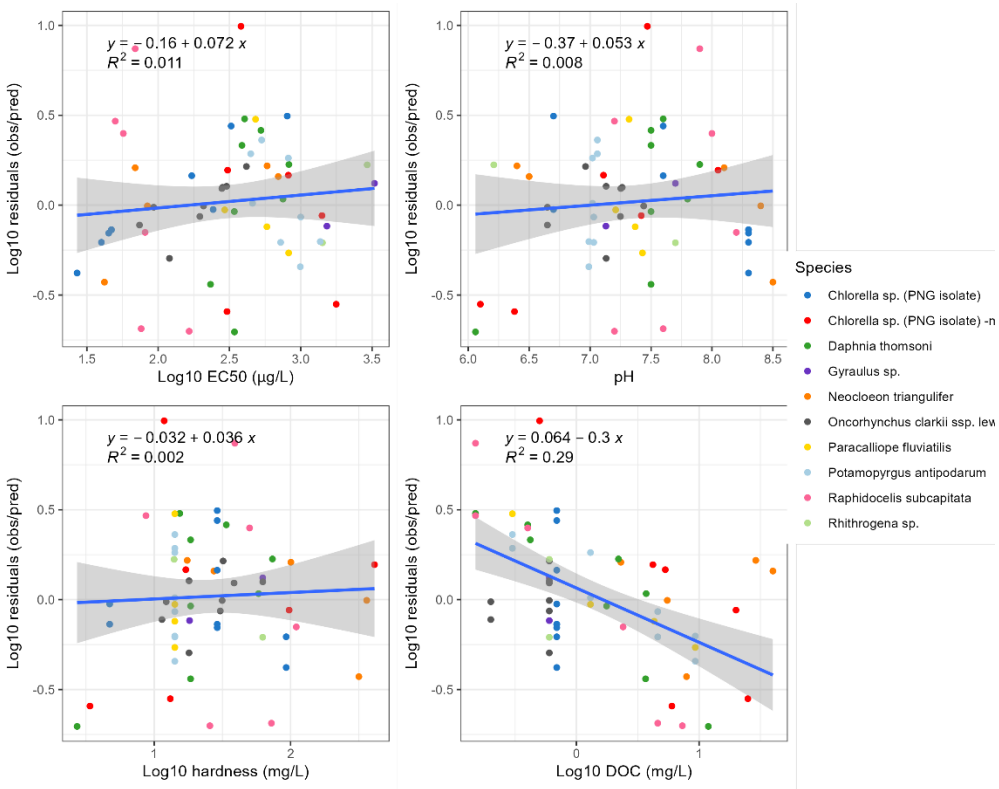


Figure F.7: Model evaluation plots for **zinc** cross-validation of trophic fish and invertebrate MLRs.

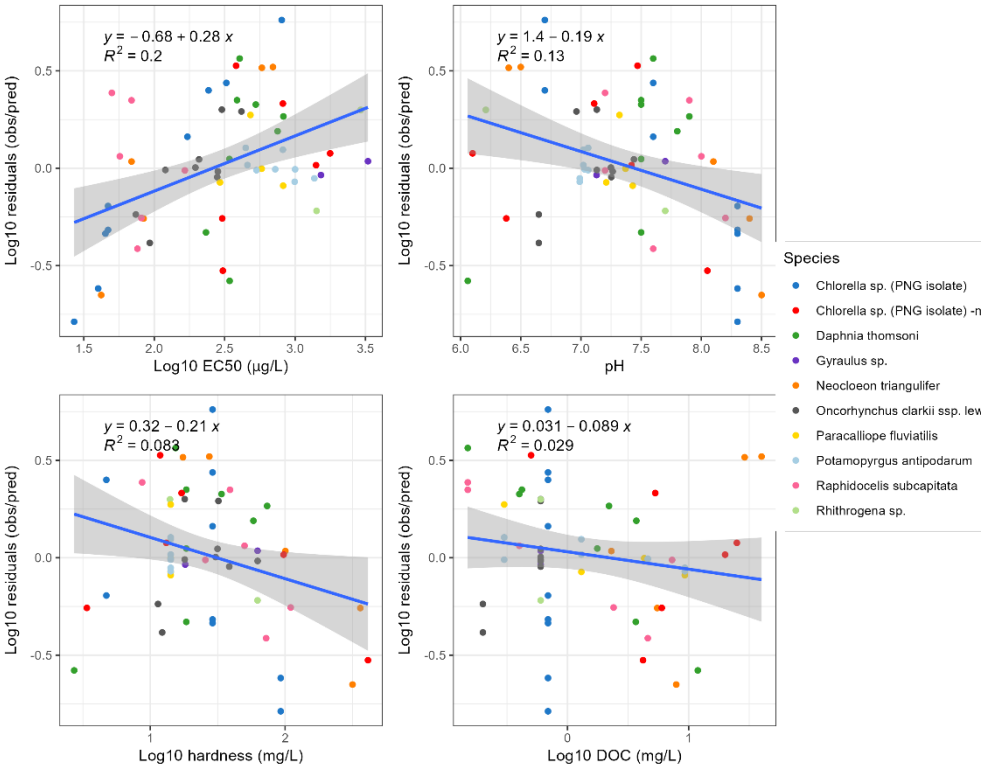


Figure F.8: Model evaluation plots for **zinc** cross-validation of pooled fish/invertebrate BLM.

F.3 Copper native species validation

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

Model	Correlation coefficient (pred.vs obs.)	RF $_{x,2.0}$ †	Slopes (p-values) of observed/predicted EC50 versus: ‡				
			Log (obs. EC50)*	pH	Log (hardness)	Log (DOC)	Log (Alkalinity)
Hardness 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/ invertebrate 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/ invertebrate 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: † Proportion of predictions within a factor of two. ‡ 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))|)[14] 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.

[14] Besser et al., 2021..

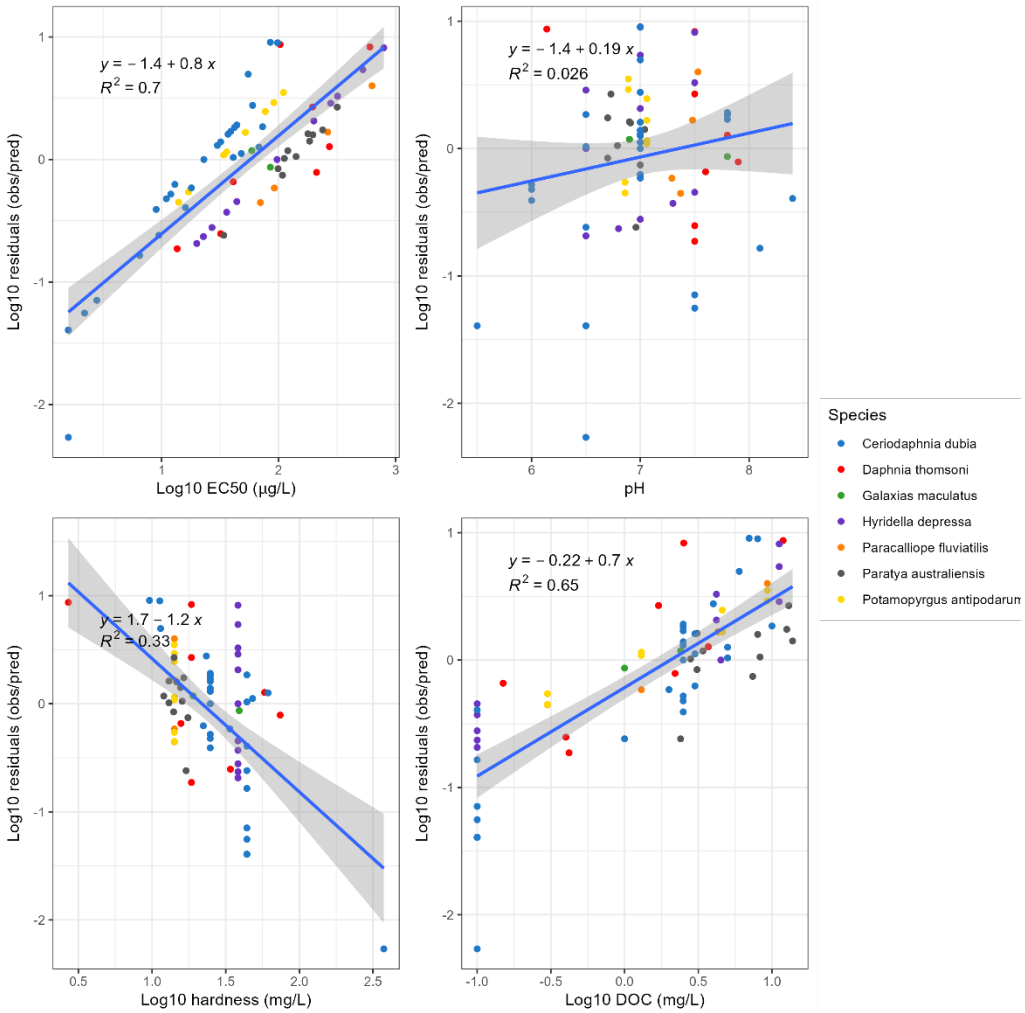


Figure F.9: Model evaluation plots for **copper** native-species validation of hardness regression.

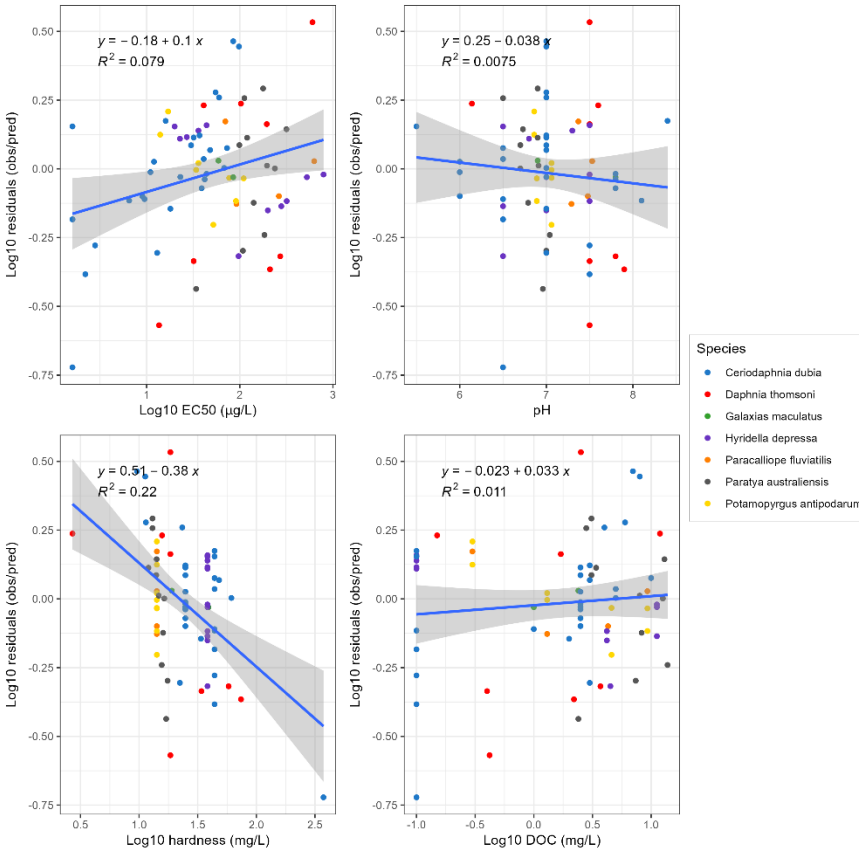


Figure F.10: Model evaluation plots for **copper** native-species validation of pooled fish/invertebrate MLR.

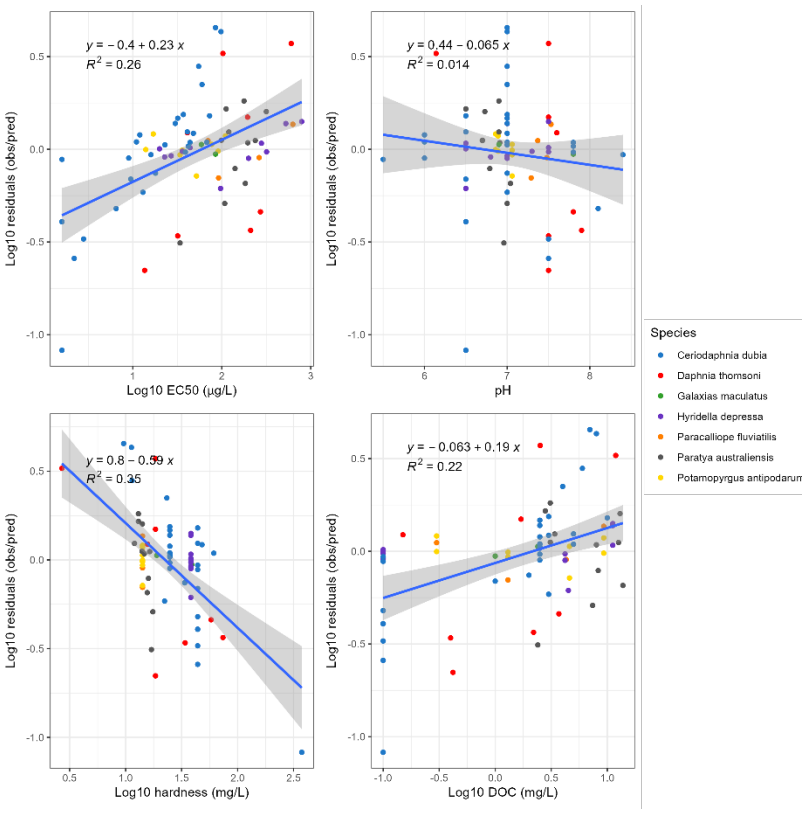


Figure F.11: Model evaluation plots for **copper** native-species validation of trophic-level MLRs.

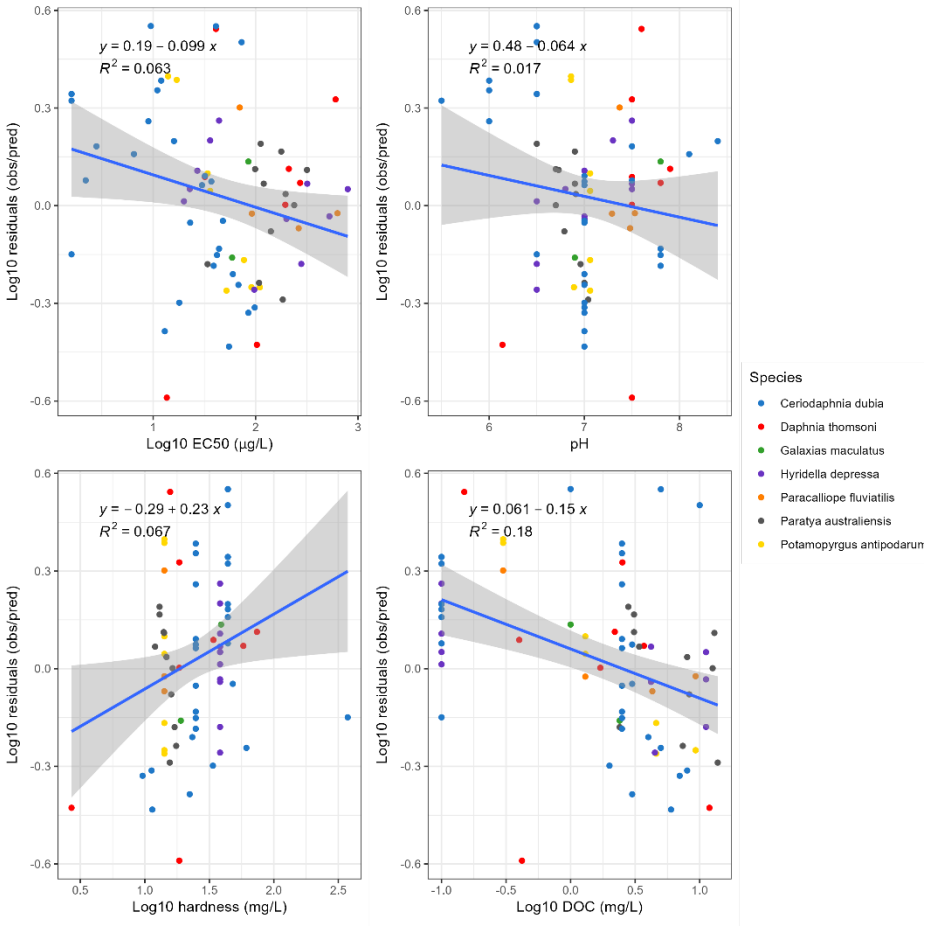


Figure F.12: Model evaluation plots for **copper** native-species validation of BLM.

F.4 Zinc native species validation

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

Model	Correlation coefficient (pred.vs obs.)	RF $_{x,2.0}$ †	Slopes (p-values) of observed/predicted EC50 versus: ‡				
			Log (obs. EC50)*	pH	Log (hardness)	Log (DOC)	Log (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/ invertebrate 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/ invertebrate 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)

Notes: † Proportion of predictions within a factor of two. ‡ 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))|)[15] 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.

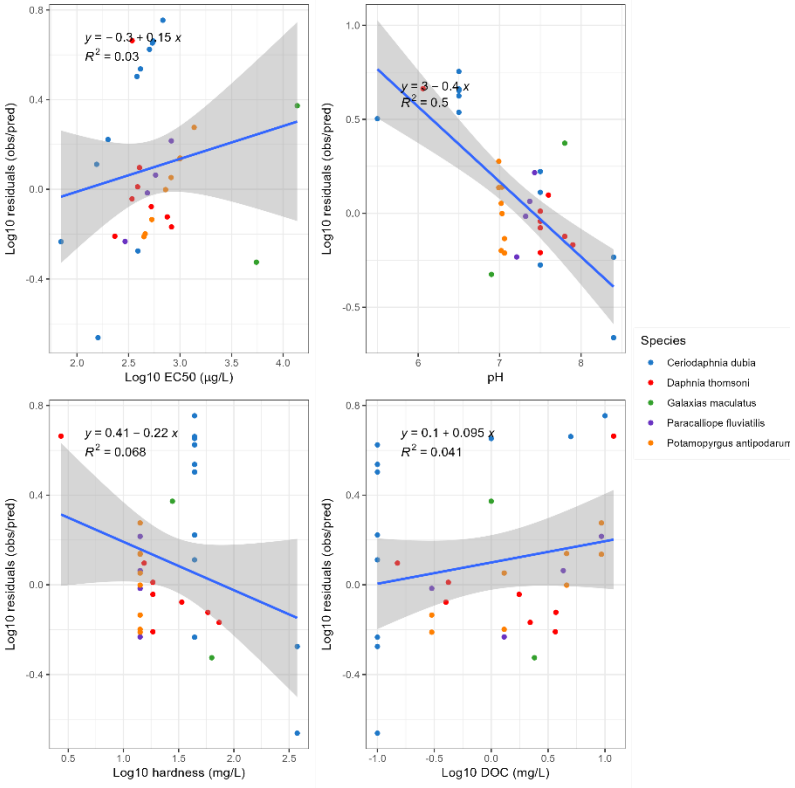


Figure F.13: Model evaluation plots for **zinc** native-species validation of hardness regression.

[15] Besser et al., 2021..

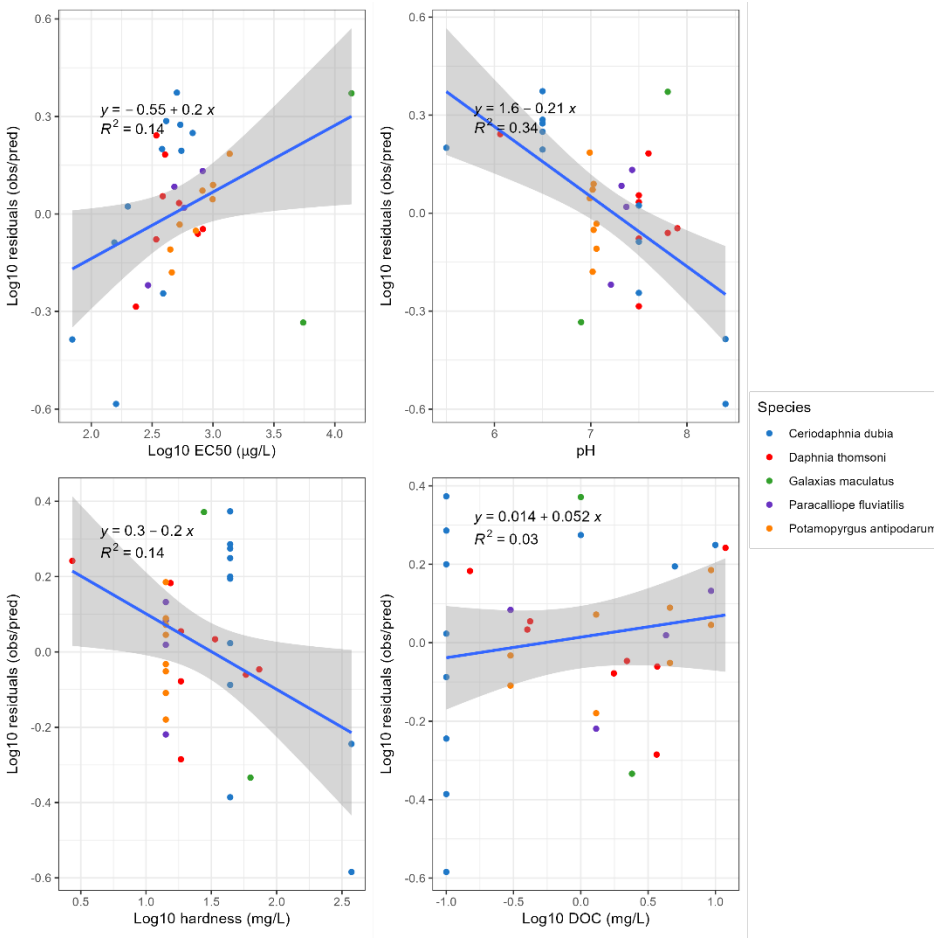


Figure F.14: Model evaluation plots for **zinc** native-species validation of pooled fish/invertebrate MLR.

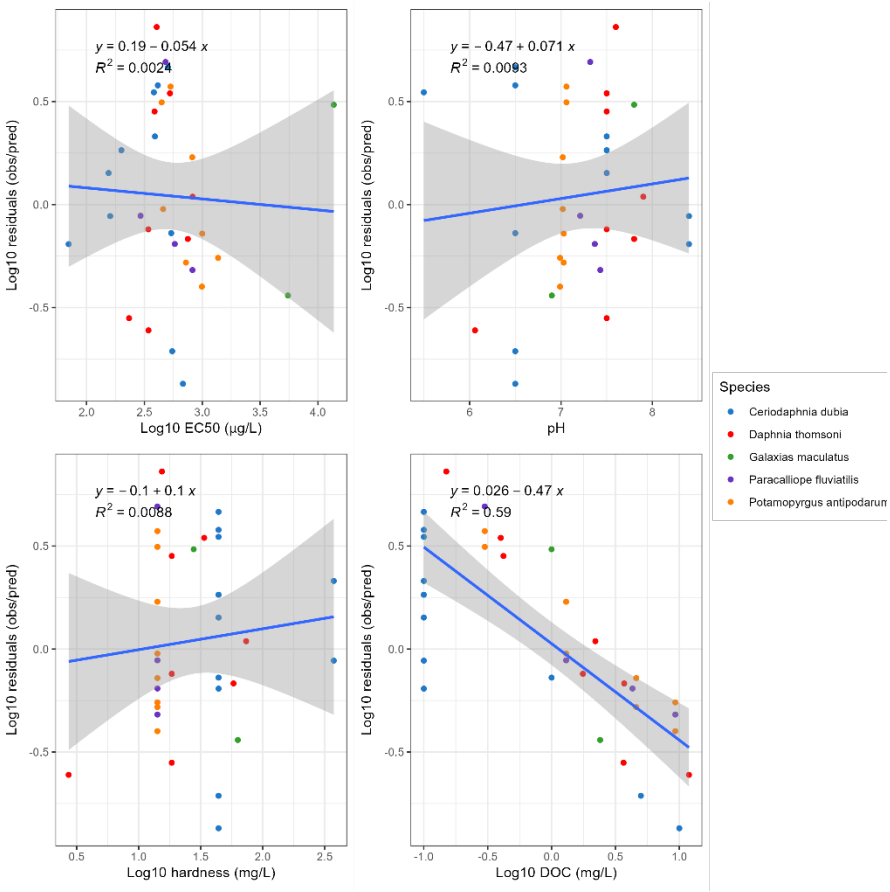


Figure F.15: Model evaluation plots for **zinc** native-species validation of trophic-level MLRs.

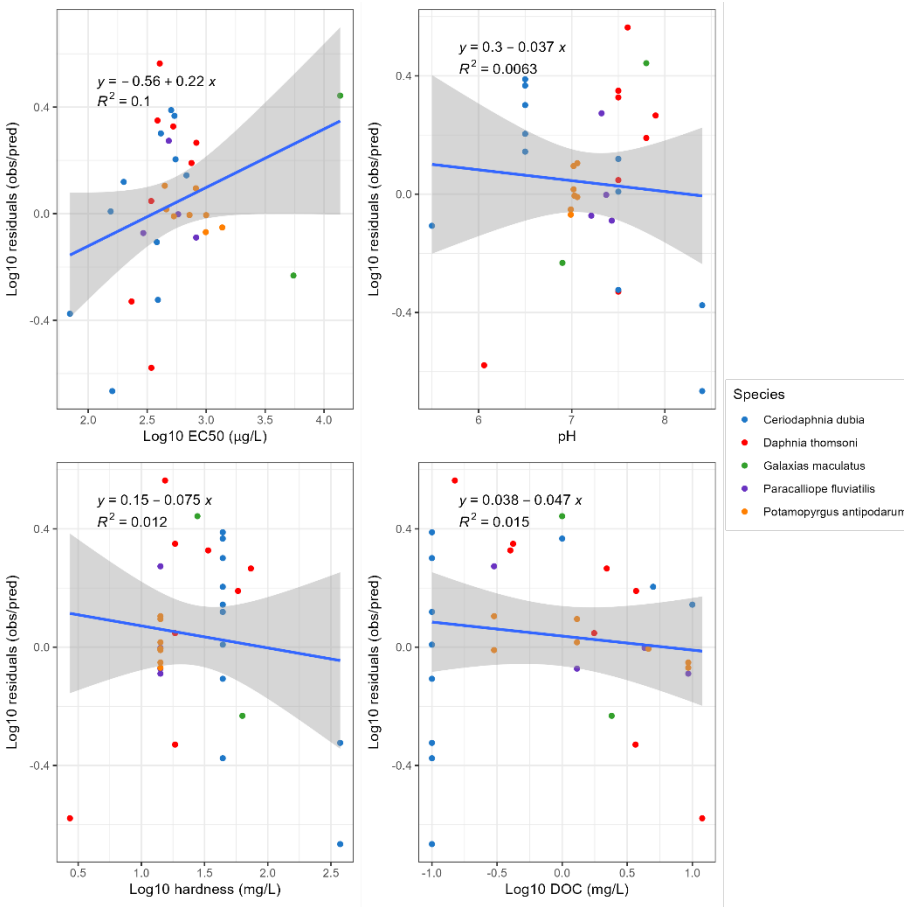


Figure F.16: Model evaluation plots for **zinc** native-species validation of BLM.

Appendix G Acute toxicity data used in the derivations

Appendix Table G.1: **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 $CaCO_3$/L and 0.5 mg/L DOC.

Tax Group	Species	Effect	Organism life stage	Exposure duration	N. data this species	Reported EC50 *	Normalised EC50	Converted EC10	Percentile rank (1-100)
Amphibian	*Lithobates clamitans*	Mortality	Embryo	96	1	160	46	25	63
Amphibian	*Lithobates catesbeianus*	Mortality	Larva	96	1	2400	7600	4200	99
Annelid	*Lumbriculus variegatus*	Mortality	Adult	48	25	110	59	36	74
Crustacean	*Scapholeberis 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 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 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 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 rostrata*	Mortality	Juvenile	48	2	99	11	6.6	27
Crustacean	*Daphnia obtusa*	Mortality	Neonate	48	53	23	11	6.7	29
Crustacean	*Simocephalus vetulus*	Mortality	Juvenile	48	18	12	11	6.8	30
Crustacean	*Hyalella azteca*	Mortality	Juvenile	96	7	20	11	6.9	33
Crustacean	*Eurycercus lamellatus*	Mortality	Juvenile	48	2	18	13	8.2	37
Crustacean	*Pleuroxus truncatus*	Mortality	Juvenile	48	2	160	17	11	42
Crustacean	*Acroperus harpae*	Mortality	Juvenile	48	2	26	17	11	43
Crustacean	*Chydorus 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 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 life stage	Exposure duration	N. data this species	Reported EC50 *	Normalised EC50	Converted EC10	Percentile rank (1-100)
Crustacean	*Paratya australiensis*	Mortality	Adult	96	11	140	63	39	78
Fish	*Acipenser transmontanus*	Growth	Larva	96	1	5.3	2.1	1.1	1
Fish	*Prosopium 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 oregonensis*	Mortality	Juvenile	96	2	20	12	6.7	28
Fish	*Etheostoma rubrum*	Mortality	NA	96	1	58	12	6.8	31
Fish	*Oncorhynchus clarkii*	Mortality	Adult/ Juvenile	96	2	67	14	7.9	34
Fish	*Oncorhynchus mykiss*	Mortality	Juvenile	96	56	23	15	8.1	36
Fish	*Oncorhynchus apache*	Mortality	NA	96	1	67	17	9.2	39
Fish	*Oncorhynchus tshawytscha*	Mortality	Juvenile	96	152	75	19	10	40
Fish	*Oncorhynchus kisutch*	Mortality	Juvenile	96	3	15	21	12	46
Fish	*Pimephales promelas*	Mortality	Larva	96	5	71	22	12	48
Fish	*Perca flavescens*	Mortality	Adult/ Juvenile	96	2	86	33	18	55
Fish	*Poeciliopsis 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 confluentus*	Mortality	Juvenile	96	5	100	52	29	67
Fish	*Scaphirhynchus platorynchus*	Mortality	NA	96	1	160	55	30	69
Fish	*Etheostoma lepidum*	Mortality	NA	96	1	250	63	34	71
Fish	*Macquaria ambigua*	Mortality	Juvenile	96	1	94	65	36	73
Fish	*Acrocheilus alutaceus*	Mortality	Juvenile	96	1	140	68	38	75
Fish	*Entosphenus tridentatus*	Mortality	Egg	96	1	46	69	38	76
Fish	*Gasterosteus aculeatus*	Mortality	Adult	96	5	330	82	45	79
Fish	*Pseudomugil tenellus*	Mortality	Adult	96	1	120	95	52	82

Tax Group	Species	Effect	Organism life stage	Exposure duration	N. data this species	Reported EC50 *	Normalised EC50	Converted EC10	Percentile rank (1-100)
Fish	*Etheostoma flabellare*	Mortality	NA	96	4	340	98	54	83
Fish	*Ptychocheilus lucius*	Mortality	NA	96	2	410	120	66	84
Fish	*Etheostoma nigrum*	Mortality	NA	96	4	510	150	81	85
Fish	*Cyprinus carpio*	Mortality	Juvenile	96	4	420	160	89	87
Fish	*Melanotaenia nigrans*	Mortality	Adult	96	1	230	180	100	88
Fish	*Mogurnda mogurnda*	Mortality	Larva	96	5	21	230	130	89
Fish	*Hypseleostris compressus*	Mortality	Adult	96	1	330	260	140	91
Fish	*Denariusa bandata*	Mortality	Adult	96	1	520	360	200	92
Fish	*Gobiomorphus cotidianus*	Mortality	Juvenile	96	1	77	390	220	93
Fish	*Porochilus rendahli*	Mortality	Adult	72	1	85	430	240	94
Fish	*Lepomis macrochirus*	Mortality	Juvenile	96	2	1700	550	300	96
Fish	*Melanotaenia splendida inornata*	Mortality	Adult	96	2	350	700	380	97
Fish	*Notemigonus 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 decorus*	Mortality	NA	48	1	740	650	410	98
Macrophyte	*Ceratophyllum demersum*	Biomass	Juvenile	96	3	9	20	12	47
Macrophyte	*Lemna aequinoctialis*	Growth	Mature	96	1	16	43	24	61
Mollusc	*Venustaconcha ellipsiformis*	Mortality	Glochidia	24	1	10	2.5	1.6	3
Mollusc	*Epioblasma 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 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 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 life stage	Exposure duration	N. data this species	Reported EC50 *	Normalised EC50	Converted EC10	Percentile rank (1-100)
Mollusc	*Villosa fabalis*	Mortality	Glochidia	24	1	6.9	9.2	5.7	25
Mollusc	*Lampsilis rafinesqueana*	Mortality	Glochidia	24	1	41	10	6.5	26
Mollusc	*Juga plicifera*	Mortality	Adult	96	1	15	13	8	35
Mollusc	*Epioblasma triquetra*	Mortality	Glochidia	24	4	24	14	9	38
Mollusc	*Obovaria subrotunda*	Mortality	Glochidia	24	1	13	17	11	44
Mollusc	*Epioblasma 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/ Juvenile	96	19	45	28	17	54
Mollusc	*Potamopyrgus antipodarum*	Mobility	NA	96	4	39	34	21	56
Mollusc	*Echyridella menziesii*	Mortality	Juvenile	48	1	33	37	23	60
Mollusc	*Actinonaias ligamentina*	Mortality	Larva	24	1	31	41	26	64
Mollusc	*Ptychobranchus fasciolaris*	Mortality	Glochidia	24	2	34	46	29	66
Mollusc	*Ligumia recta*	Mortality	Glochidia	24	1	35	52	32	70
Mollusc	*Hyridella depressa*	Duration valve opening	Adult/ 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 $CaCO_3$/L and 0.5 mg/L DOC.

Tax Group	Species	Effect	Organism life stage	Exposure duration	N. data this species	Reported EC50*	Norma-lised EC50	Converted EC10	Percen tile rank (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 williamsoni*	Mortality	Fry	96	3	420	270	140	29
Fish	*Cottus confusus*	Mortality	Juvenile	96	1	300	280	140	31
Fish	*Acipenser 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 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 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 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 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 life stage	Exposure duration	N. data this species	Reported EC50*	Normalised EC50	Converted EC10	Percentile rank (1-100)
Crustacean	*Daphnia galeata*	Mortality	<48 hr	48	1	1000	300	170	35
Crustacean	*Simocephalus exspinosus*	Mortality	<48 hr	48	2	1200	350	190	38
Crustacean	*Ceriodaphnia 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 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 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	*Capnia* 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	*Cinygmula* sp.	Mortality	Larva	96	2	69000	43000	24000	100
Mollusc	*Leptoxis ampla*	Mortality	Juvenile	96	1	67	58	32	4
Mollusc	*Lampsilis 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 capsaeformis*	Mortality	Juvenile	96	1	370	240	130	25
Mollusc	*Lampsilis straminea claibornen*	Mortality	Juvenile	96	1	290	250	140	28
Mollusc	*Cipangopaludina cathayensis*	Mortality	n.r.	96	2	3300	870	480	60
Mollusc	*Potamopyrgus 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 life stage	Exposure duration	N. data this species	Reported EC50*	Normalised EC50	Converted EC10	Percentile rank (1-100)
Mollusc	*Lymnaea luteola*	Mortality	Adult	48	3	11000	3600	2000	81
Annelid	*Limnodrilus 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 algae	*Raphidocelis subcapitata*	Population growth	exponential growth	24	5	83	54	26	1
Green algae	*Chlorella* sp. (PNG isolate)	Population growth	exponential growth	24	10	180	120	56	10