Memo

From	**Dr Chris Hickey**
To	Campbell Robertson, Bathurst Energy
CC	
Date	6 December 2018
Subject	**Environment Canterbury mudfish boron toxicity review comment**

Background

This memo responds to the review comments provided by Adrian Meredith, Environment Canterbury (dated 3 October 2018), in response to the NIWA report on boron toxicity to Canterbury mudfish (NIWA 2018). These comments followed up on a telephone conversation with Chris Hickey on 2nd October.

In the interim period the technical review comments have been received on the draft boron guideline (Binet et al. 2016), and follow-up discussions with the technical manager to resolve outstanding issues. This involved detailed responses and conferences calls with the Australian co-authors of this document. The final updated guideline derivation is included in this response. Final edits are nearing completion and the finalised document will be available shortly.

The content of Dr Meredith's email is included below together with "Response" sections to review comments. Supporting material is included in the appendix to this document.

General conclusions

I have addressed by Dr Meredith in relation to the growth performance of the Canterbury mudfish in the 40 day toxicity test. Some of the technical concerns raised relate to the initial weight and length measurements made on a representative sample of the fish. These measurements were "indicative" in that they are not part of the statistical testing procedures used in deriving toxicity effect thresholds. Initial length and weight measurements are not made on the fish to be included in the test as the anaesthetic may cause mortalities and affect feeding behaviour.

The apparent differences in performance of the Control fish compared with the lowest boron concentration (0.185 mg/L) appear related to the larger number of large fish (>60 mm) in the control treatment. It is not known whether the greater number of large fish in the Control treatments was an artefact of the random placement of fish at test initiation, or a growth/toxicant effect. However, my considered opinion is that this is more likely to be a bias at the test initiation rather than a boron toxicity effect initiating at the lowest boron concentration, as results do not show a progressive concentration-response through the 18 mg/L where the statistically significant weight and length effects occurred. I therefore concluded that the best professional judgement applied to the thresholds derived in original report were justified.

The test was definitive for long-term (40 d) survival for fish exposed to highest (55 mg/L) boron concentrations – with 100% survival at this concentration. The sub-lethal growth measures (length, weight, condition) are potentially more affected by both the initial fish condition and behaviour of the fish in the experimental system. At present we do not have these procedures standardised sufficiently for this species to optimise the chronic test for sub-lethal measures.

The revised default guideline values (DGVs) for boron have been updated as part of the technical review process. The 90th percentile DGV of 1.5 mg/L is only slightly lower than the proposed site-specific 90th percentile GV of 2.3 mg/L derived with inclusion of the Canterbury mudfish toxicity data and recommended for application to discharges to streams in this area (Hickey et al. 2018).

Responses to Dr Meredith's email:

Background to the issue is:

1. Bathurst Mining needed to develop an amended Boron toxicity trigger because their existing trigger was of only a temporary nature while a new justification was developed.

2. The consent condition said they should develop a new guideline and then it should be peer reviewed. While the condition explicitly required peer review, Bathurst was trying to get agreement to avoid "peer review". We were considering that if the results were pretty clear cut and you were 100% happy with the test results.

3. Since this all happened our Councillors have reprioritised the ECan priorities which are now 1. Freshwater Management and 2. A step change in Biodiversity improvement (particularly with reference to iconic Canterbury indigenous fauna including Canterbury Mudfish. For this reason we are particularly sensitised to anything to do with Canterbury Mudfish, because they will come under increased political scrutiny.

4. We agreed to the processes of the new Boron toxicity criteria to expedite the issues but also because we "expected" Canterbury mudfish to be tolerant of Boron like it is to most other environmental parameters (including your request to test "juvenile growth" rather than "embryology").

5. My problem with assessing your report for Bathurst is:

    a. Table 3.2 is an output of confusing results. The control had mudfish growing in length and weight; but all other treatments had them not growing in length or weight at all, except the highest concentration that had them shrinking in length and weight. That rings some alarm bells of the results either being "inconclusive" or alternatively showing them to be "sensitive to boron at low concentrations" in that boron stunted their growth? (and it was labelled as a growth trial??).

Response:

Table 3.2 is shown below. The "initial measurements" were made on a random sample of 15 fish taken from the holding tank in order to provide "indicative" measurements that the test procedures – primarily the test temperature and the feeding quantity – were achieving a level of growth over the initial fish condition to be able to measure a growth reduction effects of the toxicant relative to the Control performance. In this case, the indicative growth based on the initial average weight of 0.76 g increasing to 1.07 g was a 41% weight increase for comparison with the controls, but only about 10% for comparison with the lowest test concentration (0.187 mg/L). This level of weight increase should provide an ability to detect adverse effects attributable to the exposure to the toxicant. These initial fish weight measurements are also used to establish the daily feed ration for the fish to provide sufficient feed for growth – while minimising potential for unconsumed feed which would otherwise foul the tanks.

The initial measurements of length and weight were not made for all fish being used in the test treatments. Rather, it is our standard fish testing procedure in undertaking a chronic test to uses a separate sub-sample of fish randomly taken from the holding tank. The reason for this is that the need to anaesthetise the fish for the initial weight and length measurements rises potential issues for fish health and test performance in toxicity test. The anaesthetic may result in fish mortalities and marked changes in feeding behaviour. In our experience with galaxiids, bullies and smelt, such effects may be severe where juveniles are being used in testing procedures.

The full data for the initial fish measurements, the Control at test completion and the boron treatments at test completion are provided in Appendices 1-7. The initial fish measurements showed a relatively uniform length with low variability (CV ca. 7%), the weight variability was higher (CV 28%). Overall, length variability for the control treatment was similar for length (CV 9-10%) and weight (CV 27%); but increased for the highest boron treatment (55 mg/L; length CV 14%, weight CV 49%) – reflecting the increased boron stress on these fish. Within a treatment there were marked differences in weight (i.e., growth) variability between the replicates with CV of 20%, 25% and 32% for the controls, while the fish length CVs were all between 8-10% (Appendix 2). All CVs for weight and length were greater for the high boron treatment (Appendix 3). This relatively high variability in weight gain is a characteristic of the species where opportunistic individuals are quicker to the food and will perform better in the experimental system. The presence of one or more of these 'high achiever' individuals in a control of experimental treatment can markedly affect the trajectory of a long-term chronic growth test. Thus, while the length of the individuals may vary relatively little, their weight gain at the end of the experimental period may differ significantly. The degree to which the Canterbury mudfish exhibited opportunistic feeding behaviour was not known before this chronic test was undertaken.

I note that in each of the Control treatments there was 3 or 4 fish which were greater than 60 mm head-tail length (maximum 68 mm) while large sized fish were fewer in the other treatments (highlighted orange in Appendices). These large fish in the Control treatments bias the length and weight measurements up compared with the other treatments. It is not known whether the greater number of large fish in the Control treatments was an artefact of the random placement of fish at test initiation, or a growth/toxicant effect. However, my considered opinion is that this is more likely to be a bias at the test initiation rather than a boron toxicity effect initiating at the 0.187 mg/L concentration and then not showing a progressive concentration-response through the 18 mg/L where the statistically significant weight and length effects occurred. For this reason, the report states:

"*Based on the lack of a concentration-response, the threshold for boron effect would be considered to occur at the concentration prior to the increasing effect at 55 g boron* m^{-3}." (p16, para 1).

There is no way to retrospectively establish that the true growth (length and weight) of the treatment replicates given the lack of measurements for length and weight of all individuals when the experiment was initiated. Ideally the weights of individual fish would be measured both initially and at the test completion, however, this would require identification of individuals – which is not practical for such small fish. There is a possibility that the weight measurements could be normalised based on the measured fish length and the condition relationship for the species. The toxicity data could then be recalculated based on the "adjusted" weight values. This is not a procedure that we normally undertake in the statistical analysis.

Table 3-2 is potentially misleading in that the multiple comparison statistical testing did not include the sample of initial fish measurements. The statistical testing was only on measurements from Control treatment fish (3 replicated treatments of 10 fish) and boron treatments measuring changes in length, weight and condition of the after the 40 d exposure. The results shown in Table 3-2 cannot be interpreted as showing that the fish were "shrinking in length and weight".

Actions: (i) Modify table. Suggest shifting the 'indicative' initial measurements data to a footnote of the table. This would make clear that the statistical multiple comparisons did not include the initial measurements; (ii) provide additional text to support professional judgement applied to Control performance and interpretation of thresholds relative to concentration-response; and (iii) consider statistical reanalysis using "adjusted" weights based on length normalisation.

Table 3-2: Summary means for chronic fish growth and condition data.¶ '*' indicates statistically significant compared with control treatment (P <0.05); '[*]' indicates statistically significant result which is less than the method detection limit based on the minimum significant difference (MSD) for the test (see Table 2-1 for MSD values).

Treatment, boron concentration (g m^{-3})	Survival (%)	Length (mm) (SD)	Wet weight (g) (SD)	Condition factor (SD)
Initial measurements	-	53 (3.90)	0.76 (0.21)	0.96 (0.1)
Control 0.02	93.3	59 (5.02)	1.07 (0.29)	1.01 (0.12)
0.187	100	53 (4.37)*	0.80 (0.19)*	1.03 (0.09)
0.53	100	54 (5.31)*	0.84 (0.23)	1.04 (0.11)
5.8	96.7	54 (6.42)	0.84 (0.30)	1.04 (0.10)
18	100	53 (6.37)*	0.81 (0.28)*	1.04 (0.13)
55	100	51 (7.10)*	0.65 (0.32)*	0.91 (0.19)

b. Looking further at the CETIS tables at the back of the report the different replicates are reasonably consistent at this lack of length and weight growth, giving rise to the statistical significances in Table 3.2.

Response:
See comments to (a) and highlights for large fish on attached appendices.

c. I agree I struggle a bit with a toxicity mechanism for that lack of growth, but my reading of the results is it stunted their growth?

Response:
See response comments to (a) and suggestion "Actions".

d. Ideally, if all treatments had grown except the last one then there would be no problem.

Response:
Agree!

e. Given mudfish live and breed in-situ in these environments and Wainiwaniwa Valley is the largest remaining stronghold of Canterbury mudfish, it is not unreasonable for parties to question these results and question that if juvenile growth is stunted or halted by Boron, then will they even successfully breed (or suffer embryo death) in Boron concentrations? DOC staff are asking about this study (they haven't seen it yet while we consider it) and I am concerned they could come to these conclusions as mudfish is a priority species for them.

Response:
I do not consider that the difference between the Control performance and the low concentration of (0.187 mg/L) should be considered as definitive evidence of a causative link with boron exposure at this low concentration. Rather I think that this is potentially an experimental artefact caused by a large number of large fish in the control treatments. I note that Replicate 1 of the 5.8 mg/L boron treatment also has 4 fish

>60 mm (Appendix 5) and there were 3 large fish in Replicate 1 of the 18 mg/L treatment (Appendix 6) – indicating that these large fish can occur at elevated boron concentrations.
Overall, I consider that the absence of the concentration-response with the increasing boron concentration is the key component of the professional judgement in relation to the boron toxicity threshold for the mudfish in this test. The large fish present in many treatments shows good survival of these fish, however, it cannot be determined to what extent these fish grew during the test or were larger at test initiation.

f. For these reasons I have flagged that I am considering requiring the "peer review" clause to be actioned, although am first giving you the opportunity to discuss these somewhat anomalous results with us first.

Response:
Further clarification of methodology provided in response (a) and "growth" bias highlighted as large fish in appendix data. To some extent the lack of a chronic toxicity protocol for Canterbury mudfish is a contributor to the variability in results obtained. Until test standardisation is undertaken the optimisation of test temperature, food type, feeding regime and fish density will all be factors which contribute to the variability in the growth performance for a given duration of the chronic test. Toxicologically, the test was definitive for long-term (40 d) survival for fish exposed to high (55 mg/L) boron concentrations – with 100% survival at the maximum concentration. The sub-lethal growth measures (length, weight, condition) are potentially more affected by both the initial fish condition and behaviour of the fish in the experimental system. At present we do not have these procedures standardised sufficiently for this species to optimise the chronic test for sub-lethal measures.

g. When we then look at Table 4.2, all of the amphibian and fish species in the database are tested as "embryo life stages: embryo LC10 (mortality)" and so while we discussed early on in this process how you had not developed such tests for native (galaxiid) fish and favoured a juvenile growth test (and we agreed with that), I could understand reviewers raising this as an issue that (as a result of this stunted growth) it should have been an embryology toxicity test?

Response:
The difficulties with the egg-embryo-larval tests are greater than for juveniles in providing the standardised dietary conditions required for this life-stage. Feeding of larval fish requires large quantities of small, generally live, food and mortality during this period can often be high. Some fish species with large yoke sacs can be successfully held through a short-term "chronic" developmental period – however, the required >7 d period is often difficult for small fish species. Developing a robust test technique for larval fish development for the Canterbury mudfish would be recommended and could provide a practical approach for testing effects of multiple chemical contaminants which potentially affect their habitats.

6. Hopefully this sets out the dilemma I find myself in and am trying to work through. I understand your frustration at the technical difficulties with the tests being carried out in Christchurch remote from you. But at the end of the day we must confront the report and the results presented.

Response:
Agree.

7. What do you think the response would be if we required this report to be sent off for independent peer review to someone experienced in these ANZECC toxicity criteria development?

Response:
I have attached the scoring sheet used for reviewing the testing material for incorporation into the chronic guidelines development. The results of this test pass the acceptability criteria with a "High quality" score of 95.6% out of 100) (Appendix 8). On this basis the results of the testing would be acceptable and any further review consideration down to specific issues addressed by the reviewer based on their best professional

judgement as to whether the test results would be included in a generic guideline derivation. For a guideline the choice can be made to accept the results for species endpoints – such as survival – or the more sensitive of other sub-lethal endpoints measured. This is the procedure which has been being undertaken for the current boron revision (see discussion below).

The other aspect of this report was the site-specific guideline derivation procedure. The updated ANZECC (2000) guideline derivation procedure provides some guidance on the derivation of site-specific guidelines (Warne et al 2018). This includes consideration of the nature of the toxicity data (e.g., laboratory toxicity tests, microcosms, mesocosms, field-surveys) and chemical contaminant exposure and multi-stressor (e.g., contaminant + pH) and the adequacy of justification of the approach taken.

For this application the testing undertaken was for a site-specific application. The justifications were provided in the report for the species selections included in the species sensitivity distribution used for the derivation of the site-specific guideline values for boron. No detailed consideration was included in the report relating to the site-specific application of those values to the field site – other than the citing of field monitoring reports for chemical and biological monitoring and habitat descriptions. The receiving water stream where these site-specific guidelines would apply from a consenting perspective are biologically depauperate and subjected to ongoing drainage from historic mining, together with sediment and nutrient inputs from forestry and agricultural practices. On this basis the application of the risk-based ANZECC approach could apply a guideline for a lower protection threshold for boron (e.g., 90% or 80% protection).

The habitat for the Canterbury mudfish is located downstream of the site where the site-specific guidelines would be applied. Thus there would also be some consideration of further downstream dilution of the mine discharge waters prior to exposure at the mudfish habitat. This downstream consideration has not been incorporated into this report.

I consider that the staff of Environmental Canterbury would have sufficient expertise to review the toxicological aspects of the report and the adequacy of any assumptions and professional judgement documentation. If they desire an external reviewer to consider the adequacy of both the Canterbury mudfish testing, the site-specific guideline derivation and the receiving water application, then further information would have to be supplied to that external reviewer to provided them with the environmental context for the review. This should include information on where the populations of Canterbury mudfish are present and breeding in the streams currently receiving point and diffuse source boron inputs from the coal mine and historic workings.

General comments – updated ANZECC boron derivation

The technical review comments for the updated boron guideline (Binet et al. 2016) have been review and discussed. The reviewers and the technical manager identified the inclusion of data which were “nominal” concentrations in that the stack solution was chemically validated but the test solutions were not measured (highlighted yellow below). After consideration these were considered to have met the criteria for inclusion.

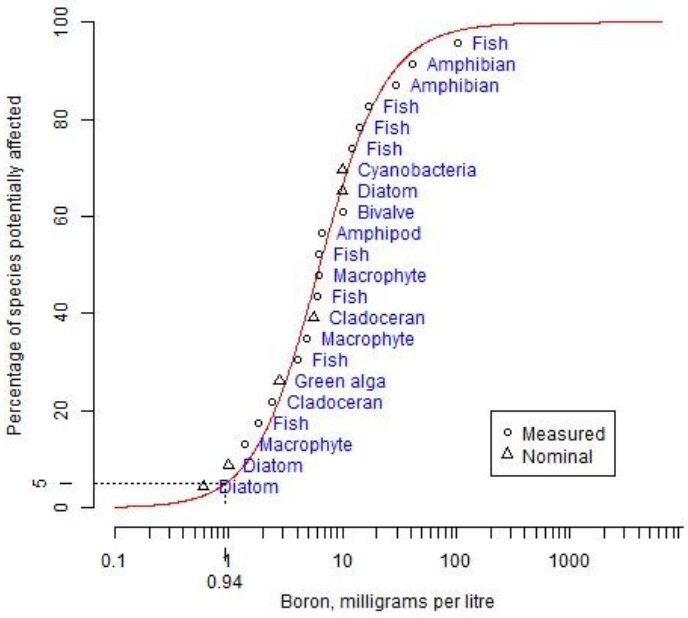
The revised default guideline values (DGVs) for boron are given below. The 90th percentile DGV of 1.5 mg/L is only slightly lower than the proposed site-specific 90th percentile GV of 2.3 mg/L derived with inclusion of the Canterbury mudfish toxicity data and recommended for application to discharges to streams in this area (Hickey et al. 2018).

The updated boron guideline report is being finalised and will soon be available.

Model: Burr type III

Protection level information

Protect. level	Guideline Value	lower 95% CI	upper 95% CI
99%	0.33	0.063	1.4
95%	0.94	0.41	2.3
90%	1.5	0.81	3.1
80%	2.5	1.4	4.5



SSD 3: Measured, with nominal values from Hickey and Macaskill (1988) and Wilkinson (1985) (22 values, 8 taxonomic groups)

Toxicity_value	General_taxa	Measured	Species
41	Amphibian	Measured	Anaxyrus fowleri
29	Amphibian	Measured	Rana pipiens
17	Fish	Measured	Carassius auratus
1.8	Fish	Measured	Danio rerio
14	Fish	Measured	Ictalurus punctatus
102	Fish	Measured	Melanotaenia spledida
6	Fish	Measured	Micropteris salmoides
6.2	Fish	Measured	Oncorhynchus mykiss
12	Fish	Measured	Pimephales promelas
10	Bivalve	Measured	Lampsilis siliquoidea
5.6	Cladoceran	Nominal	Ceriodaphnia dubia
6.6	Amphipod	Measured	Hyalella azteca
2.4	Cladoceran	Measured	Daphnia magna
6.1	Macrophyte	Measured	Egeria densa
1.4	Macrophyte	Measured	Lemna disperma
4.9	Macrophyte	Measured	Potamogeton ochreatus
10	Diatom	Nominal	Cyclotella sp
0.6	Diatom	Nominal	Navicula sp1
1	Diatom	Nominal	Navicula sp2
2.8	Green alga	Nominal	Pseudokirchneriella subcapitata
10	Cyanobacteria	Nominal	Nostoc punctiforme
4	Fish	Measured	Cirrhinus mrigala

References

ANZECC. (2000). Australian and New Zealand guidelines for fresh and marine water quality, October 2000 ed. National Water Quality Management Strategy Paper No. 4, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australia.

Binet, M.T., Batley, G.E., Hickey, C.W., Golding, L.A., Adams, M.S. (2016). Guidelines for the protection of aquatic ecosystems, toxicant trigger values: Boron – Freshwater. Australian and New Zealand guidelines for fresh and marine water quality. Draft July 2016 (revised 2018). Council of Australian Governments Standing Council on Environment and Water, Canberra, ACT, Australia.

Hickey, C.W., Thompson, K.J., Bell, S., Arnold, J. (2018). Chronic sensitivity of juvenile Canterbury mudfish (*Neochanna burrowsius*) and periphyton (*Rhizoclonium* sp.) to boron. BRL18202; 2018199HN. NIWA report prepared for Bathurst Resources Ltd.

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

Appendix 1: Summary statistical data for initial measurements

TEST SPECIES PREPARATION						
Species:		*Mudfish*				
Test date:		24-Feb-18		**Project Code:**	BRL18201/1718	
End-Point:		0		**Sample ID:**		

Sample

Fish #	Date:	Treatment	Wet weight (g)	Head-Tail Base Length (mm)	Head-Tail End Length (mm)	Condition
1		Holding tanks	1.10	53.5	60	0.98
2		Holding tanks	0.67	44	50	1.06
3		Holding tanks	1.15	55	61	0.98
4		Holding tanks	0.53	44	50	0.83
5		Holding tanks	0.99	51.5	58	0.98
6		Holding tanks	1.07	50	57	1.12
7		Holding tanks	0.74	49	55	0.87
8		Holding tanks	0.57	44	50	0.90
9		Holding tanks	0.54	44	50	0.85
10		Holding tanks	0.60	44	50	0.95
11		Holding tanks	0.67	49	55	0.79
12		Holding tanks	0.78	49	55	0.92
13		Holding tanks	0.84	48	53	1.10
14		Holding tanks	0.70	46	52	0.97
15		Holding tanks	0.45	39	44	1.06
Average			**0.76**	**47.3**	**53.3**	**0.96**
n			**15**	**15**	**15**	**15**
SD			**0.21**	**3.75**	**3.90**	**0.10**
CV			**28.0%**	**7.9%**	**7.3%**	**10.3%**
Median			0.70	48.0	53.0	0.97
5th percentile			0.51	42.5	48.2	0.82
95th percentile			1.11	54.0	60.3	1.11
Maximum			1.15	55.0	61.0	1.12
Minimum			0.45	39.0	44.0	0.79

Appendix 2: Summary statistical data for Control (0.02 mg/L boron)

TEST SPECIES PREPARATION					
Species:	*Canterbury Mudfish Neochanna burrowsius*				
Test date:	5-Apr-18			Project Code:	BRL18201
End-Point:	40 days			Sample ID:	
Sample					
Control 0.02mg/L Boron					Ling cond
				>60 mm	
Fish #	**Replicate**	**Wet weight (g)**	**Head-Tail base Length (mm)**	**Head-Tail end Length (mm)**	**Condition tail end**
1	1	1.24	57	64	0.91
2	1	1.18	55	61.5	0.98
3	1	1.51	58	65	1.06
4	1	0.83	49	54	1.03
5	1	0.59	43	58	0.58
6	1	0.84	46	52	1.17
7	1	0.80	45	53	1.05
8	1	0.72	47	53	0.95
9	1	0.78	46	53	1.03
10	1	0.65	44	50	1.02
	Average	**0.91**	**49.00**	**56.35**	**0.98**
	St Dev	**0.30**	**5.58**	**5.39**	**0.15**
	Median	**0.81**	**46.50**	**53.50**	**1.02**
	CV	32%	11%	10%	16%
11	2	1.27	58	66	0.85
12	2	1.60	59	65	1.12
13	2	1.55	59	63	1.20
14	2	1.00	51	57	1.05
15	2	1.17	55	63	0.90
16	2	0.96	50	56	1.06
17	2	1.16	54	60	1.04
18	2	0.95	48.5	55	1.11
19	2	0.72	45	53	0.95
20	2				
	Average	**1.15**	**53.28**	**59.78**	**1.03**
	St Dev	**0.29**	**4.98**	**4.71**	**0.11**
	Median	**1.16**	**54.00**	**60.00**	**1.05**
	CV	25%	9%	8%	11%
21	3	1.08	52	60	0.96
22	3	1.28	57	64	0.94
23	3	1.48	60	68	0.90
24	3	1.39	55	61	1.18
25	3	0.97	50	55	1.14
26	3	0.97	50	56	1.07
27	3	1.19	55	62	0.96
28	3	0.76	48	54	0.95
29	3	1.07	51	58	1.07
30	3				
	Average	**1.13**	**53.11**	**59.78**	**1.02**
	St Dev	**0.23**	**3.89**	**4.55**	**0.10**
	Median	**1.08**	**52.00**	**60.00**	**0.96**
	CV	20%	7%	8%	10%
	Average (All)	**1.06**	**51.70**	**58.55**	**1.01**
	Median (All)	**1.03**	**51.00**	**58.00**	**1.03**
	St Dev (All)	**0.29**	**5.14**	**5.02**	**0.12**
	CV (All)	**27%**	**10%**	**9%**	**12%**

Appendix 3: Summary statistical data for 0.187 mg/L

TEST SPECIES PREPARATION					
Species:	*Canterbury Mudfish*				
Test date:	5-Apr-18		Project Code:	BRL18201	
End-Point:	40 days		Sample ID:		

Sample
0.187mg/L Boron

				>60 mm	
Fish #	**Replicate**	**Wet weight (g)**	**Head-Tail base Length (mm)**	**Head-Tail end Length (mm)**	**Condition tail end**
1	1	0.85	52	58	0.85
2	1	1.09	53	60	0.98
3	1	0.75	46	51	1.11
4	1	0.63	47	52	0.87
5	1	0.67	44	50	1.05
6	1	0.85	48	53	1.12
7	1	0.69	47	53	0.91
8	1	0.59	44	50	0.93
9	1	0.65	43	48	1.16
10	1	0.48	40	45	1.04
	Average	**0.72**	**46.40**	**52.00**	**1.00**
	St Dev	**0.17**	**3.98**	**4.42**	**0.11**
	Median	**0.68**	**46.50**	**51.50**	**1.01**
	CV	24%	9%	9%	11%
11	2	0.86	50.5	57	0.90
12	2	1.27	55	60	1.14
13	2	1.10	51.5	58	1.10
14	2	0.96	50	57	1.01
15	2	0.73	46	50.5	1.11
16	2	0.87	50	57	0.92
17	2	0.75	45	51	1.11
18	2	0.92	49	55	1.08
19	2	1.04	57	62	0.85
20	2	0.81	48	53	1.07
	Average	**0.93**	**50.20**	**56.05**	**1.03**
	St Dev	**0.17**	**3.68**	**3.70**	**0.09**
	Median	**0.90**	**49.50**	**55.53**	**1.05**
	CV	18%	7%	7%	9%
21	3	0.51	41	47	0.97
22	3	0.73	46	51	1.08
23	3	0.94	50	56	1.04
24	3	0.61	43	48	1.09
25	3	0.61	43	48	1.08
26	3	0.74	46	52	1.03
27	3	0.86	48	55	1.01
28	3	1.05	51	56	1.17
29	3	0.86	49	54	1.06
30	3	0.50	42	47	0.95
	Average	**0.74**	**45.90**	**51.40**	**1.05**
	St Dev	**0.17**	**3.10**	**3.38**	**0.06**
	Median	**0.74**	**45.95**	**51.70**	**1.05**
	CV	23%	7%	7%	5%
	Average (All)	**0.80**	**47.50**	**53.15**	**1.03**
	Median (All)	**0.78**	**47.50**	**53.00**	**1.05**
	St Dev (All)	**0.19**	**4.10**	**4.37**	**0.09**
	CV (All)	**24%**	**9%**	**8%**	**9%**

Appendix 4: Summary statistical data for 0.53 mg/L

TEST SPECIES PREPARATION					
Species:	*Canterbury Mudfish*				
5-Apr-18			Project Code:	BRL18201	
40 days			Sample ID:		
Sample					
0.53mg/L Boron					
				>60 mm	
Fish #	**Replicate**	**Wet weight (g)**	**Head-Tail base Length (mm)**	**Head-Tail end Length (mm)**	**Condition tail end**
1	1	1.2159	55	61	1.0361
2	1	0.8371	56	61	0.7133
3	1	0.9394	50	56	1.0426
4	1	1.1388	50	57	1.1966
5	1	1.2236	57	63.5	0.9209
6	1	0.602	43	48	1.0758
7	1	0.782	43	48.5	1.3534
8	1	0.7155	46	52	0.9984
9	1	0.6166	43	49	1.0338
10	1	0.7063	47	53	0.9293
	Average	**0.88**	**49.00**	**54.90**	**1.03**
	St Dev	**0.21**	**5.08**	**5.25**	**0.17**
	Median	**0.75**	**46.50**	**52.50**	**1.03**
	CV	24%	10%	10%	16%
11	2	0.8373	47	53	1.1016
12	2	1.0625	52	58	1.0580
13	2	1.0592	51	57	1.1130
14	2	1.096	51	57	1.1516
15	2	0.9041	49	55	1.0608
16	2	0.6776	46	50.5	1.0350
17	2	0.6508	44	50	1.0252
18	2	0.6434	43	48	1.1498
19	2	0.5083	40	46	1.0360
20	2	0.5257	41	46	1.0715
	Average	**0.80**	**46.40**	**52.05**	**1.08**
	St Dev	**0.23**	**4.32**	**4.59**	**0.05**
	Median	**0.66**	**45.00**	**50.25**	**1.07**
	CV	28%	9%	9%	4%
21	3	1.00	50	57	1.0465
22	3	1.333	58	64	0.9792
23	3	0.6732	46	52	0.9394
24	3	0.6442	45	50.5	0.9840
25	3	1.1152	55	63	0.8601
26	3	0.6196	42	47	1.1816
27	3	0.7739	47	53	1.0182
28	3	0.8361	47	52.5	1.1327
29	3	0.74	47.5	53	0.9736
30	3	0.568	43	48	1.0150
	Average	**0.83**	**48.05**	**54.00**	**1.01**
	St Dev	**0.25**	**5.05**	**5.73**	**0.09**
	Median	**0.76**	**47.00**	**52.75**	**1.00**
	CV	30%	11%	11%	9%
	Average (All)	**0.83**	**47.82**	**53.65**	**1.04**
	Median (All)	**0.78**	**47.00**	**53.00**	**1.04**
	St Dev (All)	**0.23**	**4.93**	**5.31**	**0.11**
	CV (All)	**28%**	**10%**	**10%**	**11%**

Appendix 5: Summary statistical data for 5.8 mg/L

TEST SPECIES PREPARATION					
Species:	*Canterbury Mudfish*				
5-Apr-18			Project Code:	BRL18201	
40 days			Sample ID:		
Sample					
5.8mg/L Boron					
				>60 mm	
Fish #	**Replicate**	**Wet weight (g)**	**Head-Tail base Length (mm)**	**Head-Tail end Length (mm)**	**Condition tail end**
1	1	1.2721	56	63	0.9811
2	1	1.5193	58	65	1.0639
3	1	0.8225	45	51	1.2187
4	1	0.7774	45	51	1.1519
5	1	0.9333	52	58	0.9294
6	1	1.4999	58	64	1.1019
7	1	1.2146	57	64	0.8923
8	1	0.6695	48	53	0.8808
9	1	0.5295	41	46	1.0792
10					
	Average	**1.03**	**51.11**	**57.22**	**1.03**
	St Dev	**0.35**	**6.27**	**6.80**	**0.12**
	Median	**0.82**	**48.00**	**53.00**	**1.03**
	CV	34%	12%	12%	11%
11	2	1.0309	51	58	1.0266
12	2	0.5228	41	46	1.0655
13	2	1.1307	54	61	0.9635
14	2	0.532	42	48	0.9507
15	2	0.5123	41	46	1.0441
16	2	0.7303	46	52	1.0191
17	2	0.606	43	47	1.1557
18	2	0.7268	57	53	0.9562
19	2	0.6394	45	51	0.9474
20	2	0.4496	40	45	0.9807
	Average	**0.69**	**46.00**	**50.70**	**1.01**
	St Dev	**0.19**	**5.72**	**4.78**	**0.07**
	Median	**0.62**	**44.00**	**49.35**	**0.97**
	CV	28%	12%	9%	7%
21	3	0.7913	44	50	1.2465
22	3	1.2038	57	64	0.8843
23	3	0.6227	43	48	1.1128
24	3	0.8581	50	57	0.9017
25	3	0.5133	40	45	1.1197
26	3	0.7185	47	52	1.0026
27	3	1.1438	51	58	1.1390
28	3	0.7734	45	51	1.1460
29	3	0.693	45	50	1.0916
30	3	0.7367	47	53	0.9693
	Average	**0.81**	**46.90**	**52.80**	**1.06**
	St Dev	**0.22**	**4.79**	**5.51**	**0.12**
	Median	**0.76**	**46.00**	**51.50**	**1.10**
	CV	27%	10%	10%	11%
	Average (All)	**0.83**	**47.90**	**53.45**	**1.04**
	Median (All)	**0.74**	**46.00**	**52.00**	**1.03**
	St Dev (All)	**0.30**	**6.00**	**6.42**	**0.10**
	CV (All)	**36%**	**13%**	**12%**	**10%**

Appendix 6: Summary statistical data for 18 mg/L

TEST SPECIES PREPARATION					
Species:	*Canterbury Mudfish*				
5-Apr-18			Project Code:	BRL18201	
40 days			Sample ID:		
Sample					
18mg/L Boron					
				>60 mm	
Fish #	**Replicate**	**Wet weight (g)**	**Head-Tail base Length (mm)**	**Head-Tail end Length (mm)**	**Condition tail end**
1	1	1.4082	58	65	0.9861
2	1	0.7767	47	52	1.0838
3	1	0.7843	47	53	1.0319
4	1	1.6071	60	67	1.0248
5	1	1.043	54	60	0.9353
6	1	1.0345	55	61	0.8815
7	1	0.3969	39	44	0.9280
8	1	0.5468	43	49	0.9168
9	1	0.6848	41.5	46	1.3957
10	1	0.7283	47	53	0.9582
	Average	**0.90**	**49.15**	**55.00**	**1.01**
	St Dev	**0.33**	**6.51**	**7.07**	**0.15**
	Median	**0.76**	**47.00**	**53.00**	**0.95**
	CV	37%	13%	13%	14%
11	2	0.759	46	51	1.1246
12	2	0.8018	48	53	1.0549
13	2	0.6302	45	51	0.9338
14	2	0.669	43	48	1.1955
15	2	0.7132	47	52	0.9952
16	2	0.8743	50	55	1.0259
17	2	0.9215	50	56	1.0227
18	2	0.8157	45	50	1.2849
19	2	0.7303	46	52	1.0191
20	2	0.4742	42	46	0.9665
	Average	**0.74**	**46.20**	**51.40**	**1.06**
	St Dev	**0.13**	**2.66**	**2.99**	**0.11**
	Median	**0.72**	**45.50**	**51.20**	**1.02**
	CV	17%	6%	6%	10%
21	3	0.5172	40	45	1.1282
22	3	0.8511	49	55	0.9987
23	3	1.3945	60	67	0.8892
24	3	0.8592	50	56	0.9536
25	3	0.5742	41	47	1.0951
26	3	1.1664	51	58	1.1615
27	3	0.6862	49	54	0.8521
28	3	0.8816	49	55	1.0344
29	3	0.5133	39	44	1.2002
30	3	0.5877	41	46	1.1978
	Average	**0.80**	**46.90**	**52.70**	**1.05**
	St Dev	**0.29**	**6.59**	**7.21**	**0.13**
	Median	**0.77**	**49.00**	**54.50**	**1.06**
	CV	36%	14%	14%	12%
	Average (All)	**0.81**	**47.42**	**53.03**	**1.04**
	Median (All)	**0.77**	**47.00**	**52.50**	**1.02**
	St Dev (All)	**0.28**	**5.78**	**6.37**	**0.13**
	CV (All)	**35%**	**12%**	**12%**	**12%**

Appendix 7: Summary statistical data for 55 mg/L

TEST SPECIES PREPARATION

Species:	*Canterbury Mudfish*		
Test date:	5-Apr-18	**Project Code:**	BRL18201
End-Point:	40 days	**Sample ID:**	

Sample	
55mg/L Boron	Ling cond

Fish #	Replicate	Wet weight (g)	Head-Tail base Length (mm)	Head-Tail end Length (mm)	Condition tail end	Comments
1	1		47	53		
2	1	1.14	46	53	1.50	
3	1	1.12	56	62	0.91	
4	1	0.29	38	43	0.72	
5	1	0.75	48	54	0.93	
6	1	0.29	35	40	0.91	
7	1	0.47	43	47	0.90	
8	1	0.34	41	46	0.69	
9	1	0.28	37	40	0.89	
10	1	0.73	50	56	0.81	
	Average	**0.60**	**44.10**	**49.40**	**0.92**	
	St Dev	**0.35**	**6.54**	**7.34**	**0.24**	
	Median	**0.47**	**44.50**	**50.00**	**0.90**	
	CV	58%	15%	15%	26%	
11	2	1.31	59	65	0.92	
12	2	0.94	48	54	1.16	
13	2	0.76	47	51	1.12	
14	2	1.00	52	57	1.05	
15	2	0.82	51.5	58	0.81	
16	2	0.53	45	50	0.84	
17	2	0.64	47	51	0.94	
18	2	0.49	40	45	1.07	
19	2	0.59	43	48	1.06	
20	2	0.88	49	54	1.09	
	Average	**0.80**	**48.15**	**53.30**	**1.01**	
	St Dev	**0.25**	**5.27**	**5.70**	**0.12**	
	Median	**0.79**	**47.50**	**52.50**	**1.06**	
	CV	32%	11%	11%	12%	
21	3	0.78	50	56	0.86	
22	3	1.23	55	61	1.04	
23	3	0.24	35	40	0.76	
24	3	0.52	41	46	1.05	
25	3	0.73	45	60	0.65	
26	3	0.73	50	55	0.85	
27	3	0.20	35	40	0.64	
28	3	0.55	45	50	0.86	
29	3	0.32	40	45	0.71	
30	3	0.25	38	42	0.68	
	Average	**0.55**	**43.40**	**49.50**	**0.81**	
	St Dev	**0.32**	**6.79**	**8.06**	**0.15**	
	Median	**0.53**	**43.00**	**48.00**	**0.81**	
	CV	58%	16%	16%	19%	
	Average (All)	**0.65**	**45.22**	**50.73**	**0.91**	
	Median (All)	**0.64**	**45.50**	**51.00**	**0.90**	
	St Dev (All)	**0.32**	**6.38**	**7.10**	**0.19**	
	CV (All)	**49%**	**14%**	**14%**	**20%**	

Appendix 8: Scoring for chronic boron toxicity test to Canterbury mudfish (Hickey et al. 2018)

Category	Freshwater/non-metal/non- plant	*Citation No.*	0
Paper and data unique identifier(eg. 1-1,10-150)		**Species (Scientific name)**	
Citation	Hickey, C.W., Thompson, K.J., Bell, S., Arnold, J. (2018). Chronic sensitivity of juvenile Canterbury mudfish (Neochanna burrowsius) and periphyton (Rhizoclonium sp.) to boron. BRL18202; 2018199HN. NIWA report prepared for Bathurst Resources Ltd.	**Lifestage**	
		Exposure duration	
		Measure of toxicity	
		Toxicity endpoint	
		Toxicity value	

Select the appropriate answer option to each question. Comments can be added to the cells in column H.

* If the selected answer option does not work try clicking on any other cell first and then click back on the answer option again.

No.	Question	Answer Options	Scores	Answers
1	Was the duration of the exposure stated (e.g. 48 or 96 h)?	(•) Yes (10) ( ) No (0)	10	
2	Was the biological endpoint (e.g. immobilization or population growth) stated and defined (10 marks)? Award 5 marks if the biological endpoint is only stated.	(•) Stated AND defined (10) ( ) Only stated (5) ( ) Not stated OR defined (0)	10	
3	Was the biological effect stated (e.g. LC or NOEC)?	(•) Yes (5) ( ) No (0)	5	
4	Was the biological effect quantified (e.g. 50% effect, 25% effect)? **Note:** The effect for NOEC and LOEC data must be quantified.	(•) Yes (5) ( ) No (0)	5	
5	Were appropriate controls (e.g. a no-toxicant control and/or solvent control) used?	(•) Yes (5) ( ) No (0)	5	
6	Was each control and contaminant concentration at least duplicated?	(•) Yes (5) ( ) No (0)	5	
7	Were test acceptability criteria stated (e.g. mortality in controls must not exceed a certain percentage) (5 marks)? **OR** Were test acceptability criteria inferred (e.g. test method used [USEPA, OECD, ASTM etc.]) (award 2 marks). **Note:** Data that fail the acceptability criteria are automatically deemed to be of unacceptable quality and must not be used.	(•) Stated (5) ( ) Inferred (2) ( ) Not stated OR inferred (0)	5	
8	Were the characteristics of the test organism (e.g. length, mass, age) stated?	(•) Yes (5) ( ) No (0)	5	
9	Was the type of test media used stated?	(•) Yes (5) ( ) No (0)	5	
10	Was the type of exposure (e.g. static, flow through) stated?	(•) Yes (4) ( ) No (0)	4	
11	Were the contaminant concentrations measured at the beginning and end of the exposure (4 marks)? Award 2 marks if they were measured only once during the test. **Note:** If the concentrations were not measured then automatically the data cannot be high quality.	(•) Measured twice (4) ( ) Measured once (2) ( ) Not measured (0)	4	
12	Were parallel reference toxicant toxicity tests conducted?	( ) Yes (4) (•) No (0)	0	
13	Was there a concentration–response relationship either observable or stated?	(•) Yes (4) ( ) No (0)	4	
14	Was an appropriate statistical method or model used to determine the toxicity? **Note:** They should be accepted by a recognised national or international regulatory body (e.g. USEPA, OECD, ASTM).	(•) Yes (4) ( ) No (0)	4	Statistical tests applied. Best professional judgement applied to interpretation of ANOVA results relative to Control performance.
15	For LC/EC/BEC data was an estimate of variability provided? **OR** For NO EC/LOEC data was the significance level 0.05 or less?	(•) Yes (4) ( ) No (0)	4	
16	Were the following parameters measured and stated? (3 marks if measured and stated, 1 mark if just measured).			
16	pH	(•) Measured AND stated (3) ( ) Only stated (1) ( ) Not measured OR stated (0)	3	
16	Dissolved Oxygen	(•) Measured AND stated (3) ( ) Only stated (1) ( ) Not measured OR stated (0)	3	
17	Was the temperature measured and stated (3 marks)? Award 1 mark if the temperature was measured but not stated or if only the temperature settings of the room or chamber are stated.	(•) Measured AND stated (3) ( ) Measured OR stated (1) ( ) Not measured OR stated (0)	3	
18	Were test solutions, blanks and/or controls tested for contamination OR were analytical reagent grade chemicals used or the highest possible purity chemicals used for the experiment? (3 marks).	(•) Yes (3) ( ) No (0)	3	

Total score		87
Total Max. possible score for the various types of data and contaminants	91	
Quality Score = [Total score / Total possible score] * 100 =		95.6%

H = high quality — Quality score ≥ 80%
A = acceptable quality — > 50% Quality score < 80%
U = unacceptable quality — Quality score ≤ 50%