Moolarben Coal Independent Water Quality Study (IWQS)
Main Report

Report By
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Date: 10 December 2021

For
Moolarben Coal Operations Pty Ltd
ABN 59 077 939 569

Disclaimer

Acknowledgements

Dr. Rick Krassoi, Director Ecotox Services Australia, contributed to the design of ecotoxicity studies and provided supporting detail for the collection of a local Mayfly species. The handling of laboratories studies, technical input into the report, and field work in the Upper Goulburn River contributed by Fiona Henderson are acknowledged.

Dr. Rick Krassoi Ecotox Services Australia provided direction on nymph-stage Mayfly identification and loaned out equipment (waders, collection trays, buckets for Mayfly transport, battery driven aerators and D-nets) essential to successful Mayfly extraction to Sydney on day of collection. Collection of Mayflies in the field was directed by Fiona Henderson, who has experience and familiarity with the habitat , collection and identification protocols of the Mayfly species. Based on the expert advice of Dr. Rick Krassoi, Ecotox Services Australia, ecotoxicity testing of Mayflies have to be completed on Mayflies reared in the natural environment from the nymph stage (not in a laboratory setting).

Fiona Henderson assisted with the analysis of ecotoxicity data in the report, the utilization of test species responses for the generation of species sensitivity distribution plots and the derivation of site specific guideline values utilizing the BurrliOZ 2.0 software (distributed and recommended for use by CSIRO from the ANZECC Water Quality Guidelines).

Executive Summary

In July 2019 Moolarben Coal Operations (MCO) received approval (Project Approval 05_0117 and Project Approval 08_0135) for the Open Cut Optimisation Modification (Mod 14). The Modification allowed, amongst other things, installation of water treatment facilities and changes to controlled releases of water from the Moolarben Coal Complex (MCC). In response to the Modification, Project Approval (05_0117) was revised to include the requirement for an Independent Water Quality Study (IWQS) under Condition 32A, as reproduced below:

By 1 December 2021, unless the Secretary agrees otherwise, the Proponent must complete an Independent Water Quality Study in accordance with ANZECC Guidelines, in consultation with EPA and to the satisfaction of the Secretary. The study must:

- *a) be undertaken by an independent scientific organisation with suitable water expertise whose appointment has been approved by the Secretary;*
- *b) collect and utilise water quality monitoring data in the Goulburn River using locations endorsed by the EPA;*
- *c) determine appropriate background salinity and heavy metal levels for the Goulburn River upstream of the project site;*
- *d) recommend an electrical conductivity limit for treated water discharges to the Goulburn River from the Moolarben Coal Complex based on the process outlined in the ANZECC Guidelines.*

The Goulburn River is the key drainage line in the region, running from the west of MCC to the northeast, including through the Goulburn River Diversion developed by Ulan Coal in the 1980s. The Goulburn River receives tributary inputs from Moolarben Creek (which includes Ryans Creek and Lagoon Creek), Sportsmans Hollow Creek and Ulan Creek, as well as licensed discharges from MCC (directly into Goulburn River Diversion) and Ulan Coal (via Ulan Creek). See Figure ES1 for the regional conceptual site model.

Condition 32A(a) – *The study must be undertaken by an independent scientific organisation with suitable water expertise whose appointment has been approved by the Secretary.*

The Secretary of the Department of Planning, Industry and Environment approved the University of Queensland – Sustainable Minerals Institute led by Professor Barry Noller as the independent scientific organisation to undertake the IWQS via correspondence dated 18 July 2019.

Condition 32A(b) – *The study must collect and utilise water quality monitoring data in the Goulburn River using locations endorsed by the EPA.*

During the development of this study, review of the regional water setting and available water quality showed that the monitoring site UCML SW01 / SW12 was the only appropriate site that could be used to determine background water quality conditions for the upper Goulburn River.

UCML SW01 / SW12 was endorsed by the New South Wales Environment Protection Authority (NSW EPA) via correspondence received 22 May 2020 as required by Condition 32A(b).

As required by Condition 32A(c), monitoring data at UCML SW01 / SW12 (Upper Goulburn River reference monitoring site) was used to determine background salinity and heavy metals for the Goulburn River upstream of MCC following the site-specific water quality assessment decision tree in accordance with the Australian and New Zealand Water Quality Guidelines (ANZG, 2018).

Condition 32A(c) – *The study must determine appropriate background salinity and heavy metal levels for the Goulburn River upstream of the project site.*

This analysis indicated that 80th percentile background key heavy metals (filtered to <0.45 µm) and salinity concentrations were applicable for the Goulburn River.

The background concentrations (retrieved from upstream reference site UCML SW01 / SW12) were determined to be:

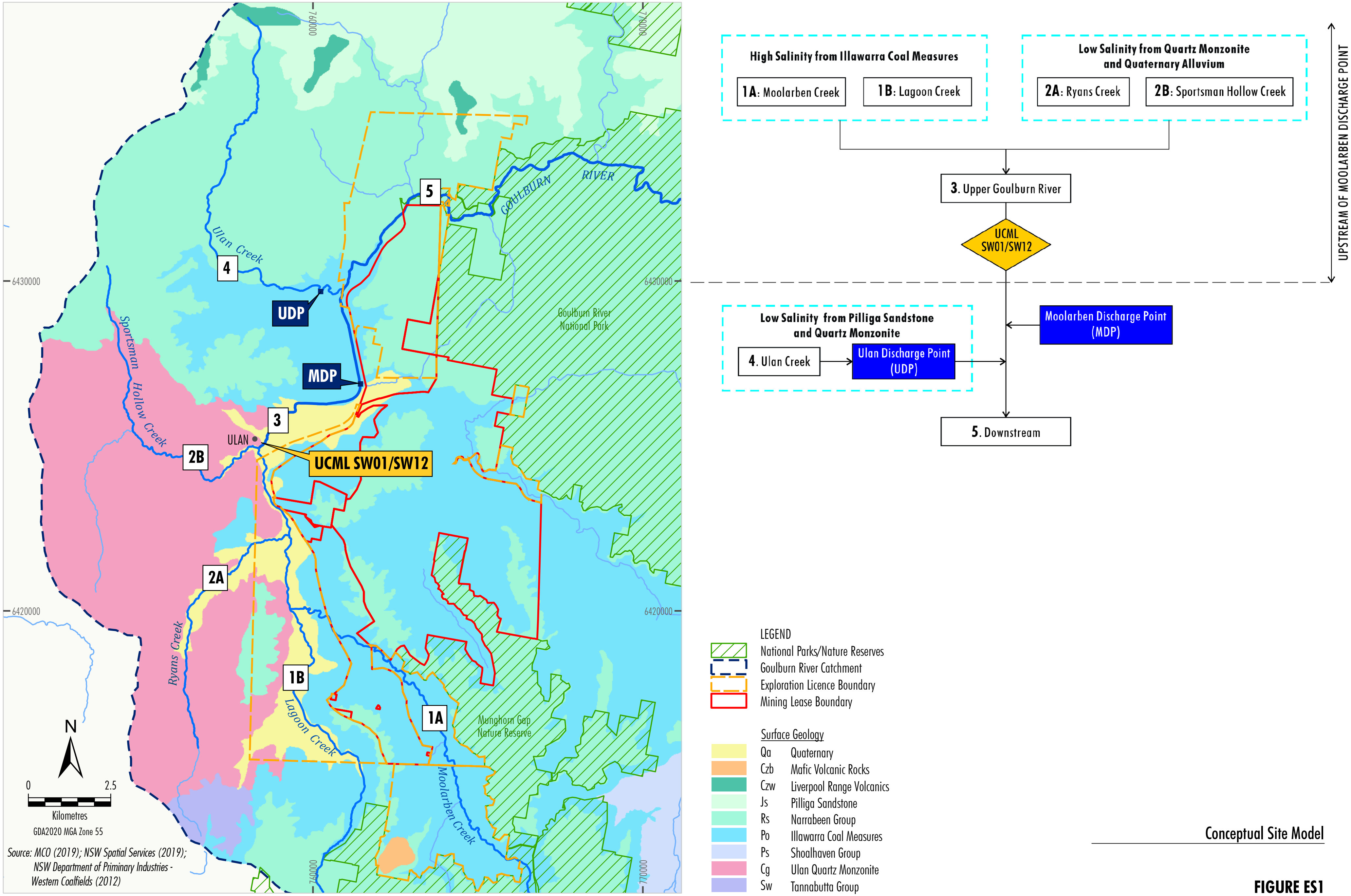
Salinity: 400 mg/L (equivalent to 619 µS/cm)

Aluminium:	126 µg/L
Manganese:	397 µg/L
Nickel:	1.0 µg/L
Zinc:	6.0 µg/L
Cadmium:	0.05 µg/L
Copper:	0.5 µg/L
Arsenic:	0.5 µg/L
Lead:	0.5 µg/L

Condition 32A(d) – *The study must recommend an electrical conductivity limit for treated water discharges to the Goulburn River from the Moolarben Coal Complex based on the process outlined in the ANZECC Guidelines.*

Based on multiple lines of evidence (including site-specific ecotoxicity studies, background concentrations, no apparent toxicity response to treatment waters and detailed salinity conversion factor considerations), the current NSW EPA limit for electrical conductivity (EC) of 685 µS/cm is considered acceptable, and given the lack of toxicity responses to salinity, a limit that reflects the historical EC limit at the MCC, as well as the current EC limit at Ulan, of 900 µS/cm could also be considered acceptable.

A summary of the multiple lines of evidence referenced in the above conclusion is discussed below.



Source: MCO (2019); NSW Spatial Services (2019); NSW Department of Primiary Industries - Western Coalfields (2012)

High Salinity from Illawarra Coal Measures

1A: Moolarben Creek

1B: Lagoon Creek

Low Salinity from Quartz Monzonite and Quaternary Alluvium

2A: Ryans Creek

2B: Sportsman Hollow Creek

3. Upper Goulburn River

UCML SW01/SW12

UPSTREAM OF MOOLARBEN DISCHARGE POINT

Low Salinity from Pilliga Sandstone and Quartz Monzonite

4. Ulan Creek

Ulan Discharge Point (UDP)

Moolarben Discharge Point (MDP)

5. Downstream

Conceptual Site Model

FIGURE ES1

Ecotoxicity Testing

Ecotoxicity testing was undertaken as a weight of evidence contribution to develop appropriate guideline limits, in accordance with the ANZG (2018) and its guidance documents. Testing was conducted on Water Treatment Plant (WTP) Filtrate, nickel, zinc and sulfate. WTP Filtrate (the product of ultrafiltration which is used as input to reverse osmosis and as a component of WTP Discharge water) was included to quantify any ecotoxicity response to mine water. The metals included in the analysis (nickel and zinc) were chosen by considering their significance for identifying concentrations that are most relevant to the protection of aquatic species in the Goulburn River, and by considering water quality monitoring data. Selection of analytes was based on measured filtered nickel and zinc concentrations when compared to other metals in samples taken from the water treatment plant. Additionally, with the relatively high pH of Goulburn River, nickel and zinc remain soluble as demonstrated by speciation modelling. As a result nickel and zinc were prioritised as heavy metal analytes. Sulfate concentration was selected as an analyte as it is representative of salinity derived from natural coal mineralisation.

Eight test aquatic species were used across ten tests to measure ecotoxicity responses to dosed analytes in diluent control water sampled at the NSW EPA-endorsed monitoring location UCML SW01 / SW12. Plant treatment waters (WTP Filtrate, WTP Permeate, and WTP Discharge) were included as additional controls in the ecotoxicity testing. Ecotoxicity testing was undertaken by Ecotox Services Australia who supplied seven of the relevant aquatic test species. The eighth test species, Mayfly (*Nousia sp.* AV1. [*Leptophlebiidae*]) was locally sourced from Ryans Creek and transported to Ecotox Services Australia for testing, as recommended by the NSW EPA.

Initial testing was undertaken on the WTP Filtrate samples (EC = 2110 µS/cm; salinity = 1593 mg/L) with the aim to develop a Species Sensitivity Distribution (SSD) from the collected data, which can be used to indicate the concentration at which 95% of aquatic species would be protected. However, it was determined that the development of an SSD for WTP Filtrate was not possible, as every ratio tested (including 100% WTP Filtrate) demonstrated no statistically significant toxicity to any aquatic test species (i.e. 90 to 100% unaffected for all species).

WTP Discharge (typical water output from the WTP post-treatment) samples were also tested as a control. The characteristics of WTP Discharge are determined by the ratio of WTP Filtrate blended with WTP Permeate in order to meet the current limits detailed in MCO's Surface Water Management Plan. WTP Discharge also showed no statistically significant toxicity response in aquatic test species (90 to 100% unaffected for all species).

As a result of the WTP Filtrate water showing no toxicity response, a direct SSD for EC could not be developed, and the sulfate SSD was instead used as an analogue to assess the toxicity response to EC. The SSDs developed using ecotoxicity testing results for nickel, zinc and sulfate are shown in Figures ES2, ES3 and ES4. Figures ES2, ES3 and ES4 show the Site Specific Guideline Values (SSGVs) for nickel, zinc and sulfate that are generated for the Goulburn River at a level of 95% aquatic species protection. Note the blue dashed lines indicate the 95% confidence interval.

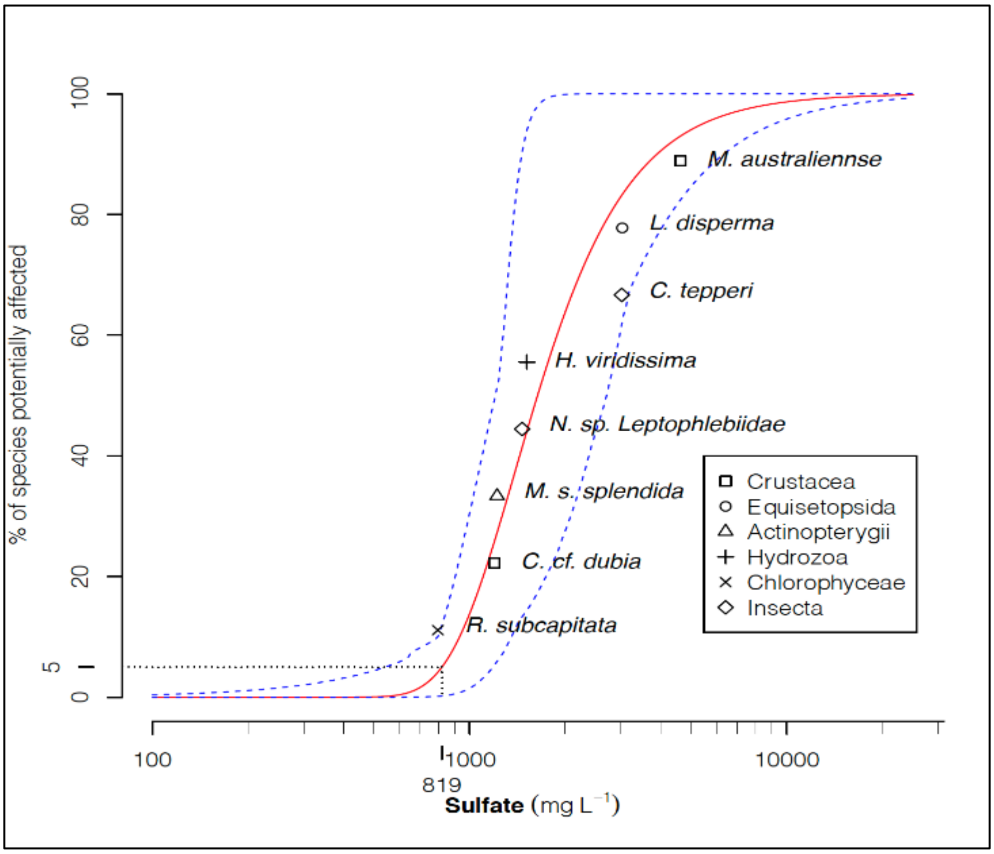


Figure ES2. SSD for Sulfate including SSGV for 95% Species Protection within the Goulburn River

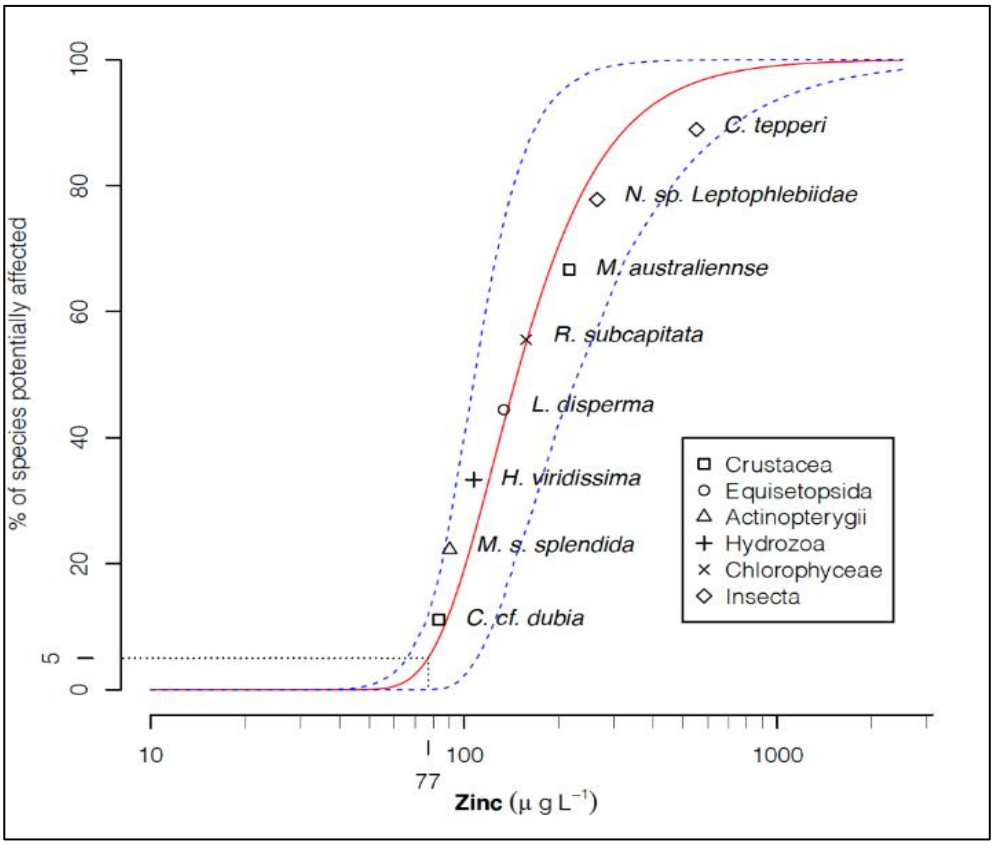


Figure ES3. SSD for Zinc including SSGV for 95% Species Protection within the Goulburn River

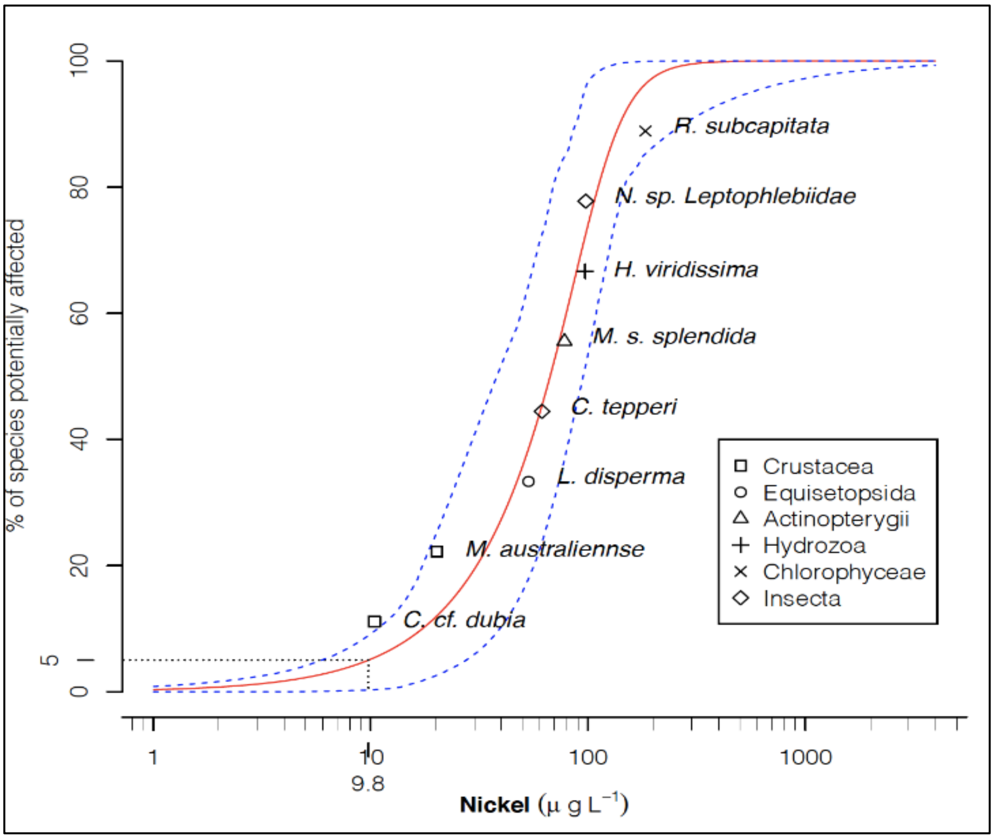


Figure ES4. SSD for Nickel including SSGV for 95% Species Protection within the Goulburn River

A summary of the SSGVs (with 95% confidence intervals) for nickel, zinc and sulfate are follows:

- Nickel: 9.8 (5.9-25.8) µg/L
- Zinc: 77 (65-112) µg/L
- Sulfate: 819 (544-1314) mg/L

Under MCO's Surface Water Management Plan, the current ANZG (2018) aquatic toxicant guideline for freshwater species (for 95% level of protection) discharge limit for zinc (8.0 µg/L) is well below the derived value, while the current discharge limit for nickel (11.0 µg/L) is within the range of the derived value.

Converting the Sulfate SSGV to Electrical Conductivity

An SSGV for EC could not be developed based on responses to WTP filtrate, as the testing demonstrated no statistically significant toxicity to any aquatic test species. To enable the derivation of an SSGV for EC, a conversion of the sulfate SSGV to EC was developed.

The salinity of the Goulburn River and Moolarben treatment waters were examined to define a relationship (salinity factor, $k = \left[\frac{TDS}{EC}\right]$, default of 0.73, Appendix B Table B.2) between EC (in µS/cm) and total dissolved solids (TDS in mg/L), a direct measure of salinity. Sodium sulfate can be considered the most toxic of any relevant component of salinity. Additionally, investigations of salinity characteristics reveal that the Goulburn River and Moolarben treatment waters have higher portions of sulfate content. As a result, a sodium sulfate SSGV can be considered representative of a salinity SSGV for the purposes of ecotoxicity testing. Using this method, the SSGV for sulfate detailed above

(Figure ES2) can be calculated as a salinity SSGV of 1211 (804.5-1943.2) mg/L, equivalent to an EC SSGV of 1670 (1110-2680) µS/cm (for a detailed explanation refer to Section 8.5).

As an additional line of evidence, a more robust, direct method of converting salinity to EC was also examined using linear regression (Section 8.5, Chart 11). Measured EC (µS/cm) data (y) associated with dosed sulfate measurements during ecotoxicity testing were plotted against sulfate concentration (x) of the same solution to give the equation $y = 2.3585x + 717.41$ with $R^2 = 0.9988$ and $P<0.001$. Using the derived regression, the ecotoxicity-derived sulfate SSGV can be estimated as an EC (with 95% confidence interval) SSGV of 2649 (2000-3816) µS/cm with a TDS calculated equivalent of 1920 (95% confidence interval: 1450-2766) mg/L.

Background Electrical Conductivity Values in the Goulburn River

Utilising the basic salinity factor method described above, 80th percentile values for background EC were determined for a number of monitoring sites on the Goulburn River and nearby tributaries, expressed with 95% CI: 619 (606-631) to 890 (872-908) µS/cm (excluding SW08 and SW06 outliers). As expected, background EC values measured in the Goulburn River sites are much lower than the toxicity threshold estimated for salinity values. Although the direct ecotoxicity testing calculated an SSGV for EC for 95% aquatic species protection of 2649 µS/cm, background concentrations were also considered with the proposed recommendations detailed above, following the guidance per ANZG (2018).

Glossary Of Terms and Acronyms

Acute toxicity – Rapid adverse effect caused by a substance in a living organism. The term can be used to define either the exposure or the response to an exposure (effect). ANZG (2018) define acute exposure as being between 24 and 96 h duration for multi-celled organisms and being between 24 and 72 h duration for single-celled organisms.

ACR $_{Toxicant}$ – Defines the acute to chronic ratio endpoint conversion for a given toxicant (nickel, zinc or sulfate).

ACR – Acute to chronic ratio. The ratio of the acute toxicity to the chronic toxicity of a species to a toxicant. The acute and chronic data do not have to have the same measure of toxicity or endpoint, but they must be for the same species and have been presented in the same paper or at least determined in the same laboratory (Warne, 2001).

Actinopterygii – Members are known as ray finned fishes and are the largest most successful group of fishes, making up half of all living vertebrates.

Acute toxicity/endpoint – test conducted to determine the short-term adverse effects of a chemical or substance on test species when administered as a single dose or in multiple doses over a period (typically over 24 hours, but also dependent on the species tested and timeframe applied to elicit a short-term response).

Analyte – A substance for which the chemical constituents are being identified and measured.

AR – Analytical reagent. A class of chemical reagents for analytical testing in qualitative or quantitative analysis, utilized to detect the presence or absence of another substance.

Anion – A negatively charged ion.

ANZECC – Australian and New Zealand Environment Conservative Council.

ANZG – Australian and New Zealand Guidelines (2018).

AM – Arithmetic mean (or average) of a set of numbers. An average, is a value obtained by dividing the sum of several quantities by their number.

ARMCANZ – Agriculture and Resource Management Council of Australia and New Zealand, 2000.

AUSRIVAS – Australian River Assessment System. A system developed under the National River Health Program funded by the Federal Government in 1994, used to predict and assess the biological health of Australian rivers and provides data on macroinvertebrate species diversity.

Bicarbonate (HCO_3) – A component of total Alkalinity. The Goulburn River is mainly composed of bicarbonate.

Blank (Laboratory) – A blank sample carried through all steps of the preparation of the sample process to analysis. Typically analysed with each sample batch, blanks are used to demonstrate that an instrument system is free of contamination by being analysed prior to a sample analysis run and following the analysis of highly contaminated samples.

BurrliOZ (2.0) – Software application (version 2.0), is underpinned by the R statistical software, which enables users to derive trigger values of toxicants in accordance with ANZG (2018) guidelines for fresh and marine waters by utilizing the inverse Weibull distribution function.

Cation – A positively charged ion.

Chlophyceae – A class of green algae, distinguished by its ultrastructural morphology.

Chronic toxicity/endpoint – An adverse biological response that is irreversible (i.e. death) or occurs due to the rate of injury being greater than the rate of repair during a prolonged exposure to a toxicant. It also refers to low level long-term effects such as reduction in growth or reproduction and adverse impacts that affect survival. The term can be used to define either the exposure of a test species or its response to an exposure (effect). ANZG (2018) define chronic exposure as being greater than 96 h duration for multi-celled organisms and being equal to or greater than 72 h duration for single-celled organisms.

Control (control treatment) – In toxicity tests, the control is that treatment in which the test organisms are not subjected to the test substance. The control is used as a standard comparison, to check that the outcome of the experiment reflects the test conditions and not some unknown factor.

CRM – Certified reference material.

Crustacea – Form a large anthropoda taxon including animals such as crabs, lobster, crayfish, shrimp, krill, prawns, woodlice, barnacles, copepods, amphipods, and mantis shrimp.

Diluent Control – A sample used to dilute toxicant stock solutions to meet nominal concentrations, often utilized as a control during ecotoxicity testing. In this report SW12 (natural upper Goulburn River sample) is utilized as a diluent control in toxicity testing.

DTA – Direct Toxicity Assessment. The use of toxicity tests to determine the acute and/or chronic toxicity of effluents and other mixtures of potential toxicants.

Duplicate (Laboratory) – an additional sample taken from the same container and analysed independently in the same instrument run and/or additional runs.

DMW – Dilute Mineral Water.

DO – Dissolved oxygen concentration.

DOC – Dissolved Organic Carbon.

EC – Electrical Conductivity measured in µS/cm, which is an estimate of the amount of total dissolved salts (TDS).

EC10 – The concentration of a chemical that is estimated to cause a response in 10% of the test organisms or causes the mean response of the organisms to differ from the control by 10%. The EC10 is usually expressed as a time-dependent value, e.g., 24 h EC10 is the concentration estimated to cause an effect on 10% of the test organisms after 24 h of exposure.

EC50 – The concentration of chemical that is estimated to cause a response in 50% of the test organisms or causes the mean response of the organisms to differ from the control by 50%. The EC50 is usually expressed as a time-dependent value, e.g., 24 h EC50 is the concentration estimated to cause an effect on 50% of the test organisms after 24 h of exposure.

Ecosystem Receptors – include biodiversity, toxicity and biomarkers as lines of evidence in ANZG (2018) water quality guidelines. Biodiversity, is often linked to management goals and considered a key line of evidence for an ecosystem receptor, while toxicity and biomarkers provide evidence for inference and causation.

EPDFC –Empirical cumulative probability distribution function used in the BurrliOZ 2.0 software to derive site specific distributions of aquatic species toxicity responses from EC10/IC10 values.

Endpoint – The biological response of test organisms in toxicity tests that is measured (e.g., lethality, immobilisation).

EPA – Environment Protection Agency. In this report NSW EPA, unless stated otherwise, refers to the New South Wales Environment Protection Agency.

EPL – Environment Protection Licence.

EPBC Act – Environment Protection and Biodiversity Conservation Act, 1999.

Equisetopsida – Class of spore bearing vascular plants. Most members of the group are extinct and only known due to discovery of fossilized remains. The only living genus is Equisetum.

ESA – Ecotox Services Australia.

Exposure – is direct or indirect contact with a toxin (natural or anthropogenic substance or chemical agent).

Fish Imbalance – Endpoint measurement of acute testing for fish. Demonstrating visible abnormalities in regard to equilibrium (e.g., loss of balance, head up or down, floating at surface or sinking) appearance (weak or dark pigmentation, exophthalmia), ventilatory behaviour (irregularity of ventilation) and swimming behaviour (hyper or hypo activity, immobility, convulsions etc.).

Fulvic Acid – A component of humic substances (natural organic matter) that remains soluble defined as either carboxylic (FA1) or phenolic (FA2) groups. Modelling of fulvic acids tend to be hypothetical and should be considered as a way to rationalise chemical behaviour.

GS – Gauging Station, used to monitor streams, rivers, lakes, canals or reservoirs collecting data on water height, chemistry, velocity and temperature.

Goodness of Fit – A statistical measure of how well a set of observations fit the predicted pattern of a probability distribution function.

Goulburn River Catchment – The Goulburn River is a major tributary and resides in the upper catchment of the Hunter River region of New South Wales. The river is situated on the eastern slopes of the Great Dividing Range, where majority of the water course (total 221 km) flows through the Goulburn River National Park. The river reaches its confluence with the Hunter River south of Denman.

Goulburn River Diversion – In the 1980's the Goulburn River was diverted by Ulan Coal Mine to allow excavation works of coal at Ulan (west of Denman). Four kilometres of the Goulburn River was diverted to the mine's southern and eastern boundaries.

GM – Geometric Mean, the average rate of return of a set of values, calculated using the product of terms.

Growth Rate – Also defined as growth inhibition, is a utilized for chronic toxicity testing. Growth is usually defined in relation to a control and attempts to evaluate growth over several generations (population growth rate) of testing or under a set of defined conditions (unaffected growth).

GV – Guideline Value.

Hardness –Measured as mg/L of calcium carbonate ($CaCO_3$).

Hunter River Catchment– A river catchment in New South Wales, situated within the Hunter Valley. The watercourse flows south and then east towards Newcastle and out to sea (total watercourse ~468 km).

Hydrozoa – distinguished from other groups by their complex life cycle, hydrozoan is a subgroup of cnidarians. Typical habitats are marine waters, but there are few that reside in fresh or brackish waters. In this report *Hydra viridissima* is a hydrozoan test species for the Goulburn River.

IC10 – The concentration that inhibits an endpoint by 10 percent (e.g., the IC10 ([reproduction]) is the concentration that inhibits reproduction by 10%). It represents a point estimate of a concentration of test material that causes a designated percent inhibition compared to the control. The IC10 is usually expressed as a time-dependent value, e.g., 24 h IC10 is the concentration estimated to cause an effect on 10% of the test organisms after 24 h of exposure.

IC50 – The concentration that inhibits an endpoint by 50 percent (e.g., the IC50([reproduction]) is the concentration that inhibits reproduction by 50%). It represents a point estimate of a concentration of test material that causes a designated percent inhibition compared to the control. The IC10 is usually expressed as a time-dependent value, e.g., 24 h IC50 is the concentration estimated to cause an effect on 10% of the test organisms after 24 h of exposure.

Insecta – Insect class holds one million named species and account for the majority of species of animals on earth. Insects are found in almost all terrestrial and freshwater habitats.

ISO/IEC – International Organization for Standardization (ISO) and International Electrotechnical Commission (IEC).

IWQS – Independent Water Quality Study.

Lagoon Creek – tributary input into Moolarben creek residing in the upper Goulburn River Catchment. Water quality of this tributary is influenced by the natural mineralisation of the underlying Illawarra Coal Measures.

LC10 – The concentration of material in water that is estimated to be lethal to 10% of the test organisms. The LC10 is usually expressed as a time-dependent value e.g., 24 h or 96 h LC10, the concentration estimated to be lethal to 10% of the test organisms after 24 or 96 h of exposure.

LC50 – The concentration of material in water that is estimated to be lethal to 50% of the test organisms. The LC50 is usually expressed as a time-dependent value e.g., 24 h or 96 h LC50, the concentration estimated to be lethal to 50% of the test organisms after 24 or 96 h of exposure.

LCS – Laboratory Control Sample.

Level of protection – The degree of protection afforded to a water body based on its ecosystem condition (current or desired health status of an ecosystem relative to the degree of human disturbance). The level of protection informs the acceptable water quality for a waterway.

LOEC – The lowest observed concentration of a toxicant used in a toxicity test that has a statistically significant ($P \leq 0.05$) adverse effect on the exposed population of test organisms compared with the controls. This is estimated by hypothesis-based statistical methods and is therefore not a point estimate.

LOP – Limit of Protection.

Matrix Spike – Often used as a form of quality control, to evaluate the effects of sample matrices on the performance of an analytical method.

Max – Maximum.

Mayfly – An aquatic insect species (macroinvertebrates) belonging to the Arthropoda phylum. These insects are very sensitive to pollution and typically found at high quality, minimally polluted sites. They are one of the most common indicators of an ecosystems health and utilized in ecotoxicity testing.

MCC – Moolarben Coal Complex.

MCO – Moolarben Coal Operations.

MCM – Moolarben Coal Mines Pty Ltd.

Min – Minimum.

ML – Mining Lease.

Moolarben Creek – Tributary to the upper Goulburn River that is has elevated concentrations of metals and salinity associated with natural mineralisation from underlying geology that encompasses the Illawarra Coal Measures.

Moolarben Dam – Located in Moolarben Creek, ~1.5 km upstream of Sportsmans Hollow Creek. Constructed in 1957 and owned by UCML, the dam has a catchment area of 109 km^2 and was used to supply water to the Ulan Power Station (decommissioned in 1968).

NATA – National Association of Testing Authorities.

NEC – No effect concentration. Typically defined by application of an arbitrary conversion factor e.g., 1,000 to an EC/IC50 value.

NOEC – The highest observed concentration of a toxicant used in a toxicity test that does not exert a statistically significant adverse effect ($P<0.05$) on the exposed population of test organisms compared to the controls. This is estimated by hypothesis based statistical methods and is therefore not a point estimate.

NSWEPA – New South Wales Environment Protection Agency.

Percentile – Percentile rank (or x-th percentile) is the value at or below which a percentage of data falls. E.g., the 50th percentile (or 50%tile) is the median, and is the score at or below which 50% of the data in the distribution may be found.

Piper Diagram – is an effective graphical procedure and tool used to segregate relevant analytical data to understand the sources of dissolved constituents (anions and cations) in water.

Pressures – External activities that affect water quality such as land-based activities (i.e., seepage associated with mining activities).

Protective concentrations (PC) – The concentration predicted by species sensitivity distribution methods that will protect a chosen percentage of species from experiencing toxic effects. For example, the PC99 should protect 99% of species in the ecosystem being considered. The toxic effects that are being prevented will depend on the type of toxicity data used to derive the PC values. Thus, if sub-lethal EC10 data are used to generate a PC95 – it will protect 95% of species from experiencing sub-lethal EC10 effects.

pH – Potential Hydrogen scale, which provides a measure of hydrogen ions in a liquid (water).

Probit Analysis – a statistical procedure and specialized form of regression analysis which is applied to binomial response variables (variables with only one of two positive outcomes – i.e. positive or negative). It is a standard method and form of analysis to evaluate dose response data.

PSER – Pressure-stressor-ecosystem receptors causal pathway is an ANZG (2018) recommended weight of evidence approach to measure indicators from multiple lines of evidence for water/sediment quality assessments.

QA/QC – Quality Assurance and Quality Control are activities that provide confidence in the accuracy and reliability of results in laboratories. QA are preventative processes that enable laboratories to achieve and maintain high levels of accuracy and proficiency through implementation of training, audits, and selection of tools. QC ensures precision and accuracy of results through quality control samples. QC allows laboratories to self-regulate testing and verify results are produced in accurate and precise manner by finding and correcting any flaws in the analytical process through controls before results are released.

RCE – Riparian, Channel and Environment is an inventory that has been developed to assess the physical and biological condition of small streams in lowland agricultural landscapes. It defines the structures of the riparian zone, stream channel morphology and biological condition of habitat.

Reproduction – Ability to produce offspring. Often used as a toxicity test evaluation of growth rate in chronic test assessments.

RO – Reverse osmosis.

Ryans Creek – Tributary input into lower segment of Moolarben Creek, just before the confluence of the upper Goulburn River. Ryans Creek has reduced salinity levels and good characteristics of water quality. Local species of Mayfly were collected in Ryans Creek for ecotoxicity testing.

Salinity Factor, k –The correlation between total dissolved solids (TDS in mg/L) and electrical conductivity (EC in µS/cm) represented as a ratio of TDS / EC.

Salinity – Measured as total dissolved solids (TDS in mg/L), salinity is defined as the dissolved salts (or total dissolved ions [TDI]) in a body of water.

SH – Stream Health, monitoring sites throughout the Goulburn River that monitor stream health.

SIGNAL –Stream Invertebrate Grade Number – Average Level – a biotic index for river macroinvertebrates for the bioassessment of water quality with set grade numbers between 1 and 10 to represent the water-quality sensitivities of taxa. The higher the SIGNAL value, the better the condition of the water quality at a site. SIGNAL is calculated as the arithmetic mean of the grade of each taxon within a sample. Pollution Tolerance Site SIGNAL scores are a component of the MCO Stream Health Monitoring Program (MCO, 2020).

SIS Control – A Swedish Standard (SIS) growth media control utilized in toxicity tests evaluating specific growth rate (toxicant effects of growth inhibition) of aquatic duckweed (*Lemna Disperma*).

SI Units – International System of Units, is the world's most widely used system of measurement.

Site-specific guideline value (SSGV) –The concentration of an analyte that should be met to avoid environmental damage occurring.

Site-specific trigger value (SSTV) –The concentration of an analyte that, once exceeded, should trigger a management response.

Speciation Modelling – Geochemical speciation modelling is completed using speciation models such as (MINTEQ 3.0 software), a tool that is used to define the potential chemical species within a water environment. This gives insight into the potential solubility of certain metals and ultimately the toxicity of metal species in the aquatic environment.

Species Sensitivity Distribution (SSD) – SSD is a statistical approach for predicting the threshold concentrations of a contaminant or effluent that will protect a specific proportion of aquatic species with a predetermined level of confidence.

Sportsmans Hollow Creek – tributary input into upper Goulburn River, has similar surface geology (granitic provenance) to that of Ryans Creek. Not much data regarding the water quality of Sportsmans Hollow Creek is available. It is expected that this tributary has similar water attributes to Ryans Creek, which has characteristically good water quality and reduced salinity levels.

Stressors – can be chemical, physical or associated with other causes (e.g., directly or indirectly toxic where guideline values are determined from background or reference site data or associated with other causes such as flow).

Surrogate Spike – A type of quality control used to measure the performance of organic testing by adding a known concentration to primary samples which are then analysed and reported.

Sub-lethal – A biological response that is less severe than death. Examples of sub-lethal effects include inhibition of reproduction, reduction in growth, reduction in population growth, inhibition of fertilisation and inhibition of development.

Survival – state of continuing to live or exist. Often a response of acute toxicity testing.

SW – Surface Water, monitoring sites throughout the Goulburn River that monitor surface water quality.

Taxonomic Groups – animal or plant group having a natural relation.

Total Alkalinity – Is a measure (in mg $CaCO_3$/L) of water to resist acidification. It is defined through the components: bicarbonate (HCO_3^- in mg/L), carbonate (CO_3^{2-} in mg/L) and hydroxide (OH^- in mg/L). The Goulburn River is primarily composed of bicarbonate (HCO_3^-).

Total Dissolved Ions (TSI) – total number of ions in a solution (i.e., water) expressed in mg/L. In most surface waters these include anions: Cl^-, HCO_3^-, SO_4^{2-} and cations: Na^+, K^+,Ca^{2+}, and Mg^{2+}.

Total Dissolved Solids (TDS) – a measure of salinity, is the mass of solutes remaining at, typically, 103.5℃ following drying of a water sample. TDS is measured as the "filterable component", which represents the concentration of dissolved substances in water, yet also includes mineral and organic matter that are not in ionic form (i.e., free silica, SiO_2 in mg/L).

Toxicant – A substance or chemical (anthropogenic or naturally occurring) that causes adverse effects in a living organism.

Toxicity – The inherent potential or capacity of a chemical to cause adverse effects in a living organism.

Toxicity test – A test that exposes living organisms to several concentrations of a substance that is under investigation, and evaluates the organism's responses.

TV – Threshold value. A toxicity threshold is the maximum exposure in which toxicity does not occur.

UCML – Ulan Coal Mine Ltd and is the operator of the Ulan Coal mine located to the north of Ulan.

Ulan – Ulan village is located northeast from Mudgee about 40 km and to the west of Moolarben Coal Complex.

Uncertainty – is a range of expected values and can be defined as the quantitative measurement of the variability within the data.

Visual MinTEQ (3.0) – A freeware chemical equilibrium model utilized for the calculation of metal speciation, solubility equilibria, and sorption (amongst other parameters) for natural waters.

Wilpinjong Creek – Tributary that borders the MCO and Wilpinjong Coal Mine. It is a headwater tributary of Wollar Creek, which joins the Goulburn River ~ 26 km downstream.

Wilpinjong Coal Mine – is located approximately 40 km north-east of Mudgee, near the village of Wollar within Mid-Western Regional Local Government Area, central New South Wales.

WTP Filtrate – Water that has undergone Ultrafiltration within the WTP. The WTP Filtrate is sent to undergo reverse osmosis treatment, or blended with WTP Permeate for discharge.

WTP Permeate – Water treatment plant reverse osmosis output water used to blend with WTP Filtrate to generate water quality conditions acceptable for release (WTP Discharge).

WTP Discharge – Treated waters released from the WTP under an Environment Protection Licence. Consists of a blend of WTP Filtrate and WTP Permeate.

WTP – Water Treatment Plant used to treat mine waters from the Moolarben Coal Complex and consists of ultrafiltration and reverse osmosis (Section 6.2).

WTW – A part of Xylem Analytics, WTW is a brand of electrical conductivity meter.

Units

h – Hour

km – Kilometre

L – Litre

m – Meter

mg – Milligram

ML – Megalitre

µL – Microlitre

µg/L – Micrograms per litre

µS/cm – Microsiemens per centimetre

Elements

Ca – Calcium

K – Potassium

Mg – Magnesium

Na – Sodium

Ni – Nickel

Zn – Zinc

SO_4^{2-} – Sulfate

HCO_3^- – Bicarbonate

$CaCO_3$ – Calcium Carbonate

Contents

List of Figures

List of Charts

1 Introduction

1.1 Background

The Moolarben Coal Complex is located approximately 40 kilometres (km) north of Mudgee in the Western Coalfields of New South Wales (NSW) (Figure 1). Moolarben Coal Operations Pty Ltd (MCO) is the operator of the Moolarben Coal Complex (MCC) on behalf of the Moolarben Joint Venture (Moolarben Coal Mines Pty Ltd [MCM], Yancoal Moolarben Pty Ltd (YM) and a consortium of Korean power companies). MCO, MCM and YM are wholly owned subsidiaries of Yancoal Australia Limited (Yancoal).

Mining operations at the Moolarben Coal Complex are currently approved until 31 December 2038 and would continue to be carried out in accordance with Project Approval (05_0117) (Moolarben Coal Project Stage 1) as modified and Project Approval (08_0135) (Moolarben Coal Project Stage 2) as modified.

In June 2019, MCO received approval from the NSW Government Department of Planning and Environment for the proposed Open Cut Optimisation Modification (Mod 14). The Modification allows for the release of water from an on-site water treatment plant (WTP), including ultrafiltration and reverse osmosis (RO), into the Goulburn River. The general arrangement of the Moolarben Coal Complex, showing the surface water setting including the licensed discharge point, is provided in Figure 2.

Project Approval (05_0117) has been modified to include Condition 32A in response to Mod 14. Condition 32A is reproduced below as follows:

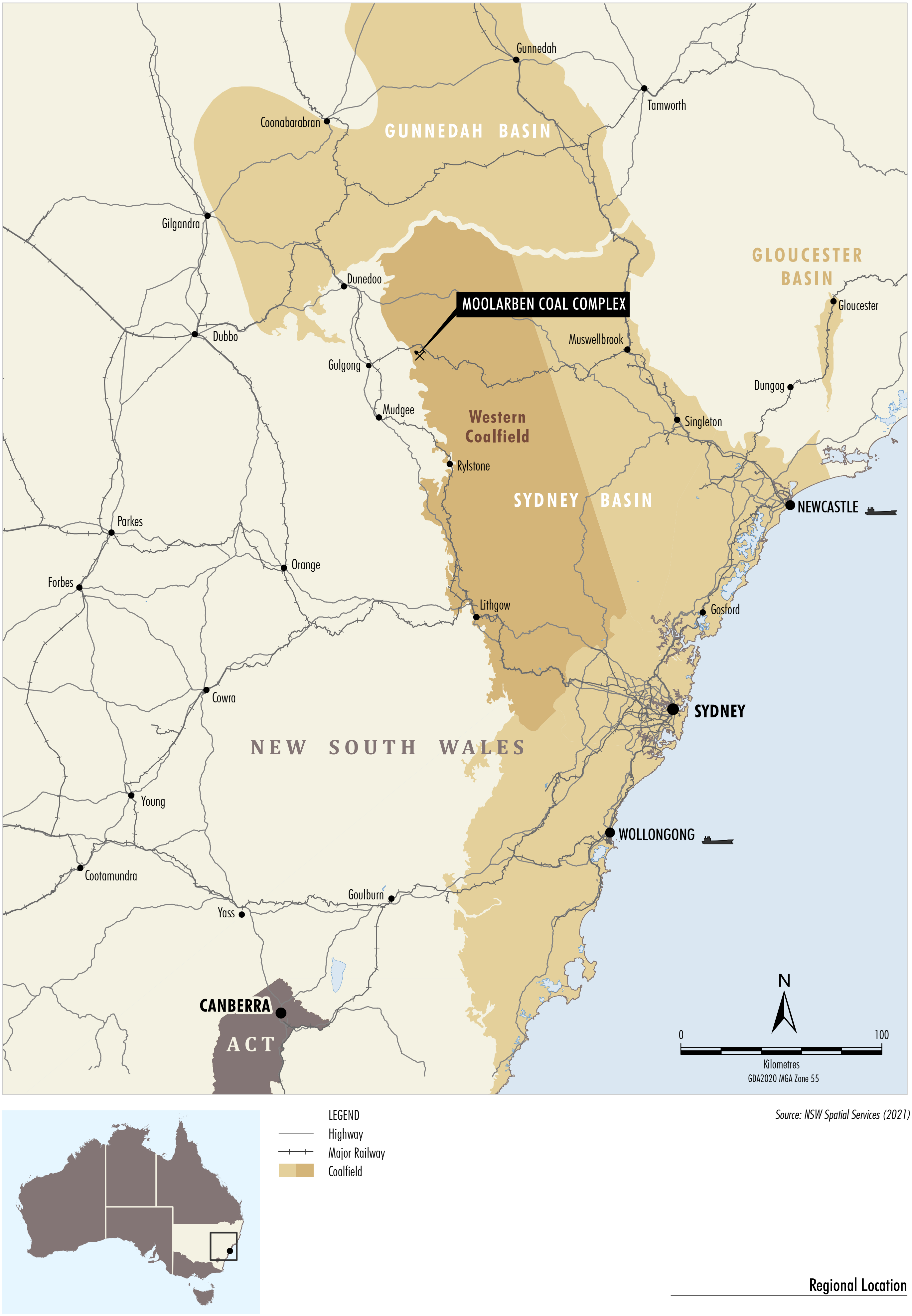
By 1 December 2021, unless the Secretary agrees otherwise, the Proponent must complete an Independent Water Quality Study in accordance with ANZECC Guidelines, in consultation with EPA and to the satisfaction of the Secretary. The study must:

- *a) be undertaken by an independent scientific organisation with suitable water expertise whose appointment has been approved by the Secretary;*
- *b) collect and utilise water quality monitoring data in the Goulburn River using locations endorsed by the EPA;*
- *c) determine appropriate background salinity and heavy metal levels for the Goulburn River upstream of the project site;*
- *d) recommend an electrical conductivity limit for treated water discharges to the Goulburn River from the Moolarben Coal Complex based on the process outlined in the ANZECC Guidelines.*

The Secretary of the Department of Planning, Industry and Environment approved the University of Queensland – Sustainable Minerals Institute led by Professor Barry Noller as the independent scientific organisation to undertake the IWQS via correspondence dated 18 July 2019.

In accordance with the ANZECC Guidelines, this report develops an understanding of the Goulburn River surface water system at the MCC and addresses Condition 32A of Project Approval (05_0117).

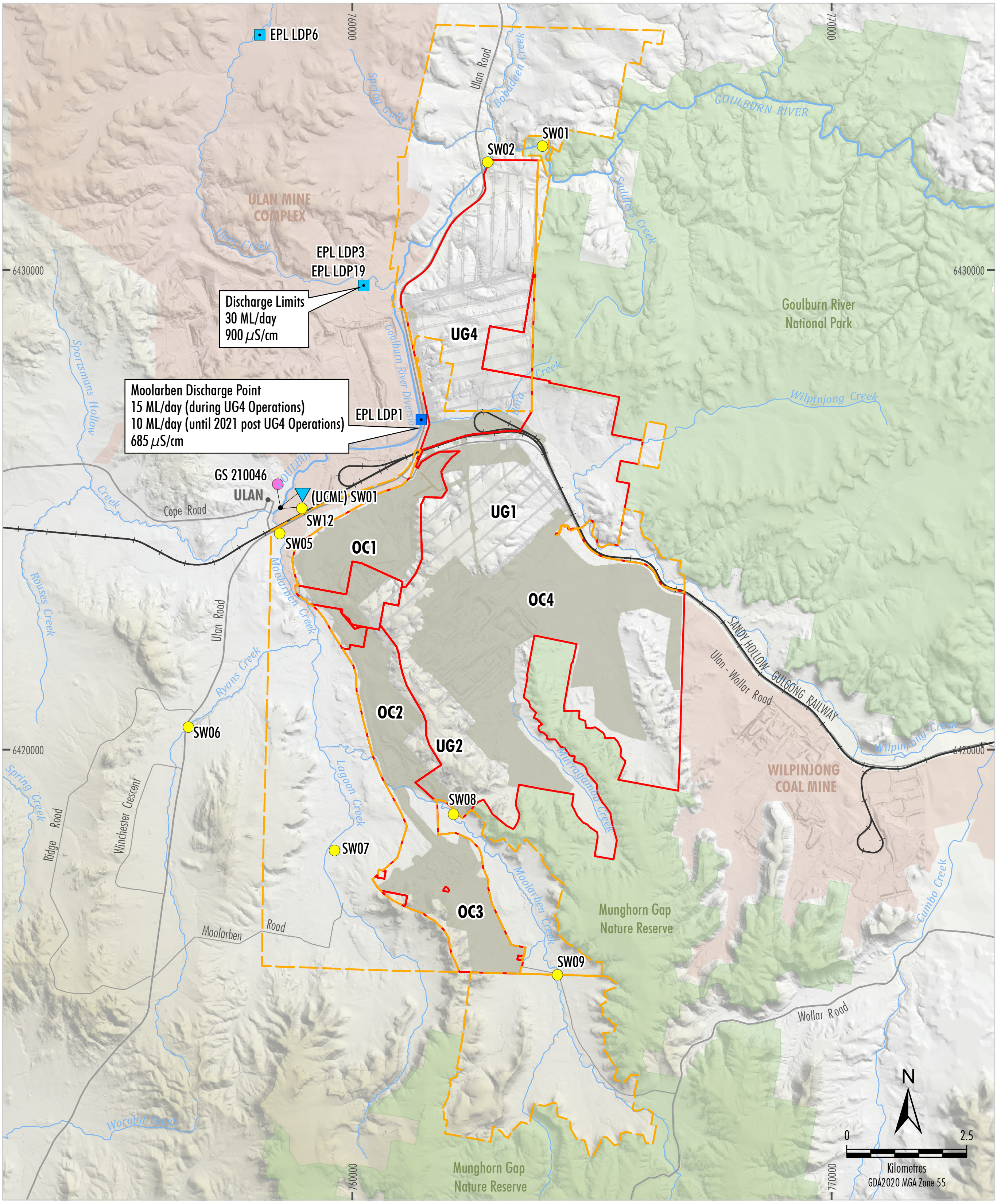
To determine the works required to satisfy Condition 32A of Project Approval (05_0117) a Scope of Works assessment was undertaken in accordance with ANZECC Guideline management frameworks (ANZG, 2018).



Source: NSW Spatial Services (2021)

Regional Location

FIGURE 1



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LEGEND

- 🟩 National Parks/Nature Reserves
- ▨ Other Mining Operations
- ▭ Exploration Licence Boundary
- 🟥 Mining Lease Boundary
- ▨ Moolarben Coal Complex Disturbance Footprint
- ▭ Underground Longwall Layout

Surface Water Monitoring

- ■ Moolarben Licensed Discharge Point
- ■ Ulan Licensed Discharge Point
- ▼ UCML SW01
- ● Surface Water Monitoring Site
- ● Government Monitoring Site GS 210046

Source: MCO (2021); NSW Spatial Services (2021)

Surface Water Setting

FIGURE 2

2 Scope of Works

A comprehensive IWQS has been undertaken by the Sustainable Minerals Institute, The University of Queensland for MCO as the NSW DPIE approved independent scientific organisation. The objective of the study was to satisfy Condition 32A of Project Approval (05_0117), including determining appropriate background salinity and heavy metal levels for the Goulburn River upstream of the project site, and deriving appropriate EC limits for release from the MCC. The IWQS has been undertaken in accordance with the requirements of the Australian and New Zealand Water Quality Guidelines (ANZG, 2018).

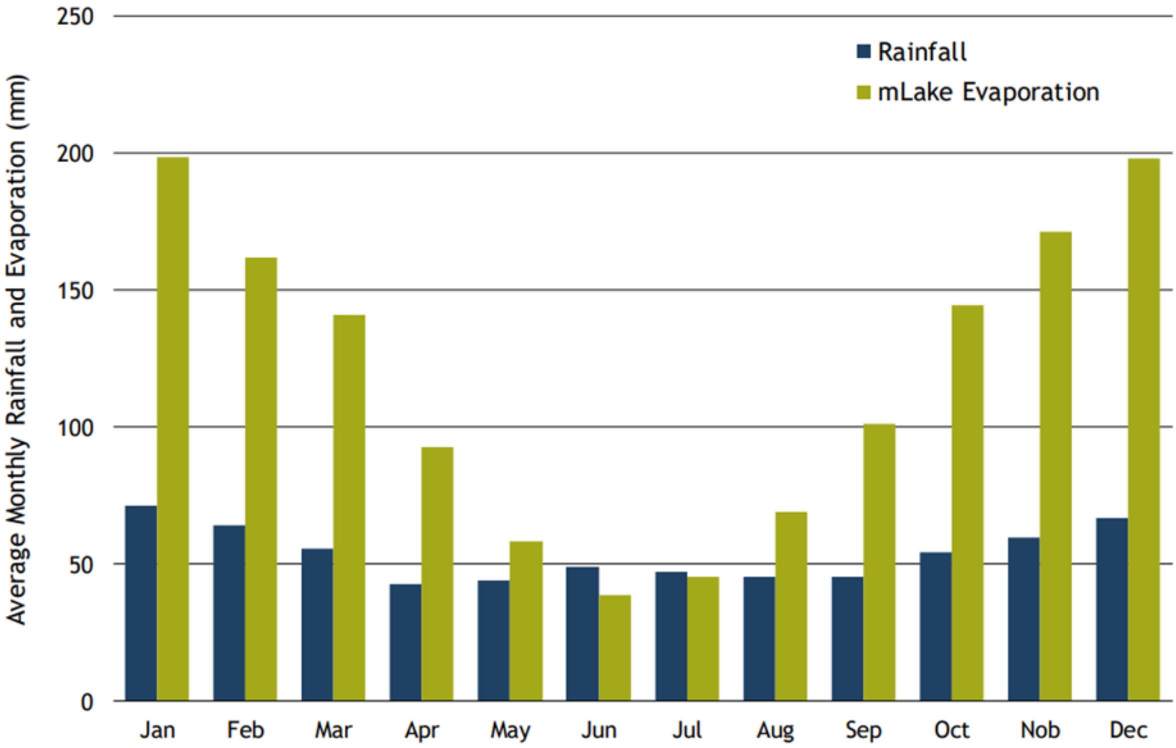
The IWQS scope included the following:

1. Review of the regional surface water setting and available water quality monitoring data.
2. Determination of the appropriate site to collect and utilise water quality monitoring data determine appropriate background salinity and heavy metal levels for the Goulburn River upstream of the project site. The site was to be endorsed by the NSW EPA.
3. Review of the current monitoring network and determine suitability to derive background salinity and heavy metal limits, and determination of whether changes to the monitoring are required.
4. Development of the approach to determine background salinity and heavy metal levels in accordance with the requirements of the Australian and New Zealand Water Quality Guidelines (ANZG, 2018) and the supporting document Revised method for deriving Australian and New Zealand water quality guideline values for toxicants (Warne et al., 2018).
5. Identification of heavy metals associated with the release or water to the Goulburn River.
6. Planning of study design for ecotoxicity assessment to support the determination of site-specific guideline values (SSGVs). The Ecotoxicity Assessment design included:
    a. control water from the upstream monitoring site.
    b. aquatic species for adequate spread of ecotoxicity values to enable derivation of site specific value for salinity and heavy metal guidelines in water including locally collected Mayflies (as per NSW EPA requirement 22 May 2020).
    c. at least four (4) different taxonomic groups and organisms.
    d. four (4) analytes consisting of nickel (Ni), zinc (Zn), sulfate (SO_4), and electrical conductivity (EC, using WTP Filtrate).
7. Consideration of the effects of water chemistry during the testing phase.
8. Consideration of analytical requirements for laboratory handling of test waters according to NATA ISO/IEC 17025 requirements for reliability of measured data and post ecotoxicity test solution analyses by Envirolabs (Chatswood, NSW) according to NATA ISO/IEC 17025 requirements for reliable measured data with uncertainties.
9. Oversight of ecotoxicology assessments, including water sampling, Mayfly collection, water analysis and ecotoxicology assessment completed by Ecotox Services Australia.
10. Development of Species Sensitivity Distribution (SSD) estimations following recommended methodology of ANZG (2018) for heavy metals, WTP Filtrate and Control water.
11. SSD data analysis for salinity in control water (UCML SW01 / SW12) and EC with WTP Filtrate and control water (Site SW12) ecotoxicity test series.
12. Determination of appropriate background salinity and heavy metal levels for the Goulburn River upstream of the project site.
13. Derivation of SSGVs for salinity and heavy metals.
14. Determine limits of electrical conductivity for treated water discharges to the Goulburn River from the MCC based on the process outlined in the ANZECC Guidelines.

3 Surface Water Setting

3.1 Climate and Hydrology of Goulburn River Catchment

The climate around the mine site at MCO is temperate, with an average annual rainfall of 646mm based on Ulan Water rainfall station from January 1889 to December 2016 (128 years) (WRM, 2017). The mean rainfall and evaporation per month is shown in Chart 1.



m Lake Evaporation = Morton's Lake Evaporation.

Source: MCO (2020).

Chart 1: Mean Monthly Rainfall and Evaporation – Patched Point Dataset (1889 – 2016)

3.2 Current Condition

The catchment of the Upper Goulburn River has been affected by historic anthropologic activity, including clearing for agriculture, timber harvesting, quarrying and coal mining at the Ulan, Moolarben and Wilpinjong Coal Mines (Figure 2).

In the early 1980s Ulan Coal created the 4.2km Goulburn River Diversion to allow for Ulan Coal Mine open cut pit operations. The diversion is located to the west and adjacent to the Ulan-Cassilis Road and to the west of the Moolarben Coal Complex (Advisian, 2017). The bed of the Diversion generally comprises a bedrock base with an average gradient of 0.3 to 1% (Hunter Land Management, 2006). The Diversion has a trapezoidal channel cross section along most of its length and has sufficient capacity to convey the 100 year Average Recurrence Interval (ARI) flood event within its banks (UCML, 2013).

Moolarben Dam, located on Moolarben Creek upstream of its confluence with Sportsmans Hollow Creek was constructed in 1957 to supply water to the Ulan Power Station (decommissioned in 1968). Moolarben Dam has a storage volume of approximately 170 ML. Environmental flows are released from the dam in accordance with the licence conditions for this structure.

It is appropriate to recognise the Goulburn River ecosystem as a slightly to moderately disturbed ecosystem (as described in ANZECC Guidelines) as it has been affected by human activity.

3.3 Regional Drainage Network

The primary tributary of the Upper Goulburn River is Moolarben creek which flows north alongside the western boundary of the MCC. Moolarben Creek meets Sportsmans Hollow Creek at Ulan Village (Figure 2). The intersection of these two tributaries form the headwater of the Goulburn River. Wilpinjong Creek drains in a south-easterly direction along the eastern boundary of the Moolarben Coal Complex and joins Wollar Creek, before joining the Goulburn River approximately 26 km downstream of the Moolarben Coal Complex.

The Goulburn River flows in an easterly direction originating in Ulan and linking with the Hunter River south of Denman 150 km downstream of the MCC. The Goulburn River has a catchment area of approximately 2,455 square kilometres (km^2) to the Ulan-Cassilis Road Bridge.

3.4 Local Drainage Network

The local drainage network in the vicinity of the MCC is shown in Figure 2. The majority of MCC Stage 1 mining operations, including OC1, OC2 and OC3, are located within the Moolarben Creek catchment. The catchment area is approximately 126 km^2 to Ulan-Cassilis Road near Ulan. Moolarben creek is located on the western boundary of OC2 and flows between OC2 and OC3. Lagoon Creek flows alongside the western boundary of OC3 and OC2 north where it meets Moolarben Creek north of OC3.

Steep, heavily forested slopes draining into a cleared and relatively flat floodplain characterise the upper reaches of Moolarben Creek. Moolarben Dam is located on Moolarben Creek approximately 1.5 km upstream of the Sportsmans Hollow Creek confluence. The dam has a catchment area of approximately 109 km^2 and a surface area of about 6 hectares (ha).

Stage 1 infrastructure including the Coal Handling and Preparation Plant (CHPP), product stockpile pad and the rail loop are located within the Bora Creek Catchment. Bora creek is a small tributary of the Goulburn River with a catchment area of approximately 6.7 km^2 to Ulan-Cassilis Road. Bora Creek drains in a westerly direction along the northern boundary of the CHPP area.

Ulan Creek is located west of the mid-western boundary of UG4 and flows east where it meets the Goulburn River just north of the Goulburn River Diversion. The Goulburn River flows north-east alongside the western boundary of UG4 where it meets Bobadeen Creek and flows east into the Goulburn River National Park.

The majority of the MCC Stage 2 mining operations (OC4) reside within the Murragamba and Eastern Creek catchments. The total catchment area of Murragamba and Eastern Creek is approximately 31.5 km^2. Both creeks drain into Wilpinjong Creek in a north easterly direction. The Murragamba and Eastern Creek catchments generally consist of steep heavily forested headwaters, draining into a flat and mostly cleared floodplain.

3.4.1 MCC Discharge

Licensed discharge from MCC into the Goulburn River is permitted in accordance with the Environment Protection Licence (EPL) 12932 and Project Approval (05_0117) conditions. The discharge is to the Goulburn River Diversion at the junction with Bora Creek as depicted on Figure 2. Current salinity discharge and flow criteria are detailed in Table 1.

Table 1: Moolarben Coal Mine EPL 12931 Discharge Criteria

Licensed Discharge Point	Parameter	Units	Value
EPL Site 1	Maximum Discharge Volume – Prior to and following the completion of mining operations in UG4 *	ML/day	10
	Maximum Discharge Volume – during mining operations in UG4*	ML/day	15
	Electrical Conductivity 100th Percentile	microsiemens/cm	685

Note: * During prolonged wet periods, with the written approval of the EPA, daily discharge volumes may be exceeded.

3.4.2 Ulan Coal Mine Discharge

Licensed discharge from the Ulan Coal Mine into Ulan Creek is permitted in accordance with EPL 394 conditions. The Ulan Coal Mine discharge is to the Ulan Creek as depicted on Figure 2. Current salinity and discharge flow criteria are detailed in Table 2.

Table 2: Ulan Coal Mine EPL 394 – Ulan Creek Discharge Criteria

Licensed Discharge Point*	Parameter	Units	Value
EPL Site 6	Discharge Volume	ML/day	15
	Conductivity 50th Percentile	microsiemens/cm	800
	Conductivity 100th Percentile	microsiemens/cm	900
EPL Site 3	Discharge Volume	ML/day	10
	Conductivity 50th Percentile	microsiemens/cm	800
	Conductivity 100th Percentile	microsiemens/cm	900
EPL Site 19	Discharge Volume	ML/day	30
	Conductivity 50th Percentile	microsiemens/cm	800
	Conductivity 100th Percentile	microsiemens/cm	900

Note: *The combined daily discharge limit from EPL sites 3, 6 and 19 is 30 ML/day.

3.5 Regional Geology

Major surface geological units within and surrounding the MCC include:

- Quaternary alluvium;
- Pilliga Sandstone;
- Permian Illawarra Coal measures;
- Narrabeen Group; and
- Quartz Monzonite.

A figure of the regional geology is depicted in Section 4.

The drainage network surrounding the MCC lies within a combination of Quaternary Alluvium, Pilliga Sandstone, Illawarra Coal Measures and Carboniferous Quartz and Granite. The river systems typically reside within thin strips of Quaternary Alluvium.

Moolarben Creek, Lagoon Creek and sections of the Goulburn River reside directly above or adjacent to the Illawarra Coal Measures. Naturally saline conditions can be expected in these river systems due to the mineralisation of surface waters from the Illawarra Coal Measures.

3.6 Goulburn River Flow Regime

Flows in the upper reaches of the Goulburn River in the vicinity of the MCC are primarily sourced from the Moolarben Creek and Sportsmans Hollow Creek catchments. Further downstream, the river receives contributions from Bora, Ulan and Bobadeen Creeks (Advisian, 2017). The flows have been altered over time by:

- the presence of Moolarben Dam, which has significantly reduced flows from Moolarben Creek into the Goulburn River and altered flow regimes;
- the construction of the Goulburn River Diversion upstream of Ulan Creek, which has altered the original flow-path, size and shape of the Goulburn River channel;
- land use changes; and
- discharges from the MCC at the Goulburn River Diversion and UMC downstream of Ulan Creek.

The following flow gauges are located on the Goulburn River in the vicinity of the MCC:

- Flow gauge 210046: The gauge was located at the headwaters of the Goulburn River approximately 100 m downstream of the confluence of Sportsmans Hollow Creek, Moolarben Creek and the Goulburn River. The gauge was operated by the Department of Primary Industries (DPI) Water from March 1956 to July 1982 (prior to the commencement of mining at the MCC). Historical flows as reported in Advisian (2017) based on information obtained from the Pinneena archive are shown in Chart 2.
- Flow gauges UCML SW01 and UCML SW02: These gauges are owned and operated by UMC and are located at UCML SW01 / SW12 site near Ulan Village and downstream of the junction with Ulan Creek respectively (Figure 2).

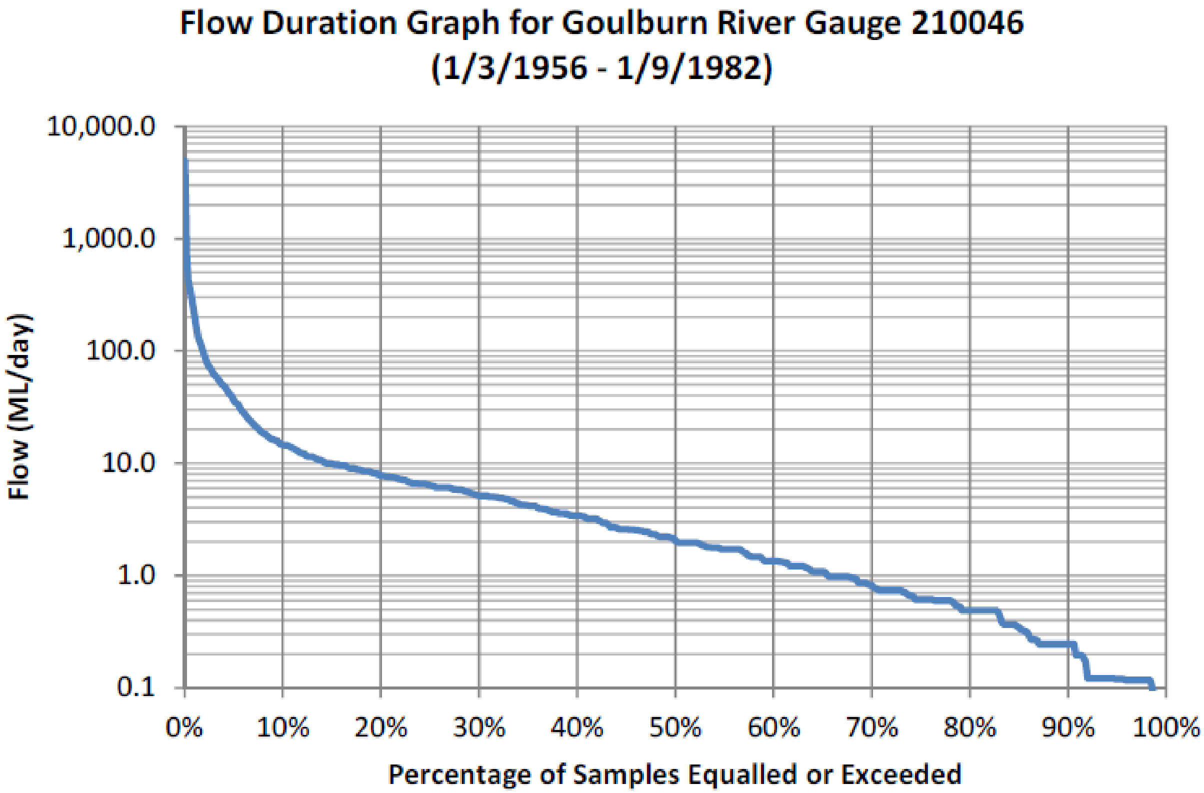
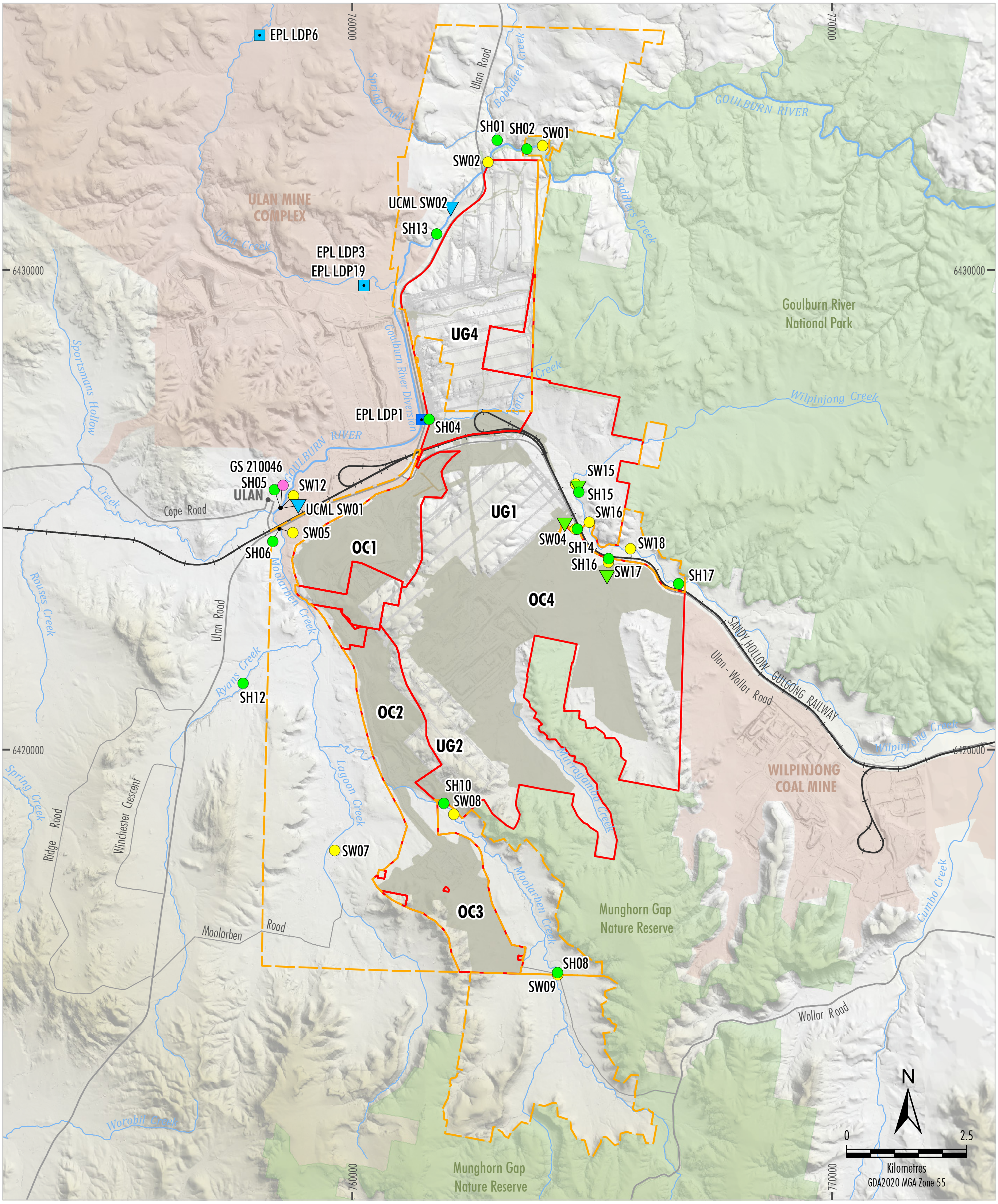


Chart 2: Flow Duration Graph for Goulburn River Gauge 210046 (1956-1982) (Advisian, 2017)

3.7 Moolarben Surface Water Monitoring Program

The Surface Water Management Plan describes the monitoring regime in place at the MCC.

Relevant monitoring sites with available salinity data based on EC are depicted on Figure 3. Figure 3 has also been annotated to include other available EC and salinity monitoring data that was considered in this Report. For the purposes of this study, the data has been supplemented with monitoring data undertaken at UCML SW01 (co-located with SW12) and from government sites as described above.



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LEGEND

- 🟩 National Parks/Nature Reserves
- 🟥 Other Mining Operations
- Exploration Licence Boundary
- Mining Lease Boundary
- ▨ Moolarben Coal Complex
- ☐ Disturbance Footprint
- Underground Longwall Layout

Surface Water Monitoring

- ● Stream Health Monitoring Site
- ● Surface Water Monitoring Site
- ▼ Stream Flow Gauging Station
- ▼ UCML Streamflow Gauging Station
- ● Government Monitoring Site GS 210046

Licensed Discharge Points

- ■ Moolarben Licensed Discharge Point
- ■ Ulan Licensed Discharge Point

Source: MCO (2021); NSW Spatial Services (2021)

Surface Water Monitoring Program

FIGURE 3

4 Conceptual Site Model

Conceptual site models are identified by the ANZECC Guidelines (ANZG, 2018) as a key step in selecting sites for the development of site-specific guidelines development for water release.

4.1 Conceptual Site Model Development

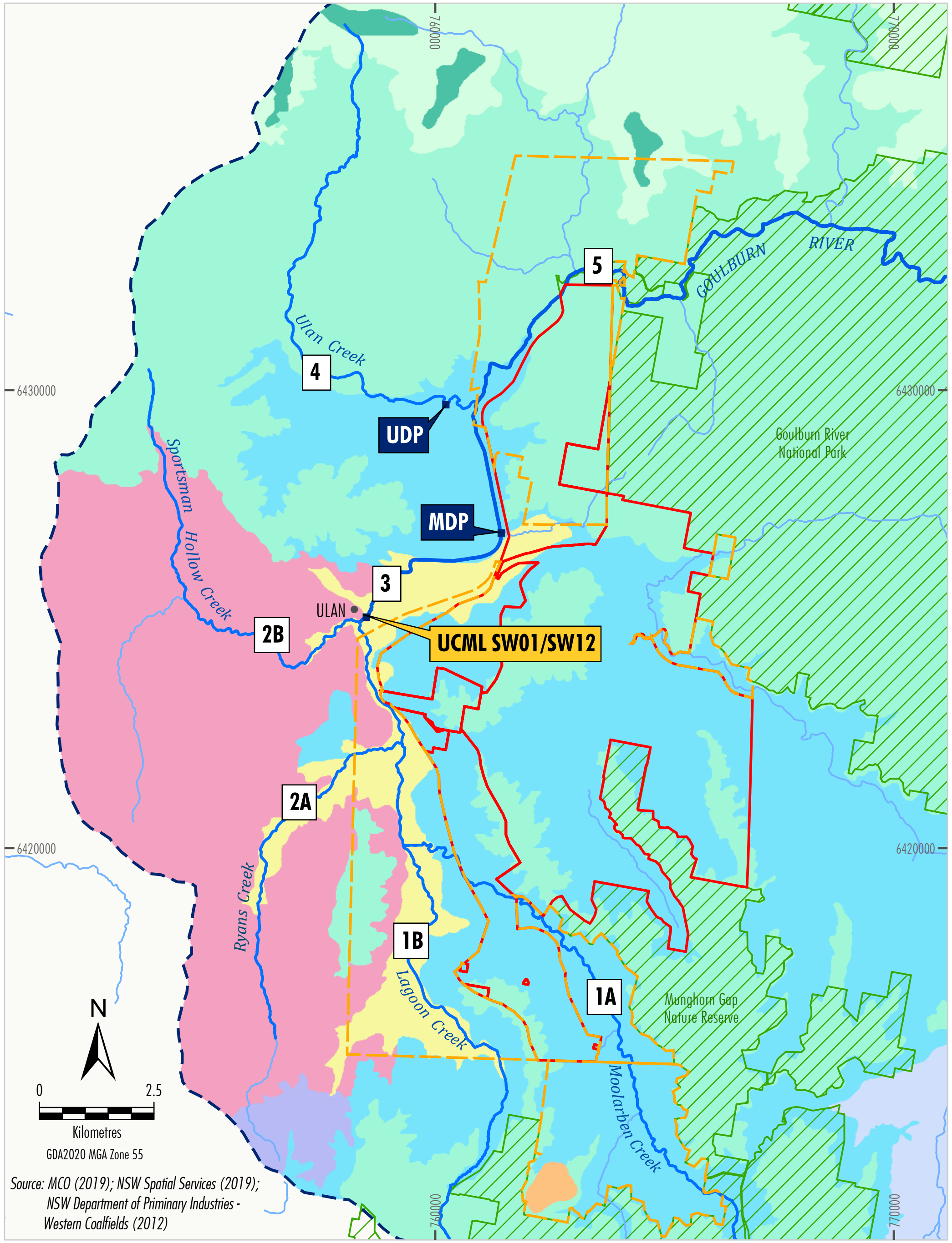
The following resources were used to develop the conceptual site model (Table 3):

- Surface Geology (Geoscience Australia, 2013);
- Government Monitoring Site Data (Australian Government Bioregions Assessments, 2019);
- Ulan Creek Monitoring Site Data within Upper Goulburn River (Ulan Coal, 2018);
- Moolarben Coal Surface Water Monitoring Program Data (Moolarben Coal, 2019); and
- ANZECC Guidelines (ANZG, 2018).

The conceptual site model considers salinity data from government and surface water monitoring sites upstream and downstream of the Moolarben Discharge Point. Table 3 provides an overview of the reference sites in the conceptual model.

Figure 4 and Table 3 demonstrate the salinity of the headwaters of the Upper Goulburn River are influenced by the underlying surface geology. Upstream of the Moolarben Discharge Point highly saline water in Moolarben Creek and Lagoon Creek (underlying Illawarra Coal Measures) mixes with relatively fresh water in Sportsmans Hollow Creek and Ryans Creek to form the Upper Goulburn River.

The Conceptual model has been reviewed in consideration of more recent monitoring data and remains valid.



Source: MCO (2019); NSW Spatial Services (2019); NSW Department of Primiary Industries - Western Coalfields (2012)

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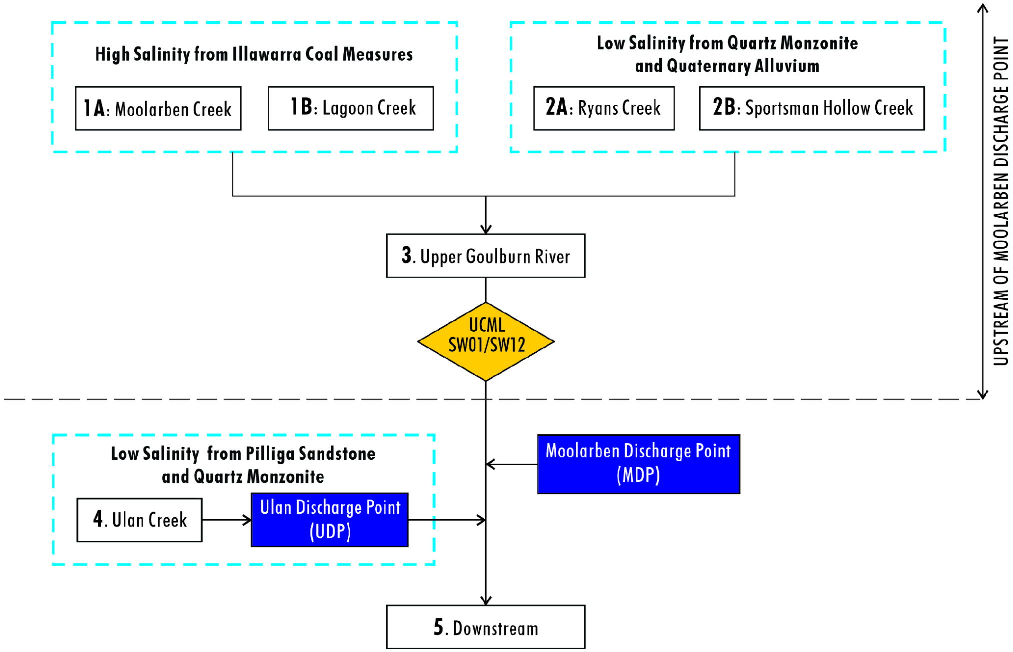


LEGEND

▨ National Parks/Nature Reserves
▭ Goulburn River Catchment
▭ Exploration Licence Boundary
▭ Mining Lease Boundary

Surface Geology

🟨	Qa	Quaternary
🟧	Czb	Mafic Volcanic Rocks
🟩	Czw	Liverpool Range Volcanics
🟩	Js	Pilliga Sandstone
🟩	Rs	Narrabeen Group
🟦	Po	Illawarra Coal Measures
🟦	Ps	Shoalhaven Group
🟥	Cg	Ulan Quartz Monzonite
🟪	Sw	Tannabutta Group

Conceptual Site Model

FIGURE 4

Table 3: Conceptual Site Model Overview

Conceptual Site Model Reference	Underlying Geology	Water Quality Overview	Indicative 80th Percentile Electrical Conductivity (µS/cm)	Indicative 80th Percentile Salinity (mg/L)
1A – Moolarben Creek (SW08)	Illawarra Coal Measures with narrow sections of Quaternary Alluvium.	High salinity. Strongly influenced by mineralisation from the underlying Illawarra Coal Measures.	4126	2600
1B – Lagoon Creek (SW07)	Illawarra Coal Measures with small narrow sections of Quaternary Alluvium.	Extremely high salinity. Strongly influenced by mineralisation from the underlying Illawarra Coal Measures	4478	2687[a]
2A – Ryans Creek (SW06/SH12)	Dominated by Ulan Quartz Monzonite and Quaternary Alluvium.	Fresh water flow of low salinity.	364	220
2B – Sportsmans Hollow Creek	Dominated by Ulan Quartz Monzonite and Quaternary Alluvium.	No site-specific data exists for Sportsmans Hollow Creek. The salinity of the Upper Goulburn River is significantly lower than the salinity upstream within Moolarben and Lagoon Creek. Ryans Creek is relatively fresh however covers a much smaller catchment area than Sportsmans Hollow Creek. The only other source of significant dilution into the Upper Goulburn River is from Sportsmans Hollow Creek. Underlying Geology of the Sportsmans Hollow Creek catchment is identical to Ryans Creek. It is likely that this stream is fresh water of low salinity.	No site-specific data. Likely similar salinity to Ryans Creek.	No site-specific data. Likely similar salinity to Ryans Creek.

Conceptual Site Model Reference	Underlying Geology	Water Quality Overview	Indicative 80th Percentile Electrical Conductivity (µS/cm)	Indicative 80th Percentile Salinity (mg/L)
3 – Upper Goulburn River (UCMLSW01/SW12)	Quaternary Alluvium with Illawarra Coal Measures to the East and Carboniferous Quartz and Granite to the West.	Moderate Salinity. Relatively fresh water from Sportsmans Hollow Creek and Ryans Creek (Pilliga Sandstone and Quartz Monzonite underlying geologies) dilutes the mineralised water from upstream Lagoon and Moolarben Creek.	619	400
4 – Ulan Creek	Dominated by Jurassic Pilliga Sandstone with a small section of Illawarra Coal Measures.	The Ulan Coal Discharge point is permitted to discharge 30 ML/day of up to 900 µS/cm water into the Goulburn River. The downstream Goulburn River will be influenced by Ulan discharges. At the confluence with the Goulburn River conductivity is influenced by discharge from the Ulan Coal Mine.	At the confluence with the Goulburn River conductivity is influenced by discharge from the Ulan Coal Mine.	At the confluence with the Goulburn River conductivity is influenced by discharge from the Ulan Coal Mine.
5 – Goulburn River (Downstream MCC) (SW01 and SW02)S	Dominated by Jurassic Pilliga Sandstone. The upstream section north of the Moolarben Discharge Point is dominated by Illawarra Coal Measures.	This section of the Goulburn River is influenced by a combination of flows from the Ulan Discharge Point, Upper Goulburn River, Ulan Creek. SW01 is also influenced by flows from Spring Gully Creek and Bobadeen Creek. The underlying Illawarra Coal Measures are a source of salinity due to natural mineralisation.	795 – 830	479-498

Note: a. EC X 0.60 converted to salinity (mg/L) using data given in Section 8.3.6 Table 34 for mean salinity factor k, for SW08. SW07 and SW09 have similar water characteristics to that of SW08.

5 Surface Water Quality Characterisation

5.1 Surface Water Data Review

A summary and analysis of available Electrical Conductivity and salinity data from these monitoring sites is detailed in Table 4. Additional water quality parameters are included in Tables 5 to 7. Available water quality data was extracted from MCOs environmental monitoring data base (MonitorPro). The database incorporates a number of QA/QC processes, including direct input of laboratory results into the data base with automatic review processes.

The surface water data was collated and reviewed for accuracy, including sampling of results against laboratory results. Water quality for each relevant site was analysed to determine the range of raw values (minimum, mean and maximum) and percentiles for the entire data set, along with the last 24 months of data. Where water quality was below the Limit of Reporting (LOR), a factor of 0.5 x the LOR was used to derive mean and percentiles. Further data review was undertaken based on timeseries graphs of relevant quality parameters, available flow data and raw statistical analysis. In order to address comments from the EPA (correspondence dated 22 May 2020), outliers due to first flush responses and no flow and anomalous values were excluded from the analysis and determination of reported values. The values used to derive the mean, 80th percentile and maximum values reported exclude these outliers and anomalous values unless otherwise noted.

A consideration may be required of loads for heavy metals and salinity carried by the Goulburn River in acknowledgement of NSW EPA's focus on load and the ANZECC/ARMCANZ (Section 3.3.2.8; 2000) describing load-based guidelines.

Traditionally, water quality guidelines are expressed in terms of the concentration of the stressor that should not be exceeded if problems are to be avoided (ANZECC/ARMCANZ, 2000). Concentration-based guidelines are primarily focussed on the prevention of toxic effects and situations and do not involve direct toxicity effects where guidelines are better expressed in terms of the flux or loading (i.e. mass per unit time) that causes a direct effect on aquatic biota, rather than concentration. For example, algal growth rate (or productivity) is related to the concentration of key nutrients in the water column but the biomass is more controlled by the total mass of these nutrients available to the growing algae (Wetzel, 1975). The water column nutrient concentration is not a good indicator of algal biomass. The dissolved oxygen concentration in a waterbody depends on the balance between the flux of bioavailable organic carbon and the rate at which heterotrophic bacteria use up oxygen in decomposing this material, and the daily inputs of oxygen by diffusion from the atmosphere (increased by mixing) and via photosynthesis by macrophytes and phytoplankton (Stumm and Morgan, 1996). Load-based guidelines are applicable also for assessing the effects of sedimentation of suspended particulate matter in smothering benthic organisms. While rate of sedimentation and the critical depth of the deposited material are load-based (Section 8.2.3 of ANZECC/ARMCANZ, [2000]) they do not give a toxicity-based assessment of response with aquatic species or effects of bioaccumulation.

Load-based triggers such as annual loads for heavy metals and salinity in Goulburn River total suspended solids serve little purpose for environmental control in the context of the Moolarben IWQS as they do not measure effects on aquatic species downstream nor a toxicity response in organisms and fates of the heavy metals in the sediment. Effects of heavy metals transported in the water column by river flow and accumulated in fluvial river sediment need to be assessed as a direct toxicity response and/or associated with the bioaccumulation of metals in a range of aquatic species according to the decision tree for the assessment of contaminated sediment (ANZECC/ARMCANZ, 2000).

Table 4: Available Salinity Data Analysis

Monitoring Site Location	80th percentile Electrical Conductivity (µS/cm)	80th percentile Salinity (mg/L)*	Data Range	Analysis
SW01	795	479	Feb 2005 – Sep 2021	Downstream of Ulan and Moolarben operations to meet EPL discharge limit below mixing zone (with further dilution to Goulburn River from Spring Gully Creek and Bobadeen Creek).
SW02	830	498	Feb 2005 – Sep 2021	Downstream Ulan and Moolarben operations alternative site to EPL discharge limit below mixing zone (without additional dilution to Goulburn River). Shows input of salinity from Ulan operations compared with upstream Goulburn River.
UCML SW01 / SW12	619	400	Dec 2009 – Sep 2021	SW12 and UCML SW01 are co-located sites on the Goulburn River, both of which are situated upstream of Moolarben and Ulan mining operations. Captures the fresh water quality inputs from Sportsmans Hollow Creek (reduced salinity) and the natural mineralization from coal measures experienced in Moolarben Creek (saline).
SW05	890	543	Feb 2005 – Sep 2021	Upstream of the Goulburn River Diversion indicating mixing of Moolarben and Lagoon Creeks with Ryans Creek. Contribution of total flow from Moolarben Creek is affected by the rate of release from Moolarben Dam.
SW06	364	220	Feb 2005 – Mar 2010	Upstream input of low salinity water to Moolarben Creek.
SW07	4478	2687[a]	Feb 2005 – Aug 2019	Indicates salinity contribution of Lagoon Creek from the influence of Permian Strata and natural coal mineralisation.
SW08	4126	2600	Feb 2005 – Sep 2021	Moolarben Creek upstream of OC2. Indicates salinity contribution of middle Moolarben Creek from the influence of the Permian Strata and natural coal mineralisation.

Monitoring Site Location	80th percentile Electrical Conductivity (µS/cm)	80th percentile Salinity (mg/L)*	Data Range	Analysis
SW09	4370	2622[a]	Feb 2005 – Aug 2019	Moolarben Creek upstream at ML 1691 boundary. Indicates salinity contribution of upper Moolarben Creek from the influence of the Permian Strata and natural coal mineralisation.
NSW Government Monitoring Sites[b]				
GS210031 [c]	862[b]	**629**	Feb 1992 – Nov 2021	Key NSW Government Monitoring site at Sandy Hollow, downstream of SW01 at Goulburn River.
GS210016 [c]	**1046** [b]	764	Jul 2002 – Nov 2021	Key NSW Government Monitoring site at Kerrabee, downstream of SW01 at Goulburn River.
GS210006[c]	**1159**	846	May 2012 – Nov 2021	Key NSW Government Monitoring site at Coggan, downstream of SW01 at Goulburn River.
GS2100046	583	423	Jun 1968 – Apr 1988	Comparative site at Ulan below Moolarben Creek input to Goulburn River.

Note: a EC X 0.60 to determine salinity (mg/L). Salinity Factor k, 0.60 given in Section 8.3.6 Table 34 as a mean salinity factor for SW08. SW09 and SW07 have similar water characteristics to SW08 monitoring site situated Moolarben Creek.

b Median (50th percentile) data is shown

c Continuous monitoring data has been reduced at these locations. Site locations not identified on figures. Included to describe the salinity at government monitoring locations downstream of the MCC

Table 5: Electrical Conductivity and Salinity (lab-measured TDS) for Goulburn River and NSW Government Monitoring Sites for the Period 2005-2021

Goulburn River Monitoring Sites	Electrical Conductivity (µS/cm)				Salinity (mg/L)			
	n	Mean	80%	Max	n	Mean	80%	Max
SW01	211	632	795	1180	269	397	479	808
SW02	225	673	830	1800	274	430	498	1120
UCML SW01 / SW12	234	446	619	971	271	331	400	628
SW05	241	673	890	1570	278	436	543	965
SW06*	59	307	364	630	65	206	220	536
SW08	192	3161	4126	5990	249	1977	2600	4200
NSW Government Monitoring Sites[b]								
GS210016 (Jul 2002 – Nov 2021)	-	1064	1252	1807	-	772	908	1310
GS210006 (May 2012 – Nov 2021)	-	994	1236	2560	-	721	896	1856
GS2100046 (Jun 1968 – Apr 1988)	50	441	583	1560	-	320	422	1131

Note: a SW06 monitoring data from 2005-2010. Field data was specifically used instead of laboratory data, to higher number of data points (n). Laboratory data did not have a sufficient count (n) to capture the historical variation at this site.

b Median (50th percentile) data is shown

- Data not available

Table 6: Hardness, pH and Bicarbonate for Goulburn River Monitoring Sites for the Period 2005-2021

Goulburn River Monitoring Sites	pH				Hardness				Bicarbonate (mg/L)[a]			
	n	Mean	80%	Max	n	Mean	80%	Max	n	Mean	80%	Max
SW01	226	7.4	8.1	9.3	5	144	153	157	74	92	122	180
SW02	234	7.4	8.0	8.7	5	147	158	170	75	101	138	190
UCML SW01 / SW12	215	7.1	7.5	8.7	35	93	138	201	96	60	95	122
SW05	238	7.0	7.6	8.7	32	155	182	338	111	71	100	190
SW06 [b]	82	6.8	7.5	8.2	1	21	-	-	65	33	35	66
SW08	225	6.8	7.3	8.2	-	-	-	-	77	102	141	350

Note: a Bicarbonate comprises all of Total Alkalinity mg/L as $CaCO_3$

b SW06 monitoring data from 2005-2010, Includes Stream Health (SH) 12 data 2011-2021.

- Data not available

Table 7: Nickel, Zinc and Sulfate Concentrations for Goulburn River Monitoring Sites

Goulburn River Monitoring Sites	Nickel Filtered (µg/L)				Zinc Filtered (µg/L)				Sulfate (mg/L)			
	2013-2021				2013-2021				2005-2021			
	n	Mean	80%	Max	n	Mean	80%	Max	n	Mean	80%	Max
SW01	18	3.9	5.0	6.0	7	3.9	2.5	12.0	69	95	112	360
SW02	22	4.5	5.8	9.0	8	2.3	2.5	2.5	70	115	140	410
UCML SW01 / SW12	75	0.9	1.0	8.0	75	4.2	6.0	30.0	72	41	68	196
SW05	44	2.3	3.0	19.0	44	5.7	7.0	63.0	42	79	116	199
SW06	1	<1	-	-	1	<5	-	-	64	15	19	25
SW08	18	8.6	8.0	35.0	18	6.8	7.0	37.0	16	300	363	481

Note: a SW06 monitoring data from 2005-2010, Includes Stream Health (SH) 12 data 2011-2021

- Data not available

5.2 Background Monitoring Location

Conditions 32A(b) and 32A(c) of Project Approval (05_0117) specify monitoring locations to be used to determine background salinity levels and heavy metals are to be within the “Goulburn River” and “upstream of the project site” (Moolarben Discharge Point). The Goulburn River begins at the confluence of Sportsmans Hollow Creek and Moolarben Creek, and flows north east where it meets the Moolarben Discharge Point within the Goulburn River Diversion. The only section of the Goulburn River that is indicative of background heavy metal and salinity levels and satisfies the constraints of Condition 32A Project Approval (05_0117) is the location represented by UCML SW01 (and other co-located sites) (Figure 3).

Condition 32A(c) Monitoring data from site UCML SW01 (and other co-located sites) is used to address the requirements of Condition 32A(c). This is because UCML SW01 is located on the Upper Goulburn River upstream of the Moolarben Discharge Point, and downstream of the confluence of:

- highly saline water from Lagoon Creek and Moolarben Creek; and
- relatively fresh water from Ryans Creek and Sportsmans Hollow Creek.

Data from locations upstream of UCML SW01 (and other co-located sites) will not be representative of “background” for the Upper Goulburn River as:

- sites upstream on Moolarben Creek and Lagoon Creek are naturally highly saline because of the underlying geology (the Illawarra Coal Measures) and do not include contributions from the fresher tributaries to the Upper Goulburn River.
- sites upstream on Ryans Creek and Sportsmans Hollow have low salinity, but do not account for the contribution of naturally higher salinity from Moolarben Creek and Lagoon Creek.

The site can be considered appropriate for background concentrations of the upstream Goulburn River, however, would not be directly representative of background concentrations (that is, concentrations with no influence from mine discharge) within the Goulburn River due to contributions such as the diversion and other tributaries.

UCML SW01 / SW12 was endorsed by the New South Wales Environment Protection Authority (NSW EPA) via correspondence received 22 May 2020 as required by Condition 32A(b).

5.3 Background Water Quality - UCML SW01 / SW12

Following the analysis presented in Sections 5.1 and 5.2, “background concentrations” for each relevant analyte were determined in order to address the requirements of Condition 32A(c). Table 8 gives the details of the background values and relevant information, followed by a summary of the concentrations.

Table 8: Measured Background Concentrations for Salinity, Nickel and Zinc

Analyte	Background Concentration (80th Percentile)	Count	No. of Measurements Below LOR
Salinity (TDS in mg/L)	400	271	0
Electrical Conductivity (µS/cm)	619	234	0
Aluminium Filtered (µg/L)	126	73	10
Manganese Filtered (µg/L)	397	75	0
Nickel Filtered (µg/L)	1.0	75	43
Zinc Filtered (µg/L)	6.0	75	50
Cadmium Filtered (µg/L)	0.05[a]	75	75
Copper Filtered (µg/L)	0.5[a]	75	71
Arsenic Filtered (µg/L)	0.5[a]	75	66
Lead Filtered (µg/L)	0.5[a]	75	74

Note: a Greater than 80% of measurements for these analytes were lower than the limit of reporting (LOR), and have therefore been reported as half (50%) of the LOR as per convention.

The background concentrations (retrieved from upstream reference site UCML SW01 / SW12) were determined to be:

Salinity: 400 mg/L (equivalent to 619 µS/cm)

Aluminium:	126 µg/L
Manganese:	397 µg/L
Nickel:	1.0 µg/L
Zinc:	6.0 µg/L
Cadmium:	0.05 µg/L
Copper:	0.5 µg/L
Arsenic:	0.5 µg/L
Lead:	0.5 µg/L

6 Mine Site Water Management

6.1 Overview

Water sources at the Moolarben Coal Complex underground and open cut operations includes rainfall run-off, inflows to open cut and underground operations including groundwater and water sharing from other operations. Water uses at the MCC can include coal processing, process water for underground operations, dust suppression, evaporation, seepage and irrigation. Water at the Moolarben Coal Complex is stored in surface dams, open cut pits, mining voids (when available) and sediment dams.

A WTP has operated since 2020 to treat surplus water stored at the MCC to meet the water quality concentration limits of EPL 12932 and also provides water for on-site use. The WTP is described in Section 6.2.

6.2 Water Treatment Plant

The MCO WTP treatment process includes filtration and reverse osmosis. Water from MCO's surface and underground operations is transferred to the dam WP19. All WTP feed water is sourced from WP19. Due to the range of water sources, the quality of the WTP feed water will vary with climate and mine progression.

The water treatment process involves filtration consisting of disc filters followed by ultrafiltration. WTP Filtrate is the end product from the ultrafiltration process. WTP Filtrate feeds the Reverse Osmosis (RO) system. Filtrate is sent to coated media filters then to the RO trains with Permeate the end product. Chemical dosing and adjustments are incorporated into the WTP operational system.

WTP Discharge water comprises a blend of WTP Filtrate and WTP Permeate with ratios of each set by the WTP control system. Blended release water is transferred via pipeline from the WTP to the discharge point (EPL ID1) at the Goulburn River Diversion. The WTP control system monitors the system performance and key water quality parameters. Real-time quality monitoring is used to maintain release water within the release criteria for Electrical Conductivity, pH and Turbidity.

Table 9 gives a percentile data summary of WTP Filtrate and WTP Permeate water quality data.

Table 9. Water quality analysis of treatment waters (WTP Filtrate and Permeate) May 2020 to September 2021

Parameters	WTP Filtrate				WTP Permeate			
	n	Mean	80%	Max	n	Mean	80%	Max
pH	13	8.01	8.13	8.31	1	7.20	7.20	7.20
Total Hardness ($CaCO_3$ mg/L)	12	525	610	623	-	-	-	-
Total Alkalinity ($CaCO_3$ mg/L)	12	124	139	161	-	-	-	-
Bicarbonate (mg/L)	12	124	139	161				
Electrical Conductivity (µS/cm)	24	1707	1774	2070	8	31	35	36
Salinity (mg/L)	70	1168	1312	1600	7	20	22	25
Nickel Filtered (µg/L)	76	37.6	45.4	56.3	12	<1	<1	<1
Nickel Total (µg/L)	20	33.6	38.0	48.0	8	<1	<1	1
Zinc Dissolved (µg/L)	76	16.6	25.0	28.0	12	<5	<5	<5
Zinc Total (µg/L)	20	13.3	14.0	34.0	8	<5	<5	<5
Sulfate (mg/L)	12	531	641	669	-	-	-	-

7 Approach

7.1 Outline of the ANZG (2018) Approach

This report is developed in accordance with Step 1 of the current ANZECC Guidelines (ANZG, 2018), which provides an approach to establish freshwater objectives for compulsory national values (ecosystem and human health) and is summarised in Table 10 and follows the site-specific water quality assessment decision tree (ANZECC/ARMCANZ, 2000) given in Figure 5.

Table 10: ANZG (2018) Water Quality Management Framework Summary

Framework Steps	Description	Report Section
Step 1 – Examine Current Understanding	Develop conceptual models of the waterway systems demonstrating the function, issues and best management.	4

ANZG (2018) provides comprehensive guidance on undertaking site-specific toxicity assessments, selection of appropriate test species of local and regional relevance to the receiving water, biological endpoints (Figure 5) and statistical methodology and other test design considerations. These include acute vs chronic testing, single chemical vs complex (whole) mixture, laboratory vs in situ, and quality assurance and quality control (QA/QC) requirements (Figure 5).

The ANZG (2018) water quality guidelines are the penultimate source of advice in Australia on managing water quality in aquatic ecosystems to assess and control addition of substance both natural and man-made which can affect aquatic ecosystems. The water quality guidelines are relevant to all kinds of aquatic ecosystems in Australia and New Zealand. The ANZECC/ARMCANZ (2000) guidelines for managing water quality were very comprehensive at an international level in 2000 at their time of introduction, and drew on the ecological risk assessment approach of the United States Environmental Protection Agency (USEPA), the Dutch and Canadian guidelines and specific USA guidelines for sediment contamination based of toxicity to aquatic species both freshwater and marine which remain in the current Australian guidelines. ANZG (2018) acknowledge that each assessment of effects of discharged substances in aquatic ecosystems needs to be considered on a site-specific basis that takes flow characteristics into account.

The revision of the ANZECC/ARMCANZ (2000) was undertaken to improve access to the supporting details by converting them to a web-based access of ANZG (2018) water quality guidelines and taking on-board corrections and additions to toxicity-testing guidance prior to 2018 (Warne and Van Dam, 2008; Batley et al., 2014; Warne et al., 2014; and Warne et al., 2015).

The revisions of ANZECC Guidelines described in Warne et al. (2018) and Batley et al. (2018) now place an emphasis on using the 'weight of evidence' approach which considers 'Pressure' 'Stressors' and 'Ecosystem Receptors' (PSER; Figure 6). The weight of evidence approach incorporated in ANZG (2018) describes the process of collecting, analysing and evaluating a combination of different qualitative, semi-quantitative or quantitative lines of evidence and making on overall assessment of water/sediment quality and its associated management (ANZG, 2018).

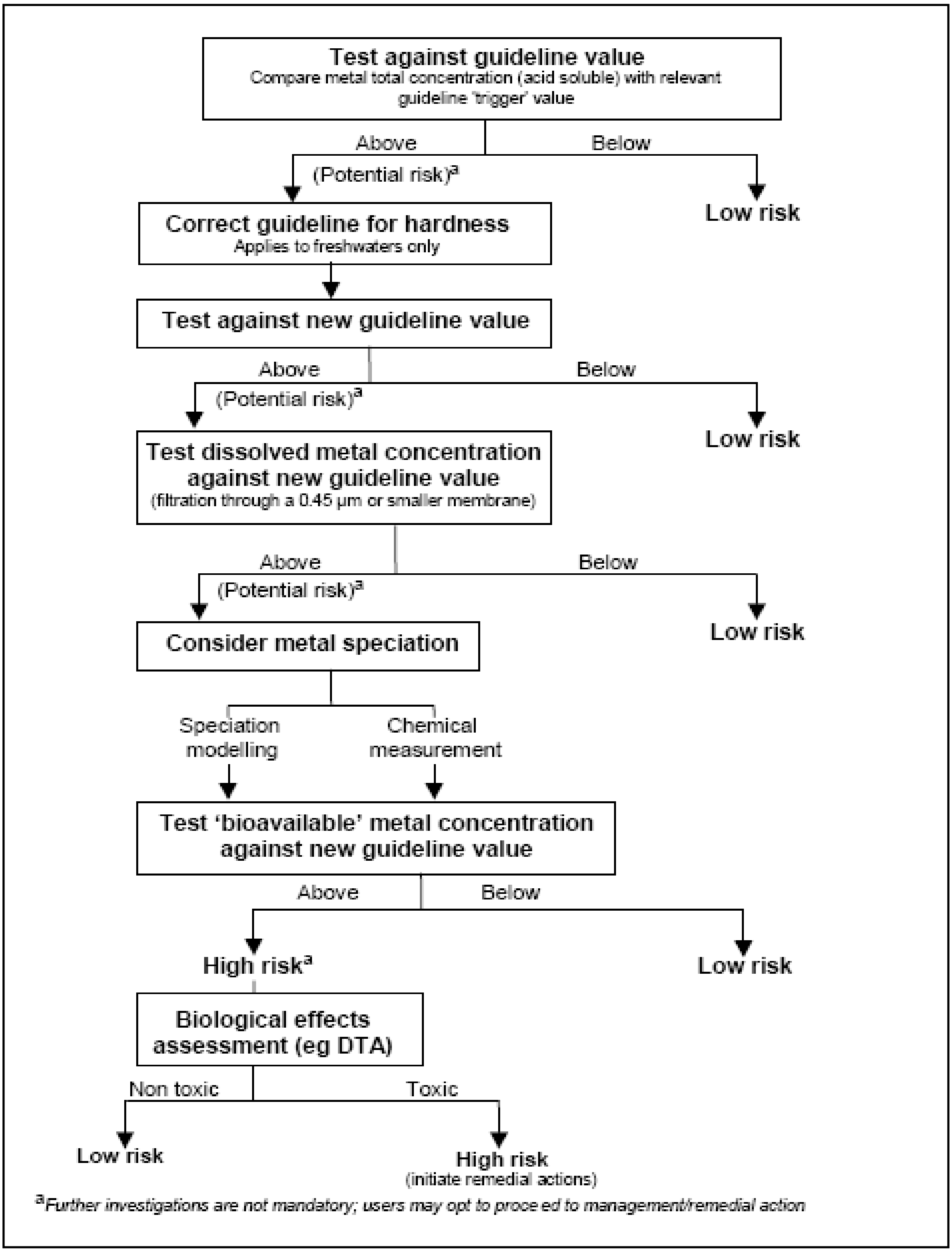


Figure 5: Site-specific Water Quality Assessment Decision Tree (ANZECC/ARMCANZ, 2000)

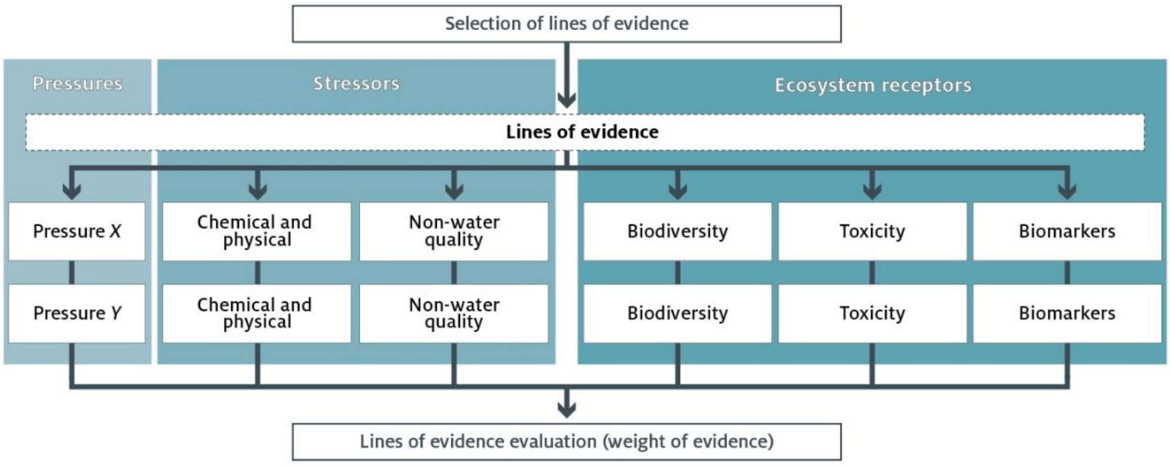


Figure 6. Weight of Evidence Process across the PSER Causal Pathway (ANZG, 2018)

In accordance with ANZECC Guidelines (ANZG, 2018) the use of biological and ecological effects data is the most robust and preferred method for site specific guideline development for the protection of aquatic ecosystems.

7.2 Weight of Evidence Approach to Developing Electrical Conductivity Limit

Condition 32A(d) requires confirmation of an electrical conductivity limit for treated water discharges from Moolarben Discharge Point and the satisfaction of Condition 32A(d) should be undertaken using the ‘weight of evidence’ approach outlined in recent revisions of ANZECC Guidelines as described in Warne et al., (2018) and Batley et al., (2017).

Figure 7 describes the weight of evidence approach for the confirmation of an electrical conductivity limit for treated water discharges from the Moolarben Discharge Point.

Ecotoxicity analysis involves the development of a SSD for site specific guideline development based on salinity using water sampled at Site UCML SW01 / SW12. The SSD is a cumulative distribution function that describes the variation in the sensitivity of a range of aquatic species (toxicity data) to a chemical (e.g. salinity).

The Upper Goulburn River corresponds to a ‘slightly-to-moderately disturbed ecosystem’ with a 95% species level of protection. Ecotoxicity testing for relevant freshwater aquatic species from at least four taxonomic groups should be used to derive the 95% species protection level for salinity.

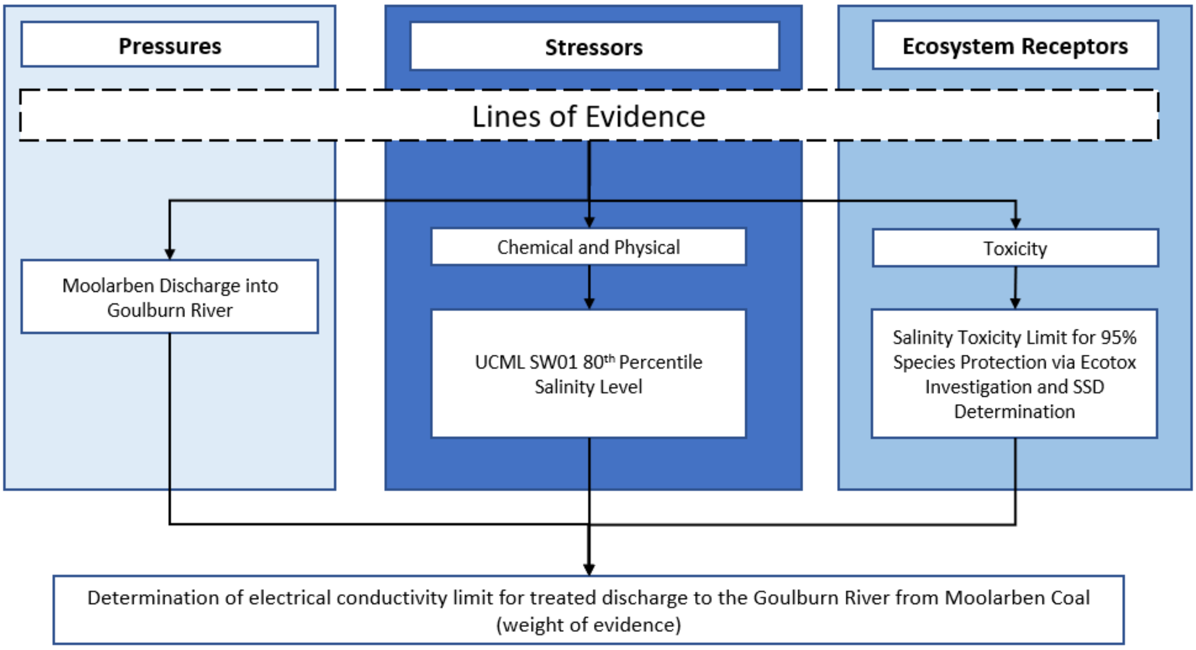


Figure 7: Recommended Salinity Assessment Weight of Evidence Approach

The weight of evidence from the combination of the 80th percentile salinity level from upstream site UCML SW01 / SW12, in combination with the results of ecotoxicity test work, will ensure the determined electrical conductivity limit by NSW EPA for treated water discharge from the Moolarben Coal Complex is justified and final.

It was recommended that MCO seeks endorsement from the EPA for the use of monitoring data from UCML SW01 / SW12 (and other co-located monitoring sites) and the results of ecotoxicity test work (using water sampled from the UCML SW01 / SW12 location) to satisfy the requirements of Condition 32A(d).

The NSW EPA replied 20 May 2020 to the ‘Moolarben Coal – Independent Water Quality Study’ seeking EPA Endorsement of Monitoring Data Location. The EPA reviewed the supplementary information and agreed that the proposed use of monitoring data from ‘UCML SW01 and other co-located sites (namely SW12 and GS 210046)’ is appropriate to be used in the IWQS to determine background salinity and heavy metals levels in the Goulburn River upstream of the MCO. Additionally, the EPA provided two recommendations for the IWQS:

1. *When determining background levels for conductivity (and possibly a range of other analytes) that it is important to understand whether the Goulburn River was flowing at the time of water quality sampling (at SW01) and to treat with caution any data that may be associated with zero flow (i.e. the potential for the sample to represent ponded/stagnant water).*
2. *Testing local aquatic species in any toxicity testing undertaken as part of the IWQS (e.g. Austrophlebioides pusillus as described by Dowse et al 2017).*

7.3 Selection of Analytes

A key part of the MCO IWQS is the ecotoxicity study, which aims to provide data to deliver the site specific guideline for EC for water discharge by MCO. The ecotoxicity study testing on waters collected from MCO WTP and the Upper Goulburn River on 24 June 2021 was undertaken by Ecotox Services Australia (Lane Cove, Sydney). Ecotoxicity testing was undertaken with aquatic species including one key aquatic test species, the Mayfly, as recommended by the NSW EPA via correspondence dated 20 May 2020. This directive appears to be based on a published study on the Lower Hunter River which used locally-collected Mayfly species as test aquatic species (Dowse et al., 2017).

The WTP Filtrate is a product of ultrafiltration used as Reverse osmosis feed and for blending WTP Discharge water, and was therefore suitable for ecotoxicity testing to identify ecotoxicity responses to mine water salinity. Selection of additional analytes for the dose response ecotoxicity testing with test aquatic species was made by considering priorities based on significant water quality monitoring features. MCO monitoring data in Goulburn River was examined at SW01, SW02, SW05, SW06, SW08 and SW12. The data from 2005 to 2021 and 2013 to 2021 at the selected monitoring sites (SW01, SW02, SW05, SW06, SW08 and SW12) showed little change with hardness (calcium + magnesium) that influences metal labile concentrations (ANZG, 2018), but increases in EC, sulfate and dissolved (filtered) aluminium concentration at SW05, SW08 and SW12. It is established that EC, sulfate and dissolved aluminium concentrations can contribute to water quality characteristics in Moolarben Creek , which is influenced by natural mineralisation of coal measures in the upper Goulburn River catchment. If tributary inputs from Ryans Creek and Sportsmans Hollow Creek are elevated, more dilution of Moolarben Creek tributary input above SW12 will occur and reduce EC, sulfate and dissolved aluminium concentrations measured at SW12 (Figures 2 and 4).

In addition to major ions contributing to salinity, the summarised data in Table 9 shows that the associated dissolved (filtered) nickel and zinc concentrations are at higher levels in their respective percentile distributions for WTP Filtrate when compared against the ANZG (2018) trigger values (TVs) for 95% protection of species. Whilst the WTP discharge dissolved (filtered) nickel and zinc concentrations does not exceed the ANZG (2018) TVs for 95% protection of species, these nickel and zinc concentrations highlight that their input to the WTP is a potential risk compared to other metal ions and salinity. The conservative approach therefore is to consider the significance of nickel, zinc and sulfate concentrations in the Goulburn River waters together with salinity.

Ecotoxicity studies were proposed to include ten tests with eight different species. The EC of WTP Filtrate was to be serially diluted in SW12 control water for ecotoxicity tests with additional controls of WTP permeate and WTP discharge waters. All test water samples and collections for supporting analyses were collected under the direction and observation of Prof. Barry Noller. The ecotoxicity tests for nickel, zinc and sulfate are added as spikes to the SW12 is control water to show the individual analyte toxicity responses to test organisms. The additional controls in each test series (WTP Permeate and WTP Discharge) are intended to confirm if the 'treated' WTP waters show any response with test organisms. This was to provide an additional check on the treatment applied to the WTP water with little additional work requirement without the extensive data analysis needed for the species sensitivity distributions of each analyte.

7.4 ANZECC Guidelines and SSGV Development

The ANZECC guidelines (ANZG, 2018) give guidance for developing guideline value (GV) derivation advice with an increased emphasis on relevance of toxicological findings to the site in question (Van Dam et al., 2014). To ensure that any GV derivation is in the appropriate context of aquatic ecosystem protection of the Goulburn River, the chosen single-species tests should demonstrate relevance to Goulburn River food webs and/or keys roles with ecosystem function. Very broad distribution of a species often indicates broad environmental tolerances, including from water quality to aquatic species. This robustness may not provide a sensitive result as may a local species.

'Site-specific' is an important consideration and can refer to the toxicant mixture, receiving environment water quality and the ecosystem in itself. However, there is growing evidence that high reliability SSGVs can be derived through toxicity tests with regionally relevant species included in the development of SSDs (Shao, 2000; Campbell et al., 2001). Van Dam et al. (2014) provides further advice on site-specific toxicity-testing guidance.

Ecotoxicity analysis is a key component of the weight of evidence approach and involves the development of a SSD for site specific guideline development of a selected analyte (ANZG, 2018). The SSD is a cumulative distribution function that describes the variation in the sensitivity of a range of aquatic species (toxicity data) to a chemical or analyte.

The SSD is a cumulative distribution function that describes the variation in the sensitivity of aquatic species (toxicity data) to a chemical (analyte). SSD is a probabilistic model of the distribution of the toxicity of particular contaminants to a defined range of species representing a specified number of taxonomic groups. It determines a contaminant concentration that is theoretically protective of a given percentage of species (i.e. 95% for slightly to moderately disturbed ecosystems) (Batley et al., 2018).

The development of an SSD requires ecotoxicity data for at least five species that belong to at least four different taxonomic groups and organisms. Taxonomic groups and organisms that are considered to be taxonomically different include: fish, amphibians, crustaceans, insects, molluscs, annelids, echinoderms, rotifers, hydra, green algae, diatoms, brown algae, red algae, macrophytes, blue green algae (cyanobacteria), bacteria, protozoans, fungi and others (Warne et al., 2018).

The approach of ANZG (2018) currently provides comprehensive guidance on managing aquatic ecosystems to both freshwater and marine the conduct of site-specific toxicity assessments including selection of appropriate regionally relevant test species, receiving water, biological endpoints and statistical methodology and other test design considerations. For example, acute vs chronic, single chemical vs complex mixture, laboratory vs in situ, and QA/QC considerations (Warne et al., 2018).

The 'weight of evidence' approach detailed in the ANZECC Guidelines (Figure 6; ANZG, 2018) is recommended to determine an EC limit for treated water discharge to the Goulburn River (Condition 32A(d) of Project Approval 05_0117). The weight of evidence approach (Figure 6) describes the process to collect, analyse and evaluate a combination of different qualitative, semi-quantitative or quantitative lines of evidence to make on overall assessment of water quality and its associated management (ANZG, 2018).

The following assessments are considered appropriate to be conducted to satisfy the weight of evidence approach for the determination of MCO's discharge electrical conductivity and other analyte limits (Table 1):

1. Confirmation of the 80th percentile salinity level at UCML SW01 (and other co-located sites including SW 12) which is recommended by ANZECC Guidelines for the derivation of low-risk trigger values for slightly to moderately disturbed ecosystems; and
2. Calculation of the salinity toxicity limit for 95% species protection recommended by ANZECC Guidelines for slightly to moderately disturbed ecosystems via ecotoxicity investigation and SSD analysis.

Figure 7 demonstrates the alignment of the recommended lines of evidence with the ANZECC Guideline PSER weight of evidence approach (Figure 6).

In accordance with the ANZECC guidelines (ANZG, 2018) for site specific guideline development it is appropriate to determine trigger values for physical and chemical stressors for slightly to moderately disturbed ecosystems, in terms of the 80th percentile values obtained from an appropriate reference system (recommended to be UCML SW01 / SW12 in this instance for the reasons outlined in Section

5.2). The weight of evidence approach considers the 80th percentile salinity level in conjunction with the ecotoxicity and SSD analysis.

In accordance with ANZECC Guidelines (ANZG, 2018) the use of biological and ecological effects data is the most robust and preferred method for site specific guideline development for the protection of aquatic ecosystems. It is recommended for site specific guideline development of a salinity trigger level, that an SSD using water sampled from site UCML SW01 / SW12 is developed.

MCO sought endorsement from the NSW EPA for the use of monitoring data from UCML SW01 / SW12 for the determination of background salinity and heavy metals as this site (Table 3):

- satisfies the constraints of Condition 32A of Project Approval (05_0117);
- is situated on the Upper Goulburn River catchment upstream of the Moolarben Discharge Point; and
- is downstream of the confluence of:
    - highly saline water from Lagoon Creek and Moolarben Creek; and
    - relatively fresh water from Ryans Creek and Sportsmans Hollow Creek.

The recommendation of ‘Endorsement of Monitoring Data Location’ was confirmed with MCO by NSW EPA 20 May 2020 (Section 1.1). Data from location(s) other than site UCML SW01 (and other co-located sites) will not be representative of ‘background’ as:

- Sites upstream on Moolarben Creek and Lagoon Creek are naturally highly saline because of the underlying geology (the Illawarra Coal Measures) and do not include contributions from the fresher tributaries to the Upper Goulburn River; and
- Sites upstream on Ryans Creek and Sportsmans Hollow have low salinity, but do not account for the contribution of naturally higher salinity from Moolarben Creek and Lagoon Creek.

The ‘weight of evidence’ approach was therefore recommended to determine an EC limit for treated water discharge to the Goulburn River using an emphasis on the ‘weight of evidence’ approach that has been provided in ANZG (2018).

The Upper Goulburn River corresponds to a ‘slightly-to-moderately disturbed ecosystem’ with a 95% species level of protection (ANZG, 2018). Ecotoxicity testing for relevant freshwater aquatic species from at least four taxonomic groups should be used to derive the 95% species protection level for salinity.

The weight of evidence from the combination of the 80th percentile salinity level from upstream site UCML SW01 / SW12 (Figure 4), and the results of ecotoxicity test work from this study will ensure that the determined EC limit for treated water discharge from MCO is justified and final.
The endorsement from the NSW EPA given to use of monitoring data from UCML SW01 / SW12 (and other co-located monitoring sites) and the results of proposed ecotoxicity test work (using water sampled from UCML SW01 / SW12 location) is intended to satisfy the requirements of Condition 32A(d).

7.5 Toxicity Test Species Selection

Current ANZECC guidance recommends that data from at least eight species are used in developing SSDs (Table 11; Warne et al., 2018). This guidance is weighed against the availability of chronic and acute ecotoxicity data from test species as indicated below. Consequently, the emphasis on the range of test species size selected means that a comprehensive dataset of non-local species are likely to be selected for ecotoxicity testing against small datasets for local species; this ‘imbalance’ of regional to local test species could lead to less robust GVs. Consequently, the order of preference for data to be used in SSDs should follow as listed in Table 11.

Table 11. Determining the reliability of guideline values using SSD derivations (Warne et al., 2018).

Sample Size [a]	Data Type	Adequacy of Sample Size	SSD Fit	Reliability
≥15		Preferred	Good	Very high
			Poor	Moderate
8–14	Chronic [b]	Good	Good	High
			Poor	Moderate
5–7		Adequate	Good	Moderate
			Poor	Low
≥15	Combined chronic and converted acute	Preferred	Good	Moderate
			Poor	Low
8–14	Combined chronic and chronic marine	Good	Good	Moderate
			Poor	Low
5–7		Adequate	Good	Moderate
			Poor	Low
≥15		Preferred	Good	Moderate
			Poor	Low
8–14	Converted acute	Good	Good	Moderate
			Poor	Low
5–7		Adequate	Good	Low
			Poor	Very Low

Note: a The sample size is assumed to comprise data from at least four taxonomic groups; b This includes all types of data irrespective of whether they are chronic NEC, BEC10, EC10 and NOEC values or estimates of chronic EC10.

Nevertheless, for SSGVs to be valid, single species ecotoxicity testing should focus on a collective of regionally relevant species, chronic exposures and non-lethal end points in relevant receiving water mixtures. Effect concentrations at a defined % relative effect (ECx) are also preferred in datasets of ≤10 (Warne et al., 2018).

Taxonomic groups are generally considered to be phyla (that is, organisms that belong to different taxonomic groups; Table 12). An exception to this is crustaceans and insects, which both belong to the phyla Arthropoda but are defined as taxonomically different for SSD purposes (Warne et al., 2018). The selection of the species in this study meets the ANZG (2018) guideline requirements for the assessment of toxicants in receiving waters by having at least five species from four taxonomic groups (Warne et al., 2018) and four trophic levels as part of the testing suite. In total, the testing suite involved ten tests using eight species from seven different taxonomic groups and four trophic levels (Table 12). All eight species test results were used for derivation of the SSGVs for nickel, zinc and sulfate. The suite of tests provided a range of acute and chronic endpoints of toxicity to use in the derivation of the SSGVs. Three of the species were defined as an acute only test (Batley et al., 2018; Warne et al., 2018) and two species were combined chronic-acute tests (Table 13). Further details are given in the summary of test species suitability in Appendix A.

Table 12. Taxonomically different organisms (Warne et al., 2018).

Major types of Organisms	Organisms considered to be taxonomically different [a]
Vertebrates	Fish, amphibians
Invertebrates	Crustaceans, insects, molluscs, annelids, echinoderms, rotifers, hydra
Plants	Green algae, diatoms, brown algae, red algae, macrophytes
Others	Blue-green algae (cyanobacteria), bacteria, protozoans, coral, fungi, and others

Note a Generally taxonomic groups are phyla. Source: modified from Warne et al. (2018)

All tests in this study were undertaken using Ecotox Services Australia laboratory cultures as broodstock, except for *Nousia sp.*AV1 *(Leptophlebiidae)* (Appendix A) which used a test organism directly sourced from a sub-catchment of the Goulburn River catchment (described below). This followed the directive from NSW EPA that MCO needed to use Mayfly as a test aquatic species sourced from the Upper Goulburn River. Mayflies were collected from the wild at Ryans Creek, placed in trays which could be aerated during transfer to the test at Ecotox Services Laboratory and delivered for preparation to undertake the subsequent ecotoxicity testing. The field collecting was undertaken 23-24 June 2021 and 6 July 2021.

The Mayfly collection location was determined following review of stream health monitoring data, consideration of catchment location and inspection of possible sites. Stream health monitoring was undertaken by MCO since 2005. This provided a readily–available data base on macroinvertebrate numbers and their variation with time. Stream health monitoring has been undertaken in the Goulburn River, Bora Creek, Moolarben Creek, Ryans Creek, Murragamba Creek, Wilpinjong Creek and Eastern Creek (Figure 3; MCO, 2018). Stream health monitoring locations designated as ‘SH’ and stream health status have been summarised in the MCO Surface Water Management Plan (MCO, 2020).

Table 13. Test species utilised for Moolarben Coal Mine SSGV derivation.

Test Species	Phylum	Class	Trophic level	Endpoint	Duration	Exposure
Freshwater Cladoceran, *Ceriodaphnia dubia*	Anthropoda	Crustacea	Primary consumer	Survival	48-hr	Acute
Freshwater Cladoceran, *Ceriodaphnia dubia*	Anthropoda	Crustacea	Primary consumer	Reproduction	7-days	Chronic
Freshwater Aquatic Duckweed, *Lemna disperma*	Tracheophyta	Magnoliopsida	Primary producer	Growth Rate (growth Inhibition)	7-days	Chronic *
Eastern Rainbowfish, *Melanotaenia splendida splendida*	Chordata	Actinopterygii	Tertiary consumer	Fish Imbalance	96-hr	Acute
Eastern Rainbowfish, *Melanotaenia splendida splendida*	Chordata	Actinopterygii	Tertiary consumer	Reproduction (embryo hatching)	12-days	Chronic
Freshwater Hydra, *Hydra viridissima* (Pilbara isolate)	Cnidaria	Hydrozoa	Secondary consumer	Growth Rate	96-hr	Chronic *
Green Alga, *Raphidocelis subcapitata* [a]	Chlorophyta	Chlorophyceae	Primary producer	Growth Rate (growth inhibition)	72-hr	Chronic
Freshwater Chironomid, *Chironomus tepperi*	Arthropoda	Insecta	Primary consumer	Survival	48-hr	Acute
Freshwater Prawn *Macrobrachium australiense*	Arthropoda	Crustacea	Tertiary consumer	Survival	96-hr	Acute
Mayfly Larval, *Nousia sp.*AV1 (*Leptophlebiidae*) [b]	Arthropoda	Insecta	Primary consumer	Survival	96-hr	Acute

Note:* Defined as chronic after Batley et al. (2018) for tropical and temperate species
a Formerly Selenastrum capricornutum
b Site specific species collection of Mayfly Larval from Ryans Creek Moolarben

The stream health monitoring program is based on the Australian Rivers Assessment System (AusRivAS) aquatic invertebrate monitoring protocol, as used for the baseline stream health study. AusRivAS is a rapid biological assessment protocol with twice yearly aquatic macro invertebrate sampling (MCO, 2020). The stream health program includes aquatic macroinvertebrate diversity and pollution tolerance SIGNAL scores.

Historical data (Table 14) for Mayfly numbers in the upper catchment of the Goulburn River and sub-catchments have been identified as one of the following three species: Baetidae, Caenidae or Leptophlebiidae (MPR, 2017). Populations typically peak in the months of March, April and May.

Based on historic surveys, the 2021 sample collection of test species focused on areas known to host higher Mayfly populations. Ryans Creek reference site (SH12; Figure 3) located at Lagoons Rd crossing, upstream of confluence with Moolarben Creek SH12 was chosen to search for Mayfly. Ryans Creek is located in granitic bedrock, without any naturally-occurring coal mineralisation, with consistent good quality water with low EC and salinity and is accessible by road crossing nearby site SH12. The Pollution Tolerance Site SIGNAL scores for SH12 to 2018 was 4.2±0.3 (MCO, 2020).

Table 14. Mayfly population distribution at creek sites nearby Moolarben Coal Mine lease area from 2004-2019.

Site	n	Mean	Max	Min
SH01	187	9	853	0
SH02	187	6	630	0
SH03	187	0	1	0
SH04	187	1	52	0
SH05	187	3	126	0
SH06	187	1	43	0
SH07	187	0	1	0
SH08	187	0	6	0
SH10	187	0	9	0
SH12	187	6	399	0
SH13	187	5	285	0
SH14	187	0	6	0
SH16	187	0	5	0
SH17	187	0	1	0
SH18	187	0	1	0
SH19	187	0	1	0
SH20	187	0	0	0

Thus SH12 being upstream of any potential influence from UCML and MCO releases was the preferred location for mayfly collection in the upper Goulburn River catchment (Figure 3). Ryans Creek water quality was measured at SH12 and at SW06, 1 km upstream from SH12 (Figure 3). Table 4 shows the 80th percentile EC for Ryans Creek water at SW06 and SH12 was 238 µS/cm, while 80th percentile salinity was 220 mg/L from 14 April 2005 to 2021 (n=65). Thus SW06 and SH12 can be considered a suitable site for mayfly collection due to its good water quality. Over 400 Mayfly were collected at the SH12 site 23-24 June and 6 July 2021, confirming its suitability as a collection site. The mayfly class of species was established by Ecotox Services Australia during the ecotoxicity testing phase (Appendix A).

7.6 Natural Diluent Water Quality

Additional details of requirements for analytical chemistry testing including additional steps from Ecotox Services aquatic species testing on each analyte/species/concentration step or blank test are described here.

Diluent water for control in the ecotoxicity testing was selected for collection from the Goulburn River reference monitoring site UCML SW01 / SW12 (2x25 L: Figure 4) above the mine influence, and WTP Filtrate (1x25 L) as the treatment water at MCO for EC and salinity toxicity evaluation were collected in 5 L acid-rinsed containers on 24 June 2021. All containers were triple rinsed prior to filling. The bulk water samples were transported to the Ecotox Services Australia laboratory, Lane Cove in Sydney, NSW where they were filtered (0.45 µm) and refrigerated until use. The Chain of Custody page for these test waters is included in the Report by Ecotox Services Australia (Appendix G) and indicates that sampling of the test waters was directed by Prof. Barry Noller.

Triplicate samples of both the diluent water for control in the ecotoxicity testing collected from the Goulburn River reference monitoring site UCML SW01 / SW12 as diluent control water and the WTP Filtrate as the treatment water at MCO, both collected 24 June 2021 and sent directly to ALS Environmental laboratories for a range of water quality parameters; the details are given in Appendix B.

The Diluent Control (UCML SW01 / SW12) and a USEPA control (where applicable) were tested concurrently for ecotoxicity with the test samples. A subsample of each solution taken at the commencement (t=0 h) and termination of each test was sent by Ecotox Services Australia to Envirolab Services Pty Ltd for chemical analysis. The measured (dissolved, 0.45 µm filtered) concentrations of each analyte at t=0 h were used for the calculation of ecotoxicity statistical endpoints (Appendices C and G Ecotox Services Australia Report).

Batley et al. (2018) describe a reference toxicant as ‘a reference chemical (toxicant) used in a toxicity test to assess the sensitivity of a test organism and to demonstrate the repeatability of a test and the laboratory's ability to perform the test consistently’ (p38), concentration as ‘The quantifiable amount of a substance in water, biota, soil or sediment’ (p40), and gives Question 18 stating ‘Were test solutions, blanks and/or controls tested for contamination or were analytical reagent grade chemicals or the highest possible purity chemicals used for the experiment?’ (p12). Batley et al. (2018) in Section 12 (p34) also describe ‘Uncertainties associated with guideline values’ and identify ‘To minimise variability, good quality assurance and quality control of toxicity testing is required including: (i) analysis of all toxicant concentrations used in the toxicity test at the beginning and end of the test; (ii) use of a reference toxicant; (iii) appropriate control and measurement of experimental conditions, for example, temperature, pH; (iv) quality control of the test containers, for example water changes, organism feeding; and (v) a reproducible control response.’ These details support the use of an approach and philosophy of undertaking reliable measurements of water quality for ecotoxicity testing purposes.

There is no mention in Batley et al. (2018) or Warne et al. (2018), and presumably ANZG (2018), that the analytical testing of toxicants in water for toxicity testing should or need to be undertaken by accredited laboratories. However, this can be assumed to be necessary because the organised laboratory accreditation process in Australia is that laboratories are accredited by The National Association of Testing Authorities (NATA) as the national accreditation body. The roles of NATA are recognised by the Australian Government in a Memorandum of Understanding (MOU) (DIIS, 2018). NATA accredits laboratories in Australia against the current ISO/IEC 17025 – Testing (ISO/IEC, 2017). The ISO 17025 accreditation is intended to confirm that ‘a particular laboratory is able to produce precise and accurate test results and calibration data’, including ‘traceability of measurements and calibrations to national standards'. Thus, NATA accreditation 'provides a means of determining, formally recognising and promoting the competence of facilities to perform specific types of testing, inspection, calibration, and other related activities'. To maintain accreditation, laboratories must be re-assessed regularly.

Data generation by analytical laboratories undertaking water analysis from the ecotoxicity testing is thus underpinned by the laboratory meeting the requirements of accreditation to ISO/IEC 17025 – Testing (ISO/IEC, 2017). The 'general requirements for the competence of testing and calibration laboratories' are to demonstrate an ability to generate valid results. ISO/IEC 17025 (ISO/IEC, 2017) specifies the general requirements for the competence, impartiality and consistent operation of laboratories and also conforms operating generally in accordance with the principles of ISO9001.

'Metrological traceability' is described in Section 6.5 of ISO/IEC 17025 (ISO/IEC, 2017).

Then the following: Section 6.5.1 describes that 'the laboratory shall establish and maintain metrological traceability of its measurement results by means of a documented unbroken chain of calibrations, each contributing to the measurement uncertainty, linking them to an appropriate reference'; Section 6.5.2 describes 'that the laboratory shall ensure that measurement results are traceable to the International System of Units (SI) through: a) calibration provided by a competent laboratory; b) certified values of certified reference materials provided by a competent producer with stated metrological traceability to the SI; or reference material producers fulfilling the requirements of ISO 17034 are considered to be competent; c) direct realisation of the SI units ensured by comparison, directly or indirectly, with national or international standards'; Section 6.5.3 describes that 'when metrological traceability to the SI units is not technically possible, the laboratory shall demonstrate metrological traceability to an appropriate reference, e.g.: a) certified values of certified reference materials provided by a competent producer; b) results of reference measurement procedures, specified methods or consensus standards that are clearly described and accepted as providing measurement results fit for their intended use and ensured by suitable comparison'.

A supporting NATA document, aligning with accreditation to ISO/IEC 17025 (ISO/IEC, 2017), provides specific definitions and details on precision, trueness, and measurement uncertainty, and other complementary details that does not need complete elaboration here (NATA, 2018).

Detailed water quality analytical results of the MCO ecotoxicity test waters are given in Appendix B (Table B-1). The UCML SW01 / SW12 diluent water (filtered <0.45 µm) and WTP Filtrate water (filtered <0.45 µm) were both sampled and analysed in triplicate at the ALS Environmental (NATA Accredited Laboratory Number: 825) following standard methods. The test report results in Appendix B are both compared against 95% freshwater aquatic ecosystem protection guidelines for toxicity (ANZG, 2018) in Appendix B.

Filtered (<0.45 µm) water was used for the MCO ecotoxicity testing to derive SSGVs for the 3 selected analytes and EC. The water collected 24 June 2021 from the Upper Goulburn River reference monitoring site UCML SW01 / SW12 (Figure 4), upstream of the mine releases, the WTP Filtrate, WTP Permeate and WTP Discharge samples for EC was dispatched to the Ecotox Services Australia laboratory in Sydney and received on 25 June 2021, having a temperature of 12 °C on receipt, and 'in apparent good condition'. The received waters, comprising SW12 Diluent control and WTP filtrate in the 5 and 25L containers, were filtered (<0.45 µm-filter) and kept refrigerated until use. The following details are given in each test report from Ecotox Services Australia included in Appendix G: 'An aqueous sample of the test water from UCML SW01 / SW12 was tested in the Ecotox Services Australia laboratory and gave pH 7.0, electrical conductivity 786 µS/cm, total ammonia <2.0 mg/L; An aqueous sample of the test water from WTP Filtrate was tested in the Ecotox Services Australia laboratory and gave pH 7.9, electrical conductivity 2110 µS/cm, total ammonia <2.0 mg/L; An aqueous sample of the test water from WTP Permeate was tested in the Ecotox Services Australia laboratory and gave pH 6.9, electrical conductivity 64 µS/cm, total ammonia <2.0 mg/L; and an aqueous sample of the test water from WTP Discharge was tested in the Ecotox Services Australia laboratory and gave pH 7.1, electrical conductivity 295 µS/cm, total ammonia <2.0 mg/L'.

The water quality data for UCML SW01 / SW12 diluent water in Table B.1 lists electrical conductivity (EC) 783 µS/cm (corresponding to total dissolved solids [TDS] 568 mg/L) and exceeds the ANZECC/ARMCANZ (2000) in ANZG (2018) default trigger values for salinity (EC) at South-east Australian 'Upland rivers' (30-350 µS/cm); the pH 7.07 lies within the ANZECC/ARMCANZ (2000) default trigger values for South-east Australia (NSW) 'Upland river' (6.5-8.0) with moderate total alkalinity of 46.3 mg/L $CaCO_3$ and hardness of 152 mg/L (as $CaCO_3$) described as hard (120-179) by ANZECC/ARMCANZ (2000). Dissolved organic carbon 9.0 mg/L is high for Australian Upland rivers; and may complex with heavy metals.

The UCML SW01 / SW12 Diluent control water was of good quality, slightly alkaline and hard. Both EC and hardness were greater than the 80th percentile, but less than maximum values (Tables 5 and 6), while pH and total alkalinity (bicarbonate) were both less than mean values (Table 6). Filtered nickel and zinc concentrations remained low, slightly below mean values. Sulfate concentrations are between the 80th percentile and maximum historic values, aligning with EC. Although the turbidity (21.6 NTU) was above guidelines, all other parameters met guidelines for 95% aquatic ecosystem protection. Both turbidity and Fe (total 0.943 mg/L) were expected to be significantly reduced by 0.45 µm filtering for diluent preparation. The UCML SW01 / SW12 is considered suitable as a control for the ecotoxicity testing purposes; the control water was representative of the geochemical background of the upper Goulburn River.

The WTP Filtrate for dosing into UCML SW01 / SW12 Diluent water, and UCML SW01/SW12 diluent water collected 24 June 2021 for ecotoxicity tests was intended to enable the derivation of an electrical conductivity GV. Triplicate samples of the UCML/SW12 and WPT Filtrate water was received 24 June 2021 (Lab ID ME2101037) at by ALS. The triplicate aqueous samples of WTP Filtrate gave pH 8.03, electrical conductivity 2067 µS/cm and total ammonia <2.0 mg/L. The triplicate aqueous samples of UCML SW01 / SW12 Diluent water gave pH 7.07, electrical conductivity 783 µS/cm and total ammonia <2.0 mg/L .

The hardness modified triggers applied to nickel and zinc (Warne et al., 2018) in Appendix B are all valid against the notional hardness of 30 mg/L as $CaCO_3$ in Warne et al. (2018) as the measured hardness in SW 12 was 152 mg/L as $CaCO_3$ and in WTP Filtrate was 622 mg/L as $CaCO_3$ and categorised as 'extremely hard' by ANZG (2018).

The dates for commencing the aquatic toxicity testing using the processed filtered water at Ecotox Services Australia is documented in the test reports for each analyte were: 2 July 2021 and 8 July 2021.

7.7 Quality Control/Quality Assurance

The quality control/quality assurance of the toxicity tests and water quality data is primarily focussed on the accuracy or 'trueness' of the analyte concentrations and the uncertainty of their respective measurements (NATA, 2018). The measured analyte concentrations that become the values of the ecotoxicity endpoints and the GVs from the SSD plotting are of necessity highly accurate. All steps from processing of solutions before, during and following the toxicity testing introduce their own contribution to measurement uncertainty. The data generation is underpinned by the analytical laboratories undertaking water analysis from the ecotoxicity testing, including the details given in Section 3.3 for accreditation to ISO/IEC 17025 – Testing (ISO/IEC, 2017).

Ecotox Services Australia is NATA Accredited Laboratory Number: (14709) for ecotoxicity testing. The scope of their laboratory accreditation for weighing and volume container and solution make up may be part of the laboratory NATA accreditation but are not listed in the scope of their NATA Accredited Laboratory. Ecotox Services Australia uses a Sartorius Model ED124S with 'readability of 0.0001g'.

The analytical balance calibration reports performed externally by National Weighing and Instruments, Girraween, NSW 2145 (Report number U2105-9235 28 May 2021) and covers the 12-month period until November 2021 show satisfactory balance readability (0.0001 g) that brackets when stock solutions of analytes were prepared. The 6 monthly single point calibration check was done 29 June 2021 and gave satisfactory uncertainty (0.00005 g) and readability (0.0001 g).

Boeco pipettors covering volumes dispensed of 10 µL (Report No: 2011-0963), 50 µL (Report No: 2011-0961) , 200 µL (Report No: 2011-0962), 1000 µL (Report No: 2011-0965) and 5000 µL (Report No: 2011-0964) were calibrated 27 November 2020 by National Weighing and Instruments, Girraween, NSW 2145, and are due for re-calibration next on 27 November 2021. The uncertainties measured 27 November 2020 for each pipettor (maximum volume) were as follows: 10 (0.07 µL), 50 (0.16 µL) , 200 (0.41 µL), 1000 (1.60 µL) and 5000 (8.20 µL) µL. Pipette calibrations made 27 November 2020 covered the dates when stocks were prepared at Ecotox Services and spiked as described in Appendix D.

Ecotox Services Australia also advised regarding the preparation of stock solutions for each analyte that 'We did not record exactly what we weighed out each time, partly as I usually weigh out exactly to 4 decimal places, and also (as we have discussed), it does not matter if we are out as we rely on measured concentrations, not nominal.' This is technically correct as the concentrations of each of 3 analyte solutions, made up by weighing the approximate amount of chemical, is measured independently by an external NATA registered laboratory Envirolab Services Pty Ltd, Chatswood (see Section 7.7.1). However, the measurement uncertainty for weighing the analyte for preparation of stock solution is expected to be a minor source of uncertainty.

Ecotox Services Australia prepared the chemical stock solutions for ecotoxicity testing using the following procedure given in each test report for each respective analyte:

'A stock solution of the analyte was prepared using the AR grade analyte (Table 15). The highest test concentration was prepared by adding a volume of the analyte stock into filtered (<0.45 µm) water from SW12 Diluent water from upstream Goulburn River. The remaining test concentrations were achieved by serially diluting the highest test concentration with SW12. The Diluent Control ('SW12') and a Dilute Mineral Water (DMW) control, together with WTP Filtrate, WTP Permeate and WTP Discharge, were tested concurrently with the samples. A subsample of each solution taken at the commencement (t=0h) and termination of each test was sent to Envirolab Services Pty Ltd, Chatswood for chemical analysis. The measured (dissolved, 0.45 µm filtered) concentrations at t=0 h were used for the calculation of statistical endpoints'.

Chemical stock solutions for dosing control water with nickel, zinc and sulfate, respectively were prepared by Ecotox Services Australia laboratory using analytical grade (AR) grade hydrous nickel sulfate ($NiSO_4.5H_2O$), hydrous zinc sulfate ($ZnSO_4.7H_2O$) and hydrous sodium sulfate ($Na_2SO_4.10H_2O$) chemicals, respectively (Table 15). The highest test concentrations for each analyte solution were prepared by adding a volume of the corresponding stock into diluent. The remaining test concentrations were achieved by serially diluting the highest test concentration with diluent.

A summary of the characteristics of analytes and AR grade chemicals used in the ecotoxicity testing by Ecotox Services Australia is given in Table 15. The chemical name and formula, mole weight of chemical and solubility; details and data for respective analytes in Table 15 were obtained from the Pubchem internet site (National Center for Biotechnology Information, 8600 Rockville Pike, Bethesda, MD, 20894 USA; PubChem® is a registered trademark of the National Library of Medicine https://pubchem.ncbi.nlm.nih.gov/).

Comments on the details and driving processes of environmental chemistry on toxicology are given as follows for respective analytes:

Nickel (Ni): The analyte compound Nickel sulfate hexahydrate ($NiSO_4.6H2O$) is very soluble 775 g/L at 30 °C as $NiSO_4.6H_2O$ or 173.04 g/L as Ni at 30 °C; at pH 7.07 it may be affected by the hardness 152 mg/L $CaCO_3$.

Zinc (Zn): The analyte compound Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) is very soluble with 540 g/L as $ZnSO_4.7H_2O$ or 123 g/L as Zn (20 °C); at pH 7.07 it may be affected by the hardness 152 mg/L $CaCO_3$.

Sulfate (SO_4): The analyte compound sodium sulfate (Na_2SO_4) is very soluble with 281 g/L as Na_2SO_4 and 236 g/L as SO_4 (25 °C), including at pH 7.07 and the hardness of 152 mg/L $CaCO_3$.

Table 15. Characteristics of Analytes and AR Grade Chemicals used in Testing

Analyte	AR Grade Chemical and Formula	Mole Weight of Chemical (g/mol)	Solubility (g/L)
Nickel	Nickel sulfate hexahydrate $NiSO_4.6H_2O$	262.85 (Ni=58.69)	775 g/L (30 °C) as $NiSO_4.6H_2O$ or 173.04 g/L as Ni (30 °C)
Sulfate	Sodium sulfate $Na_2SO_4.10H_2O$	322.20 (SO_4=96.05) (Na_2SO_4=142.05) (SO_4/Na_2SO_4=0.6762)	281 g/L as Na_2SO_4 or 236 g/L as SO_4 (25 °C) 440 g/L as $Na_2SO_4.10H_2O$ (20 °C) (Note $CaSO_4$ is 2.1 g/L at 20 °C)
Zinc	Zinc sulfate heptahydrate $ZnSO_4.7H_2O$	287.6 (Zn=65.38)	540 g/L as $ZnSO_4.7H_2O$ or 123 g/L as Zn (20 °C)

In order to determine stability of toxicant concentration and consequent test organism exposure during the test period, water quality samples for EC, pH, Dissolved Oxygen (% saturation), dissolved analytes as <0.45 µm filtered and total for each analyte were analysed at T0 h, T48 h, T72 h and T96 h, T7 d, T12 d and T14 d for all relevant test concentrations (Appendix G). Water quality samples from ecotoxicity test solutions were analysed by Envirolab Services Pty Ltd, Chatswood.

Water quality analysis is undertaken to determine stability of toxicant concentration and consequent test organism exposure during the test period, for EC, pH, dissolved oxygen (% saturation), dissolved analytes as <0.45 µm filtered water quality samples were analysed at T0 h, T48 h, T72 h and T96 h, T12 d and T14 d for all relevant test concentrations. Water quality samples were analysed by Envirolab Services Pty Ltd, Chatswood (Appendix D).

The nominal and measured test water concentrations are summarised in Appendix C. The specific observations for nominal and measured analyte concentrations are described as follows:

Nickel (Ni) Test concentrations did not change substantially throughout the course of the experiments, excepting at lowest concentrations.

Sulfate (SO_4) Test concentrations did not change substantially throughout the course of the experiments, excepting at lowest concentrations.

Zinc (Zn) Test concentrations did not change substantially throughout the course of the experiments, excepting at lowest concentrations.

Electrical conductivity (EC) EC was measured in all test solutions (Appendix D, Ecotox Services Australia Report). However, statistical endpoints were only determined for WTP Filtrate, Permeate and Discharge waters (as controls). Comparisons between EC and WTP water types were used to develop comparisons for EC, salinity by conversion and guideline values where appropriate.

7.7.1 Quality Control Definitions

Envirolab Services Pty Ltd, Chatswood undertook analysis of ecotoxicity test solutions and diluent water for Ecotox Services Australia have NATA Accreditation Number 2901. Envirolab Certificates of Analysis for all test solutions are included as appendices in the Ecotox Services Australia Report (Appendix D). Each Envirolab Services Certificate of Analysis gives the following details:

Blank 'This is the component of the analytical signal which is not derived from the sample but from reagents, glassware etc, can be determined by processing solvents and reagents in exactly the same manner as for samples**'.**

Duplicate 'This is the complete duplicate analysis of a sample from the process batch. If possible, the sample selected should be one where the analyte concentrate is easily measurable'.

Matrix Spike 'A portion of the sample is spiked with a known concentration of target analyte. The purpose of the matrix spike is to monitor the performance of the analytical method used and to determine whether matrix interferences exist'.

LCS (Laboratory Control Sample) 'This comprises either a standard reference material or a control matrix (such as a blank sand or water) fortified with analytes representative of the analyte class. It is simply a check sample'.

Surrogate Spike 'Surrogates are known additions to each sample, blank, matrix spike and LCS in a batch, of compounds which are similar to the analyte of interest, however are not expected to be found in real samples'.

7.7.2 Laboratory Acceptance Criteria

'Duplicate sample and matrix spike recoveries may not be reported on smaller jobs, however, were analysed at a frequency to meet or exceed NEPM requirements. All samples are tested in batches of 20. The duplicate sample RPD and matrix spike recoveries for the batch were within the laboratory acceptance criteria.'

Matrix Spikes, LCS and Surrogate recoveries: '*Generally 70-130% for inorganics/metals*.'

Envirolab Services Pty Ltd advise that 'In circumstances where no duplicate and/or sample spike has been reported at 1 in 10 and/or 1 in 20 samples respectively, the sample volume submitted was insufficient in order to satisfy laboratory QA/QC protocols' and that 'Measurement Uncertainty estimates are available for most tests upon request'. Thus, the Quality Control details for each analyte summarised by codes in the respective Envirolab Certificates of Analysis are not included directly, but actual details of measurement uncertainties with a single result for each Spike recovery % may be supported by '*Measurement Uncertainty estimates available for most tests upon request*'. These single numbers are the only outputs given in the Certificate of Analysis of quality control (generally in the range 70-130% for inorganics/metals) for each analytical batch. These quality control samples are indicative of the trueness of the measured concentrations of analytes.

The Envirolab Services Pty Ltd Certificate of Analysis in the Appendices of the Ecotox Services Australia Laboratory toxicity test reports (Appendix G) display a variety of LCS (LCS-W1, LCS-W2, LCS-W3, LCS-W4, LCS-W5, L LCS-W6,) and 'surrogate spikes' (276720-1, 276720-15, 276720-3, 276720-23, 276721-13, 276721-33, 276723-3, 276721-33, 276723-2, 276730-2, 276730-22, 276731-11, 276731-31,) with the comment that a single result for each Spike recovery % may be supported by a '*Measurement Uncertainty estimates available for most tests upon request*'. Appendix D has the statement of Envirolab Services Pty Ltd giving uncertainty values and copies of certificates of analysis for certified reference material used in the analytical work to meet the requirements of laboratory accreditation following ISO/IEC 17025 – Testing (ISO/IEC, 2017).

The Ecotox Services Australia Laboratory report (Appendix G) includes details of Envirolab Certificates of Analysis 276879, 276720, 276721, 276723,276730 and 276731 are listed together with measurement uncertainties (MU% range 3-15 at 95% confidence, Appendix D) for all analytes.

Test certificates for certified reference materials are details that underpin the 'trueness' of the concentration results for test solution analyses from the ecotoxicity testing which are then used as data input for the assessment of the decision on the SSD model fitting that gives the SSGV values of the analyte. The test certificates for standards with date of expiry for each analyte should be retained by the laboratory as part of their NATA accreditation process following ISO/IEC 17025 (ISO/IEC, 2017). The test certificate details can be included in the test report but the laboratory may not want to provide them, and are not described in the scope of the NATA accreditation listing.

Envirolab have provided test certificates for certified reference materials for nickel, sulfate and zinc (see Appendix D, except EC where the Ecotox Services Australia certified reference material could be used to provide comparative data).

Ecotox Services Australia also measured EC on the Diluent water UCML SW01 / SW12 used in ecotoxicity tests using a certified reference material for EC measurement (Merck Potassium Chloride Solution- see Appendix D). Although not endorsed by NATA for EC measurement, however Ecotox Services Australia advised that both the procedure and calibration records are reviewed by NATA at each and every assessment. Ecotox Services Australia measure EC in-house using a WTW Cond 330i meter with WTW Tetracon 325 probe, calibrated at the first use each week using the Merck KCl standard at 1410 µS/cm.

Table 16. Comparison of EC analyses in Certipur Certified Reference Material (Potassium Chloride Solution) for each Laboratory

	Envirolab Services	Ecotox Services Australia	Certipur Certified Reference Material for measurement of Electrical Conductivity[b]
Date reported	01/09/2021	2-30/08/2021[a]	Potassium Chloride Solution (nominal 1410 µS/cm)

	Envirolab Services	Ecotox Services Australia	Certipur Certified Reference Material for measurement of Electrical Conductivity[b]
N	3	10	
Mean	1400	1427	
Sd	1	2.2	
se=sd/√n	0.58	0.68	
Mean±se	1399-1401	1426-1428	Certified value and uncertainty 1412 µS/cm (25.0 °C±20 µS/cm (±1.41%)

Note: a. Measurements were made on 2, 9, 13, 16, 17, 18, 20, 23, 25 and 30 August 2021.
b. Merck KGaA, Frankfurter Str. 250, 64293 Darmstadt, Germany. Certified version issued on 2019/11/19.

Table 16 shows the summary data for EC measurements on the same Certipur Certified Reference Material for measurement of Electrical Conductivity water by the two laboratories. The individual laboratory results show agreement with the certified value confidence interval but do not quite overlap with a difference of 27 µS/cm between laboratory means, corresponding to differences of 1.89-1.92% between laboratories. Nonetheless, the mean results are considered acceptable as they are <1% different from the Certified Reference Material (CRM) value (Table 16; Appendix D).

Batley et al. (2018) in Section 12 (p34) describe 'Uncertainties associated with guideline values' and identify 'To minimise variability, good quality assurance and quality control of toxicity testing is required including: (i) analysis of all toxicant concentrations used in the toxicity test at the beginning and end of the test; (ii) use of a reference toxicant; (iii) appropriate control and measurement of experimental conditions, for example, temperature, pH; (iv) quality control of the test containers, for example water changes, organism feeding; and (v) a reproducible control response.'

Very few details of these steps are documented and limit assessing uncertainty of concentration data from making up solutions through to measuring concentrations of test solutions ultimately used in the estimation of analyte SSGVs.

Each toxicity testing series undertaken by Ecotox Services Australia uses a range of test contribution, each with a set of species being exposed. The processing of each toxicity testing series is performed using probit analysis with specific statistical corrections using well-defined tests. Maximum Likelihood Probit analysis (Finney, 1971) or Log-Logit Interpolations (USEPA, 2002) are used depending on which method was considered appropriate (Appendix G Ecotox Services Australia). The output data gives defined ecotoxicity characteristics that can be inputted directly into the SSD software for further plotting. These concentration numbers are virtually single numbers without any indication of uncertainty.

The variability that may arise during solution preparation is not considered as such for uncertainty, but the detail of possible variation of concentrations in the test solutions were identified in each Ecotox Services Australia test report and described above.

7.8 Salinity Characterisation

Salinity refers to dissolved salts or major cations and anions that are in solution (Hem, 1985). Electrical conductivity (EC in µS/cm) and total dissolved solids (TDS in mg/L) are key water quality parameters used to describe levels of salinity. Salinity is a measure of dissolved salts or TDS in water. Electrical conductivity (EC in µS/cm) is the capacity of a solution to conduct an electric current, which is directly related to the dissolved salts in solvents, such as water. Usually the TDS and EC relationship can be described by the following Equation (1), with k representing the salinity factor or ratio (TDS/EC):

$$TDS\,\frac{mg}{L} = k \times EC \tag{1}$$

Electrical conductivity is a relatively inexpensive and easy in-situ measurement to perform by meter, while TDS is labour-intensive and requires more equipment and time to quantify. Due to the difficulties in reproducing TDS measurements and its relevance as a measure of salinity in water quality investigations, correlation between these two parameters have often been used to obtain a TDS value from EC. Typically, the mathematical relationship of these two parameters is easily described in freshwater river environments (Equation 1), with salinity factor, k , varying between 0.5 ≥1.00 (McNeil and Cox, 2000).

However, this statistical correlation can vary and a simple linear relationship does not always apply to waters that are heavily influenced by contaminants. It is expected that the higher the salinity level or material contents, the more complex the mathematical relationship between TDS and EC, outlining the importance of TDS measurements for complex water matrices (McNeil and Cox, 2000).

Where TDS values are not measured in monitoring data for the Goulburn River, EC measurements may be used to provide TDS values by using a site-specific Goulburn River specific salinity factor, k, based on historic TDS and EC measurements. A salinity factor is also proposed to derive TDS equivalent toxicity limit, for sulfate ecotoxicity measurements, which are established as site specific guideline values from species sensitivity distributions (refer to Sections 8.5).

7.9 Statistical Data Evaluation for SSD Input

Warne et al., (2018) advise that 'chronic rather than acute toxicity are preferred to derive guideline trigger values for toxicants, as they are more appropriate to achieve the overall aim to provide life-long protection for aquatic organisms and therefore, for aquatic ecosystems'. Therefore, SSDs are preferably based on chronic rather than acute toxicity data. Acute test data only need conversion to chronic data via an acute to chronic ratio (ACR) described by Warne et al. (2018). However, the ANZG (2018) guidance recommends using chronic toxicity test results over acute; even of sensitive life-stages.

Because the sample size (8 test species) used in the MCO SSGVs, effect concentrations at a defined % relative effect (EC/ICx) were advised as preferred to the use instead of no observed effect concentration data (NOEC) (Warne et al., 2018). The most accepted replacements of NOECs are indicated to be low effect measures of toxicity such as the concentration that causes a 10% effect (EC/IC10) and that causes 10% lethality (LC10) (Warne and Van Dam, 2008).

The EC/IC10 (the effective concentration giving 10% reduction in the endpoint compared with the controls) and used by Ecotox Services Australia (Appendix G) were calculated using Trimmed Spearman-Karber analysis (Hamilton et al., 1977), Maximum Likelihood Probit analysis (Finney, 1971) or Log-Logit Interpolations (USEPA, 2002), depending on which method was considered appropriate.

7.10 SSD Derivation

All SSDs are based on data from toxicity testing with EC/IC10 chronic endpoint. Some toxicity tests do not have chronic data available, and required acute to chronic endpoint conversions to deliver chronic endpoint estimations. Additional information regarding ACR derivation is described in the following section.

7.10.1 Acute to Chronic Ratios (ACR) Application

Instead of using a default assessment factor of 10, Warne et al. (2018) advises that toxicant specific ACRs are the preferred method to estimate chronic responses from tests with acute only endpoints. Three test species had acute only endpoints available: *(1) Chironomus tepperi,* (2) *Macrobrachium australiense*, and (3) *Nousia sp. AV1 (Leptophlebiidae)*.

Equations (2) and (3) demonstrate the preferred method for toxicant specific ACR derivation and chronic response estimates (Warne et al. 2018):

$$ACR_{Toxicant} = \frac{EC/IC50acute}{EC10chronic}$$

(2)

$$EC/IC10_{Chronic} = \frac{EC/IC50}{ACR\ Toxicant}$$

(3)

ACRs were derived from tests with paired acute to chronic endpoint data. Paired acute to chronic endpoints were available for two test species: *Ceriodaphnia dubia* and *Melanotaenia splendida splendida*. ACRs were developed for three toxicants Nickel, Zinc and Sulfate (Tables 17 to 19). Refer to test species used in Table 13 for values (Section 7.5).

Table 17: ACR Toxicant derived data from *C. dubia* acute and chronic endpoints.

Toxicant	EC/IC50 acute/ EC10 Chronic	ACR Toxicant
Nickel	=44.2/11	4.02
Zinc	=98.3/87.7	1.12
Sulfate	=1961/1270.7	1.54

Table 18: ACR Toxicant derived data from *M. splendida sp.*

Toxicant	EC/IC50 acute/ EC10 Chronic	ACR Toxicant
Nickel	=137.2/78	1.76
Zinc	=252.9/90	2.81
Sulfate	=4320.7/1222.3	3.53

Due to the similarity of *C. dubia* and *M. australiense* taxonomic groups (both Crustacea), the ACR toxicant values derived from *C. dubia* acute/chronic paired data can be used directly to estimate *M. australiense* EC/IC10 chronic response.

Table 19: EC/IC10 chronic response estimates for *M. australiense*

Toxicant	EC/IC50 / ACR Toxicant	EC/IC10 Chronic
Nickel	=81.1/4.02	20.2 µg/L
Zinc	=242.8/1.12	216.6 µg/L
Sulfate	=7137.5/1.54	4625 mg/L

Both *C. tepperi* and *N. sp* AV1 (*Leptophlebiidae*) do not have ACR toxicant data available from a similar taxonomic group. Based on Warne et al. (2018) recommendations, the geometric mean of all ACR values derived for Nickel, Zinc and Sulfate can be used as the ACR toxicant specific value for *C. tepperi* and *N. sp AV1 (Leptophlebiidae*) (Equation 4) (Table 20).

$$Geometric\ Mean\ (GM) = \sqrt{X_1, X_2 \dots X_n}$$
(4)

Table 20: ACR-toxicant specific values based on geometric means of *C. dubia* and *M. splendida sp.*

Toxicant	X1	X2	GM - ACR Toxicant
Nickel	4.02	1.76	2.66
Zinc	1.12	2.81	1.77
Sulfate	1.54	3.53	2.34

Table 21 and 22 below are estimated chronic responses for *C. tepperi* and *N. sp* AV1 *(Leptophlebiida*e). All acute converted values are utilized for SSD and SSGV derivation.

Table 21: EC/IC10 chronic response estimates for *C. tepperi*

Toxicant	EC/IC50 / ACR Toxicant	EC/IC10 Chronic
Nickel	=163.6/2.66	61.5 μg/L
Zinc	=980/1.77	552.2 μg/L
Sulfate	= 7059.1/2.34	3022.3 mg/L

Table 22: EC/IC10 chronic response estimates for *N. sp AV1 (Leptophlebiida*e).

Toxicant	EC/IC50 / ACR Toxicant	EC/IC10 Chronic
Nickel	=260/2.66	97.8 μg/L
Zinc	=472/1.77	266 μg/L
Sulfate	= 3423.7/2.34	1465.9 mg/L

7.10.2 SSD Plotting and Fitting

Toxicity test chronic EC/IC 10 data is inputted into the BurrliOZ 2.0 software (CSIRO, 2015) and is fitted to a SSD. The BurrliOZ software SSD is an empirical cumulative probability distribution function (ECPDF) and the software utilizes an inverse Weibull distribution. A total of 8 toxicity chronic and acute to chronic converted test-species values are applied to generate a distribution curve. BurrliOZ estimates the response of a species based on the concentrations of a toxicant (nickel, zinc and sulfate) at the level of protection applied (95% protection level) and calculates 95% confidence Intervals for the level of protection.

The derivation of toxicant SSGVs is derived from single-species toxicity tests utilizing SSD Weibull distribution. Figure 8 gives a guide to statistical distributions used to derive site-specific guideline values for toxicants.

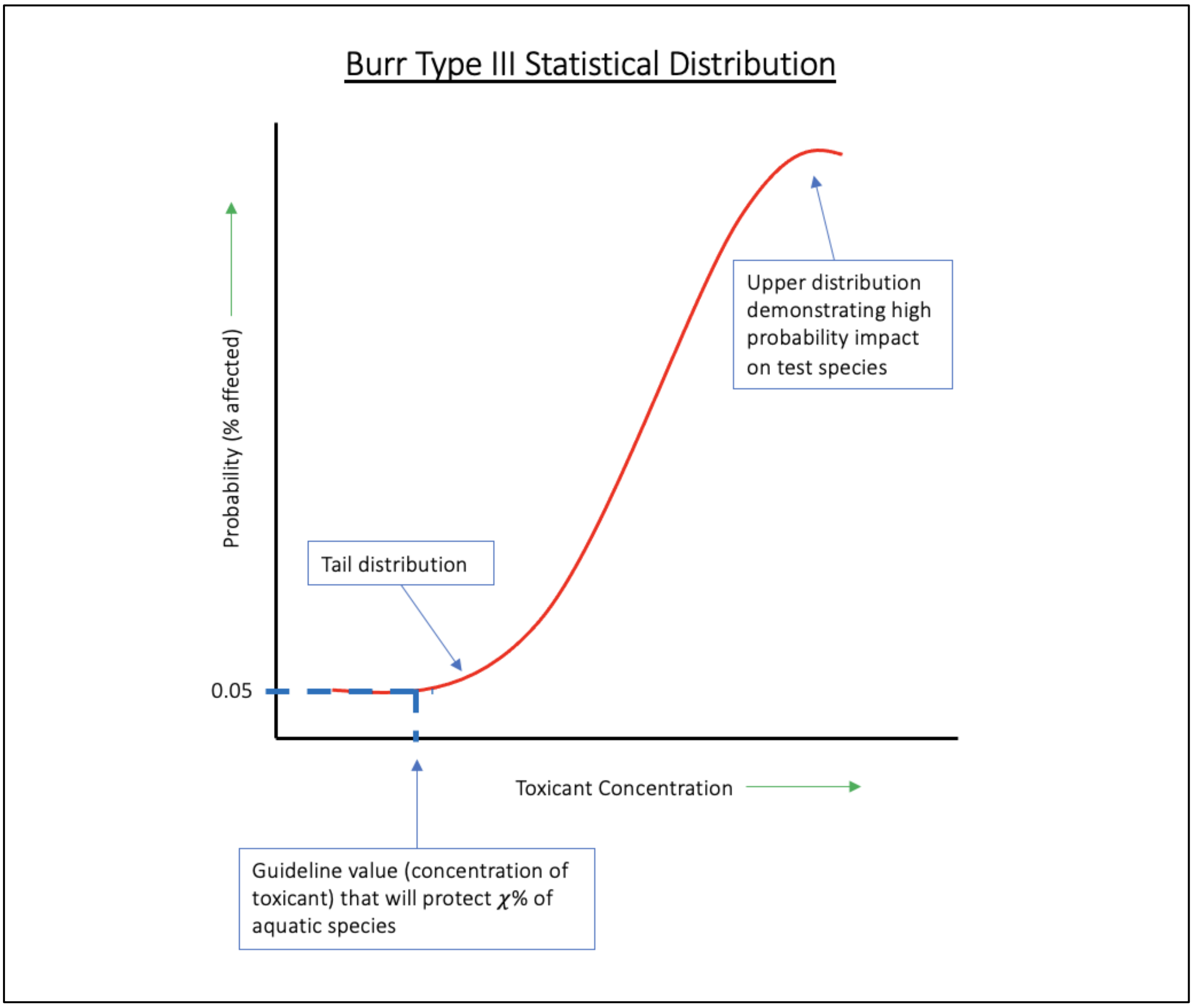


Figure 8: Guide to the Statistical Distributions Used to Derive Toxicant Guideline Values (in this case χ = 95% is used for aquatic species protection).

7.10.3 SSGV Repeatability

The robustness of the SSGVs for nickel, zinc and sulfate is primarily reliant on the EC10/IC10 values determined from ecotoxicity testing. Ultimately the repeatability of the SSGVs is dependent on the repeatability in the laboratories conducting toxicity testing (Ecotox Services Australia) and analytical laboratory tests of analyte concentrations (Envirolab Services, Chatswood) to provide reliant and robust data sets following QA/QC required for NATA registered laboratories meeting the requirements of laboratory accreditation following ISO/IEC 17025 – Testing (ISO/IEC, 2017; Section 7.7 and Appendix D). Seven out of the eight species utilised for these toxicity tests are regional species, common to NSW aquatic environments and relevant to the study area (Appendix A). One local species was utilized for ecotoxicity testing (*Nousia* sp. AV1), which aids the specificity of the guideline values and relevance to local mayfly populations in the Goulburn River (Appendix A). Following ANZECC guidelines (Warne et al 2018) a suitability check of SSGVs was followed and evaluated against toxicity test data (Section 8.6).

Electrical conductivity (µS/cm) and salinity (mg/L) SSGVs are not directly measured from ecotoxicity response data but calculated or derived from measured values (sulfate ecotoxicity testing and electrical conductivity associated with dosed sulfate tests) from ecotoxicity testing to which EC or salinity is associated. WTP Filtrate, a control water utilized in ecotoxicity testing was intended to demonstrate the toxicity of salinity from waters associated with MCO, however WTP Filtrate was below the level of no observed effect concentration, demonstrating no statistically significant toxicity to aquatic test species (Section 8.2).

Sulfate is commonly associated with saline mine water discharge. This report includes a detailed characterisation of the type of salinity of water from MCO (Section 8.3.4-8.3.6). This involved determining the proportions total dissolved ions in MCO waters and determining the suitability of using sodium sulfate as an analogue for salinity (Dowse et al. 2017; Dunlop et al. 2016; Mann et al.2014; Prasad et al. 2014). Comparison with these studies shows that variations in salinity composition do occur between different locations and ‘sources’ of salinity, e.g. marine for seawater compared with coal mineralisation.

The approach to deriving a salinity SSGVs followed the ANZG (2018) given in Section 8. This report goes further by providing a detailed investigation into the characterisation of salinity and deriving a salinity factor relationship in the Goulburn River and to MCO waters, including contribution of metals to the aquatic toxicity of test waters (Section 8.3.4-8.3.6, 8.5). Adaptations to the SSGV for sulfate enabled a robust salinity SSGV value to be derived. Final recommendations on salinity discharge limits for MCO release waters (WTP Discharge) are based on a thorough process of investigating ecotoxicity effects to the aquatic species and expert advice on natural background salinity levels in the upper Goulburn River (Section 10.1).

8 Site-Specific Guideline Values

8.1 Toxicity Test Data For Use As SSGVs

Raw laboratory data from ecotoxicity tests are shown in Table 23 (Appendix G). The dose-response data and its statistical analyses are provided in Appendix G (Ecotox Services Australia report) for the respective analytes. Comments are provided on tests showing sensitivity and tolerances based on the dose-response details summarised in Appendix A.

Table 24 gives the ACR converted values utilizing ACR values generated from acute and chronic tests from *M. splendida sp.* and *C. dubia* as described in Section 7.10.1. These converted acute values (estimated chronic) were utilized in SSDs for SSGV derivation (Section 8.4).

Table 23. Nickel, Zinc and Sulfate concentrations EC/IC10 and EC/IC50 endpoints for test species for Moolarben Coal Mine SSGV derivation.

Test Species	Exposure	Endpoint	Nickel (µg/L)		Zinc (µg/L)		Sulfate (mg/L)	
			EC/IC10	EC/IC50	EC/IC10	EC/IC50	EC/IC10	EC/IC50
Freshwater Cladoceran, *Ceriodaphnia dubia*	Acute	Survival	33.6	44.2	70.0	98.3	1293.4	1961.0
	Chronic	Reproduction	11	14	87.7	106.34	1270.7	1623.4
Freshwater Aquatic Duckweed, *Lemna disperma*	Chronic	Growth Rate	53.3	300	134	191.9	3032.9	5548.4
Eastern Rainbowfish, *Melanotaenia splendida splendida*	Acute	Fish Imbalance	87.5	137.2	161.1	252.9	2873.3	4320.7
	Chronic	Reproduction	78.0	117.6	90	137.1	1222.3	2820.6
Freshwater Hydra, *Hydra viridissima* (Pilbara isolate)	Chronic	Growth Rate	97.1	>300	107.5	317.8	1518.1	3731.8
Green Alga, *Raphidocelis subcapitata*	Chronic	Growth Rate	183.9	>300	157.9	794.9	794.5	3738.6
Freshwater Chironomid, *Chironomus tepperi*	Acute	Survival	104.2	163.6	847.6	>980	6467.2	7059.1
Freshwater Prawn, *Macrobrachium australiense*	Acute	Survival	39.2	81.1	142	242.8	5084.1	7137.5
Mayfly Larval, *Nousia* sp.AV1 (*Leptophlebiidae*)	Acute	Survival	120	>260	407.6	472	2356.4	3423.7

Table 24. Acute to chronic converted values utilizing ACR values generated from acute and chronic tests from *M. splendid sp.* and *C. dubia.* These converted acute values (estimated chronic) were utilized in SSDs for SSGV derivation.

Test Species	Nickel (µg/L)	Zinc (µg/L)	Sulfate (mg/L)
Freshwater Chironomid, *Chironomus tepperi*	61.5	552.2	3022.3
Freshwater Prawn, *Macrobrachium australiense*	20.2	216.6	4625
Mayfly Larval, *Nousia* sp.AV1 (*Leptophlebiidae*)	97.8	266	1465.9

8.2 Treatment Waters

WTP Filtrate was proposed as the 'mine test water' (Appendix B) in the IWQS ecotoxicity study, together with WTP Discharge and WTP Permeate (Section 6.2). WTP Discharge consists of WTP Filtrate blended with WTP Permeate. The WTP Filtrate response to individual analyte toxicity responses to test organisms is measured as EC (2110 µS/cm) to give the salinity (1590 mg/L) in Appendix B. In this case EC of the WTP Filtrate is measuring the contributions of both salinity from major cations and anions and dissolved metals. It is also convenient to use EC as a measurement of salinity as it can be related directly to the percent of 'mine water' in the ecotoxicity test solution.

Application of respective hardness corrections to the default filtered nickel (11.0 µg/L) and zinc (8.0 µg/L) TVs (ANZG, 2018; Markich et al., 2001) based on the observed hardness category of 'extremely hard' for WTP Filtrate (Appendix B) gave the following hardness modified threshold values (HMTVs) for 95% protection of aquatic species compared with WTP Filtrate concentrations : (i) Nickel 11.0x9 = 99.0 µg/L cf. WTP 54.5 µg/L; and (ii) Zinc 8.0x9 = 72.0 µg/L cf. WTP 25.7 µg/L. Thus both filtered nickel and zinc in WTP Filtrate are not expected to show any toxicity to aquatic species for the 95% protection level.

Table 25 gives the ecotoxicity response for each of the test species using % Unaffected, Growth Rate testing, EC (µS/cm) and salinity (mg/L) in measured in each test solution comprising DMW Control, Diluent Control (SW12), WTP Permeate, WTP Discharge and WTP Filtrate. All test species showed no statistically significant response to each of the DMW Control, Diluent Control (SW12), WTP Discharge and WTP Filtrate in the additional controls (Table 25). However WTP Permeate with lowest EC and salinity (Table 25) showed effects on all the test aquatic species suggesting effects arising from low nutrient levels rather than metal or salinity induced toxicity responses. Both nickel and zinc HMTVs (ANZG, 2018; Markich et al. 2001) were not exceeded for WTP Filtrate, WTP Discharge, WTP Permeate and Diluent control SW12, although the filtered concentrations of nickel and zinc in WTP Filtrate were closer to TVs without hardness corrections than other heavy metals. Both nickel and zinc were considered to be candidates for SSGV development using SSD from ecotoxicity measurements from dose response of the selected analytes with test aquatic species. Sulfate was also selected for dose response studies to be representative of salinity associated with a coal formation origin of the control water.

Table 25. Comparison of control water ecotoxicity for each of the test species using % Unaffected, Growth Rate testing, conductivity (µS/cm) and salinity (mg/L)

Test Species	Toxicity Testing	Conductivity (µS/cm)		Salinity (mg/L)	
Ceriodaphnia dubia (Acute)	% Unaffected	t=0h	t=48h	t=0h	t=48 h
DMW Control	100	176	173	133	131

Test Species	Toxicity Testing	Conductivity (µS/cm)		Salinity (mg/L)	
Diluent Control (UCML SW01 / SW12)	100	786	790	593	596
WTP Permeate	0	64	65	48	49
WTP Discharge	100	295	299	223	226
WTP Filtrate	100	2110	2110	1593	1593
Ceriodaphnia dubia (Chronic)	% Unaffected	t=0h	t=7d	t=0h	t=7d
DMW Control [c]	100	176	181	133	137
Diluent Control (UCML SW01 / SW12)	100	786	793	593	599
WTP Permeate	0	64	67	48	51
WTP Discharge	100	295	300	223	226
WTP Filtrate	100	2110	2110	1593	1593
Lemna disperma (Chronic)*	Specific Growth Rate [a]	t=0h	t=7d	t=0h	t=7d
SIS Control [d]	0.29	291	299	220	226
Diluent Control (UCML SW01 / SW12)	0.29	786	794	593	599
WTP Permeate	0.24	64	79	48	60
WTP Discharge	0.29	295	302	223	228
WTP Filtrate	0.29	2110	2110	1593	1593
Melanotaenia splendida sp. (Acute)	% Unaffected	t=0h	t=96h	t=0h	t=96h
DMW Control	95	176	180	133	136
Diluent Control (UCML SW01 / SW12)	95	786	790	593	596
WTP Permeate	15	64	68	48	51
WTP Discharge	95	295	300	223	226
WTP Filtrate	95	2110	2110	1593	1593
Melanotaenia splendida sp. (Chronic)	% Unaffected	t=0h	t=12d	t=0h	t=12d
DMW Control	95	176	181	133	137
Diluent Control (UCML SW01 / SW12)	90	786	791	593	597
WTP Permeate	0	64	67	48	51
WTP Discharge	90	295	300	223	226
WTP Filtrate	90	2110	2110	1593	1593
Hydra viridissima (Chronic)*	Population Growth Rate [b]	t=0h	t=96h	t=0h	t=96h

Test Species	Toxicity Testing	Conductivity (µS/cm)		Salinity (mg/L)	
WW-DMW Control [e]	0.29	785	794	593	599
Diluent Control (UCML SW01 / SW12)	0.30	786	794	593	599
WTP Permeate	0	64	69	48	52
WTP Discharge	0.29	295	304	223	229
WTP Filtrate	0.30	2110	2110	1593	1593
Raphidocelis subcapitata	Cell Yield x10^4 cells/mL [a]	t= 0h	t=72h	t=0h	t=72h
USEPA Control [f]	22.80	125	-	94	-
Diluent Control (UCML SW01 / SW12)	24.90	786	-	593	-
WTP Permeate	11.80	64	-	48	-
WTP Discharge	21.70	295	-	223	-
WTP Filtrate	22.80	2110	-	1593	-
Chironomus tepperi (Acute)	% Unaffected	t=0h	t=48h	t=0h	t=48h
DMW Control	100	176	184	133	139
Diluent Control (UCML SW01 / SW12)	100	786	795	593	600
WTP Permeate	0	64	74	48	56
WTP Discharge	100	295	304	223	229
WTP Filtrate	100	2110	2110	1593	1593
Macrobrachium australiense (Acute)	% Unaffected	t=0h	t=96h	t=0h	t=96h
DMW Control	95	176	182	133	137
Diluent Control (UCML SW01 / SW12)	95	786	790	593	596
WTP Permeate	0	64	69	48	52
WTP Discharge	95	295	298	223	225
WTP Filtrate	95	2110	2110	1593	1593
Nousia sp. AV1 (*Leptophlebiidae*) (Acute)	% Unaffected	t=0h	t=96h	t=0h	t=96h
DMW Control	n.a.	n.a.	n.a.	n.a.	n.a.
Diluent Control (UCML SW01 / SW12)	100	786	799	593	603
WTP Permeate	60	64	74	48	56
WTP Discharge	100	295	301	223	227

Test Species	Toxicity Testing	Conductivity (µS/cm)		Salinity (mg/L)	
WTP Filtrate	100	2110	2120	1593	1600

* Defined as chronic after Batley et al. (2018) for tropical and temperate species
a Ecotoxicity tests observing toxicant effects on growth inhibition (categorically growth rate testing)
b Ecotoxicity tests observing toxicant effects on growth rate
c DMW Control: Dilute Mineral Water control
d SIS Control: SIS growth media control
e WW-DMW Control: conductivity-adjusted to 780 µS/cm Dilute Mineral Water control
f USEPA control: United States Environmental Protection Agency culture media control
(-) No data available

Table 26 gives a further summary of water sample toxicity responses to the aquatic test species. It is important to note that WTP Filtrate and WTP Discharge waters of the treatment plant facility showed no toxicity responses to test species. It was anticipated that test species would show a response to WTP Filtrate waters, however, high concentrations of hardness and alkalinity (bicarbonate) in WTP Filtrate mostly likely has influenced metal speciation to an extent which ameliorates metal toxicity (Markich et al., 2001). The effect of water chemistry on metal speciation is further investigated in Section 8.3.

Table 26. Summary of water sample toxicity data.

Sample	Toxicity Response	Comments
UCML SW01 / SW12: Goulburn River Control Grab sample: -lower than average Ni and Zn concentrations	No effects	• Fluctuating metal concentration and salinity due to natural seepage from naturally-occurring coal measures and variation in sub-catchment contributions and flow regimes • SW12 grab sample for testing mostly resides above 80th percentile values for historic EC, salinity, hardness, and SO_4 values • Ni and Zn concentrations are slightly below historical means (Ni = 0.9 µg/L and Zn = 4.2 µg/L)
WTP Filtrate Grab Sample: -Above historic 80 percentile values	No effects	• An unexpected finding: no effects • Elevated metal concentration range expected to show adverse effects • Ni, Zn above historic 80th percentile values • EC, Salinity, Hardness, SO_4 above historic 80th percentile values • This shows the influence of water chemistry on metal speciation: hardness may reduce metal solubility and thus reduce toxicity
WTP Permeate (Diluted with WTP Filtrate to generate WTP Discharge)	Affected	• WTP permeate alone is toxic to aquatic species • This is due to the lack of nutrients within the water – impacting test specie survivability • WTP permeate is not released to the Goulburn River – this toxicity test result is not of concern

WTP Discharge	No effects	- WTP has taken out most of the metals and reduced salinity - Expected result

WTP Permeate alone gave toxic responses to various aquatic test species. This appears to be due to the lack of nutrients within the water itself, impacting test species survivability. *L. disperma* response to WTP Permeate was a significantly lower specific growth rate compared to the SIS Control (Homoscedastic t Test, 1 tailed, P = 0.05). *R. subcapitata* has a significantly lower cell yield to WTP Permeate compared with the USEPA Control. WTP Permeate on its own is not released from the MCO lease to the Goulburn River and is kept within the treatment plant cycle. Therefore the test results conducted on WTP Permeate relates to a lack of ions and other nutrients rather than a direct toxicity effect, and is not of concern.

8.3 Environmental Chemistry and Aquatic Toxicology of the Analytes

During ecotoxicity testing, it is important that insoluble compound formation is minimised, and soluble compounds do not reach saturation and precipitate, as this results in a lower actual concentration of the analyte than expected based on the weight of solute and the volume of solvent. Due to the nature of elevated alkalinity and hardness of the WTP Filtrate and the natural condition of the Goulburn River waters, metal speciation, with specific reference to Ni and Zn, was investigated further to determine the potential influences on toxicity in ecotoxicity testing studies. This follows the observation that the HMTVs were not exceeded for both filtered nickel and zinc concentrations in WTP Filtrate and the Diluent control SW12 (Markich et al. 2001).

Geochemical speciation modelling of the analytes nickel and zinc was undertaken using Visual MINTEQ 3.0 (Gustaffson, 2013), but not sulfate as its speciation is relatively constant for oxidizing conditions in natural waters. The outputs of the speciation modelling are given in Tables 27 to 31.

Table 27. Visual MINTEQ summary for UCML SW01 / SW12 water with change in hardness, alkalinity (HCO_3^-), EC and TDS with constant nickel and zinc concentration

Parameters	SW12 (24/6/21 Mean)[a]	SW12 (80%tile)[b]	SW12 (Max)[b]
Hardness ($CaCO_3$)	152 mg/L	138 mg/L	201 mg/L
Alkalinity (Bicarbonate)	46 mg/L	95 mg/L	122 mg/L
Electrical Conductivity (EC)	783 µS/cm	619 µS/cm	971 µS/cm
Total Dissolved Solids (TDS)	568 mg/L	400 mg/L	628 mg/L
Dissolved Organic Carbon (DOC)	9 mg/L	9 mg/L	9 mg/L
Nickel (Ni)[c]	0.001 mg/L	0.001 mg/L	0.001 mg/L
Zinc (Zn)[c]	0.001 mg/L	0.001 mg/L	0.001 mg/L
Ni Speciation	**%**	**%**	**%**
Ni^{2+}	77.5	64.0	43.1
(6) $NiHCO_3^+$ + D (aq)	-	0.01	-
$NiOH^+$	0.04	0.09	0.93
$Ni(OH)_2$ (aq)	-	-	0.68
NiF^+	-	0.02	0.01
$NiCl^+$	-	0.06	0.06

$NiSO_4$ (aq)	5.16	3.55	5.49
$NiCO_3$ (aq)	0.61	2.69	28.6
$NiHCO_3^+$	4.01	6.62	4.56
(6)Ni+2D(aq)	1.79	1.99	0.86
FA1-Ni(6)(aq)	10.8	-	15.6
FA2-Ni(6)(aq)	0.02	-	0.05
Zinc Speciation	%	%	%
Zn^{2+}	88.3	81.6	22.6
$ZnOH^+$	0.37	0.90	3.66
$Zn(OH)_2$ (aq)	0.11	0.72	45.0
$Zn(OH)_3^-$	-	-	0.08
ZnF^+	0.01	0.02	-
$ZnCl^+$	-	0.57	0.24
$ZnSO_4$ (aq)	6.41	4.93	3.14
$Zn(SO_4)_2^{2-}$	0.04	0.03	0.05
$ZnCO_3$ (aq)	1.08	5.31	23.2
$ZnHCO_3^+$	1.17	2.16	0.61
$Zn(CO_3)_2^{2-}$	-	-	0.37
(6) Zn+2D (aq)	2.03	2.53	0.45
FA1-Zn(6) (aq)	0.53	0721.21	0.49

Note: a Mean data retrieved from ALS analysis of SW12 diluent-control water sample collected 24/06/2021 for ecotoxicity studies (Appendix B, Table B.3.).

b 80 percentile (80%tile) and maximum (Max) data retrieved from historic monitoring site data Table 5 to 7

c Nickel and zinc concentrations are not reflective of what is found in 80 percentile (80%tile) or maximum (Max) historic values (Table 5-7). These concentrations for nickel and zinc parameters are reflective of mean concentrations values for SW12 and are kept constant.

Table 27 shows the changes in filtered water nickel and zinc speciation for the SW12 monitoring data (Max and 80th percentile) and Mean of sampling on 24 June 2021 with change in hardness, alkalinity (HCO_3^-) and EC/salinity gives marginal fluctuation in in hardness and EC/salinity and decreasing alkalinity (bicarbonate concentration). Dissolved organic carbon (DOC) is constant at 9 mg/L and nickel (0.001 mg/L) and zinc (0.001 mg/L) filtered concentrations are constant inputs. Decreasing alkalinity with marginal change in hardness gives a large change in free nickel present in solution, Ni^{2+} (43.1%, 64.0% and 77,5%) and is accompanied by a reduction in nickel carbonate $NiCO_3$ (aq) (28.6%,2.69% to 0.61%), a complex that is a precursor to precipitation. This shows that hardness alone, does not control free nickel (Ni^{2+}) concentration, but alkalinity is also a driving factor, especially for hard waters. Nickel binding to fulvic acids from DOC, FA1-Ni(g) (aq) shows a slight increase (10.8% and 15.6%) with increasing alkalinity and hardness. Zinc in solution, Zn^{2+}, also increases dramatically (22.6%, 81.6% to 88.3%) following a similar trend with Ni^{2+}. Also zinc hydroxide $Zn(OH)_2$(aq) (45.0%, 0.72% and 0.11%) and zinc carbonate $ZnCO_3$ (aq) (23.2%, 5.31% and 1.08%) decreases. While any bonding of zinc to fulvic acids, FAI-Zn (6) (aq), is minor.

Table 28. Visual MINTEQ summary for UCML SW01 / SW12 water with change in nickel and zinc concentration (increasing in magnitude) for fixed hardness, alkalinity (HCO_3^-), EC and TDS

Parameters	SW12 (24/6/21 Mean)	SW12 (24/6/21 Mean)	SW12 (24/6/21 Mean)
Hardness ($CaCO_3$)	152 mg/L	152 mg/L	152 mg/L
Alkalinity (Bicarbonate)	46 mg/L	46 mg/L	46 mg/L
Electrical Conductivity (EC)	783 µS/cm	783 µS/cm	783 µS/cm
Total Dissolved Solids (TDS)	568 mg/L	568 mg/L	568 mg/L
Dissolved Organic Carbon (DOC)	9 mg/L	9 mg/L	9 mg/L
Nickel (Ni)[a]	0.001mg/L	0.010mg/L	0. 100 mg/L
Zinc (Zn)[a]	0.001mg/L	0.010mg/L	0. 100 mg/L
Nickel Speciation	%	%	%
Ni^{2+}	77.5	82.6	85
$NiOH^+$	0.04	0.05	0.05
NiF^+	-	0.01	0.01
$Ni(OH)_2$ (aq)	-	-	-
$NiSO_4$ (aq)	5.16	5.5	5.66
$NiCO_3$ (aq)	0.61	0.65	0.67
$NiHCO_3^+$	4.01	4.27	4.39
(6)Ni+2D(aq)	1.79	1.9	1.95
FA1-Ni(6)(aq)	10.8	5.03	2.28
FA2-Ni(6)(aq)	0.02	-	-
Zinc Speciation	%	%	%
Zn^{2+}	88.3	88.5	88.6
$ZnOH^+$	0.37	0.37	0.37
$Zn(OH)_2$ (aq)	0.11	0.11	0.11
$Zn(OH)_3^-$	-	-	-
ZnF^+	0.01	0.01	0.01
$ZnSO_4$ (aq)	6.41	6.42	6.43
$Zn(SO_4)_2^{2-}$	0.04	0.04	0.04
$ZnCO_3$ (aq)	1.08	1.08	1.08
$ZnHCO_3^+$	1.17	1.17	1.17
$Zn(CO_3)_2^{2-}$	-	-	-
(6)Zn+2D(aq)	2.03	2.04	2.03
FA1-Zn(6)(aq)	0.53	0.27	0.13

Note: All data retrieved from ALS analysis SW12 diluent control water sample collected 24/06/2021 for ecotoxicity studies (Appendix B, Table B.3.).

a Values not reflective of nickel or zinc concentrations found in SW12 control or historic data analysis. Increase in magnitude demonstrates the potential influence concentration has on speciation.

Table 28 provides a Visual MINTEQ summary for UCML SW01 / SW12 (Mean of bulk diluent control water in Appendix Table B-1) with change in nickel (0.001, 0.01, and 0.100 mg/L) and zinc (0.001, 0.01, and 0.100 mg/L) concentrations for fixed hardness, alkalinity (HCO_3^-) and EC/salinity. SW12 has fixed hardness 152 mg/L, alkalinity (bicarbonate) 46 mg/L, EC/salinity 783 µS/cm or 568 mg/L for salinity and DOC 9 mg/L. Free nickel in solution (Ni^{2+}) increases (77.5%, 82.6% and 85.0%) while nickel sulfate, NiSO4 (aq) (5.16%, 5.50% and 5.66%) and nickel bicarbonate, $NiHCO3^+$ (4.01%, 4.27% and 4.39%) are almost constant. Fulvic acid from DOC, FAI-Ni(6) (aq) declines (10.8%, 5.03% and 2.28%) with increasing Ni concentration. Free zinc in solution, Zn^{2+} is almost constant (88.3%, 88.5% and 88.6%). Zinc sulfate, ZnSO4 (aq) (6.41%, 6.42% and 6.43%) is constant, but minor contributor in the overall complexation of zinc. Zinc binding to fulvic acids from DOC, FA1-Zn (6) (aq), is minor but declines.

Table 29. Visual MINTEQ summary for WTP Filtrate with change in hardness, alkalinity (HCO3-), EC and TDS and fixed Nickel and Zinc and DOC concentrations

Parameters	WTP Filtrate (24/6/21 Mean)[a]	WTP Filtrate (80%tile)[b]	WTP Filtrate (Max)[b]
Hardness ($CaCO_3$)	621 mg/L	610 mg/L	623 mg/L
Alkalinity (Bicarbonate, HCO_3^-)	153mg/L	139 mg/L	161 mg/L
Electrical Conductivity (EC)	2067 µS/cm	1774 µS/cm	2070 µS/cm
Total Dissolved Solids (TDS)	1560 mg/L	1312 mg/L	1600 mg/L
Dissolved Organic Carbon (DOC)	0.5 mg/L	0.5 mg/L	0.5 mg/L
Nickel (Ni)[c]	0.04mg/L	0.04mg/L	0.04 mg/L
Zinc (Zn)[c]	0.02mg/L	0.02mg/L	0.02 mg/L
Nickel Speciation	%	%	%
Ni^{2+}	62.2	59.2	54.3
$NiOH^+$	0.26	0.63	0.86
NiF^+	0.03	0.03	0.03
$NiCl^+$	0.07	0.068	0.06
$Ni(OH)_2$ (aq)	0.04	0.06	0.12
$NiSO_4$ (aq)	18.9	17.7	16.0
$NiNO_3^+$	-	-	0.01
$NiCO_3$ (aq)	10.3	13.3	20.1
$NiHCO_3^+$	7.97	8.20	8.26
(6)Ni+2D(aq)	1.79	0.03	0.02
FA1-Ni(6)(aq)	0.03	0.31	0.24

FA2-Ni(6)(aq)	0.19	0.39	-
Zinc Speciation	%	%	%
Zn^{2+}	56.8	51.3	42.1
$ZnOH^+$	1.8	4.36	5.34
$Zn(OH)_2$ (aq)	4.56	6.32	11.7
ZnF^+	0.03	0.03	0.02
$ZnCl^+$	0.49	0.46	0.37
$ZnSO_4$ (aq)	18.8	16.8	13.6
$Zn(SO_4)^{2-}$	0.98	0.78	0.65
$ZnCO_3$ (aq)	14.6	17.8	24.2
$ZnHCO_3^+$	1.87	1.82	1.6
$Zn(CO_3)_2^{2-}$	0.08	0.13	0.31
(6)Zn+2D(aq)	0.03	0.02	0.02
FA1-Zn(6)(aq)	0.01	0.02	0.01

Note: a Mean data retrieved from ALS analysis of WTP Filtrate control water sample collected 24/06/2021 for ecotoxicity studies (Appendix B, Table B.3.).

b 80%ile and Max values retrieved from WTP Statistics summary data Table 9.

c Nickel and zinc concentrations are not reflective of what is found in mean, 80 percentile (80%tile) or maximum (Max) values. These concentrations for nickel and zinc parameters are kept at low concentrations and constant to determine how other parameters may influence speciation of nickel and zinc

The Visual MINTEQ summary for WTP Filtrate (Mean, 80th percentile and Maximum) demonstrates the speciation outputs of a model run that focuses on fixed nickel (0.04 mg/L), zinc (0.02 mg/L) and DOC (0.5 mg/L) concentrations, with changing alkalinity, hardness and electrical conductivity. In solution, Ni^{2+} (62.2%, 59.2% and 54.3%) show a slight decrease in concentrations with increasing hardness and alkalinity. The remaining 38-46% is comprised of nickel sulfate, $NiSO_4$ (aq) (18.9%, 17.7% and 16%), nickel carbonate, $NiCO_3$ (aq) (10.3%, 13.3% and 20.1%) and nickel bicarbonate, $NiHCO_3^+$(7.97%, 8.20% and 8.26%). Collectively these nickel complexes show only slight variations. Free zinc in solution, Zn^{2+} (56.8%, 51.3% and 42.1%) show similar decreasing concentration values to nickel. The remaining 49-58% of zinc speciation is comprised of zinc hydroxides, $ZnOH^+$ (aq) (1.80%, 4.36% and 5.34%) showing small increase and $Zn(OH)_2$ (aq) (4.56%, 6.32% and 11.7%) showing greater increase. Zinc sulfate, $ZnSO_4$ (aq) (18.8%, 16.8% and 13.6%) shows a decrease and zinc carbonate $ZnCO_3$(aq) (14.6%, 17.8% and 24.2%) shows an increase. Collectively these complexations only show slight variations. Speciation modelling of nickel and zinc metals in WTP Filtrate waters (characterised by Mean, 80% and max) demonstrate limited changes are likely to occur in speciation when comparing the control water sample chemistry to that of historic shifts of 80% to maximum conditions in alkalinity and hardness. Due to hardness and alkalinity conditions remaining characteristically high in WTP Filtrate waters, it is expected that little change in nickel and zinc speciation will occur.

Table 30. Visual MINTEQ summary of WTP Filtrate (Mean of Triplicate sample 24 June 2021) with change in nickel and zinc concentration and fixed hardness, alkalinity (HCO_3-), EC, TDS and DOC.

Parameters	WTP Filtrate (24/6/21 Mean)[a]	WTP Filtrate (24/6/21 Mean) [a]	WTP Filtrate (24/6/21 Mean) [a]
Hardness ($CaCO_3$)	621 mg/L	621 mg/L	621 mg/L

Alkalinity Bicarbonate (HCO_3)	153mg/L	153mg/L	153mg/L
Electrical Conductivity (EC)	2067 µS/cm	2067 µS/cm	2067 µS/cm
Total Dissolved Solids (TDS)	1560 mg/L	1560 mg/L	1560 mg/L
Dissolved Organic Carbon (DOC)	0.5 mg/L	0.5 mg/L	0.5 mg/L
Nickel (Ni)[b]	0.04 mg/L	0.5mg/L	5 mg/L
Zinc (Zn)[b]	0.02 mg/L	0.5 mg/L	5 mg/L
Nickel Speciation	%	%	%
Ni^{2+}	62.2	60.7	61.1
$NiOH^+$	0.26	0.51	0.51
NiF^+	0.03	0.03	0.03
$NiCl^+$	0.07	0.07	0.07
$Ni(OH)_2$ (aq)	0.04	0.04	0.04
$NiSO_4$ (aq)	18.9	17.8	17.7
$NiCO_3$ (aq)	10.3	11.7	11.5
$NiHCO_3^+$	7.97	9.15	9
(6)Ni+2D(aq)	1.79	-	-
FA1-Ni(6)(aq)	0.03	-	-
FA2-Ni(6)(aq)	0.19	-	-
Zinc Speciation	%	%	%
Zn^{2+}	56.8	54.7	55
$ZnOH^+$	1.8	3.64	3.66
$Zn(OH)_2$ (aq)	4.56	4.18	4.2
$Zn(OH)_3^-$	-	-	-
ZnF^+	0.03	0.03	0.03
$ZnCl^+$	0.49	0.48	0.48
$ZnSO_4$ (aq)	18.8	17.5	17.5
$Zn(SO_4)^{2-}$	0.98	0.82	0.82
$ZnCO_3$ (aq)	14.6	16.3	16
$ZnHCO_3^+$	1.87	2.11	2.08
$Zn(CO_3)_2^{2-}$	0.08	0.1	0.1
(6)Zn+2D(aq)	0.03	-	
FA1-Zn(6)(aq)	0.01	-	

Note: a Mean data retrieved from ALS analysis WTP Filtrate water sample collected 24/06/2021 for ecotoxicity studies (Appendix B, Table B.3.).

b Values are not reflective of nickel or zinc concentrations found in WTP Filtrate summary data. Increase in magnitude demonstrates the potential influence concentration has on speciation.

Table 30 gives the Visual MINTEQ summary of WTP Filtrate (Mean of 24 June 2021 WTP triplicate samples for ecotoxicity studies),) with change in nickel (0.04, 0.5 and 5 mg/L) and zinc (0.02,0.5 and 5 mg/L) concentration and fixed hardness, alkalinity (HCO_3^-), EC/salinity and DOC. Free nickel, Ni^{2+}, shows a small decrease for large percent of free ion (62.2%, 62.4%, 60.7 and 61.1%). Nickel sulfate, nickel carbonate and bicarbonate ($NiSO_4$ (aq), NiCO3 (aq), $NiHCO_3^+$) contribute approximately 30% of nickel and show slight changes with nickel sulfate, NiSO4 (aq) and nickel bicarbonate, $NiHCO_3^+$decreasing, and nickel carbonate NiCO3 (aq) increasing. Free zinc in solution, Zn^{2+}, shows a small decrease of free ion (56.8%, 56.8% , 54.7% and 55.0%). The remaining zinc (43.9-45.3%) is comprised of zinc hydroxides ($ZnOH^+$, $Zn(OH)_2$ (aq)), which increases, zinc sulfate, ZnSO4 (aq), which decreases and zinc carbonate, $ZnCO_3$ (aq) which increases.

Table 31. Visual MINTEQ summary of downstream Goulburn River site, SW01, under mean, 80th percentile and maximum water quality conditions reflecting the impact of metal speciation with increasing hardness, alkalinity (HCO_{3-}), EC/salinity, DOC, nickel and zinc concentrations.

Parameters	SW01 (Mean)	SW01 (80%tile)	SW01 (Max)
Hardness ($CaCO_3$)	144 mg/L	153 mg/L	157 mg/L
Alkalinity (Bicarbonate)	92 mg/L	122 mg/L	180 mg/L
Electrical Conductivity (EC)	632 µS/cm	795 µS/cm	1180 µS/cm
Total Dissolved Solids (TDS)	397 mg/L	479 mg/L	808 mg/L
Dissolved Organic Carbon (DOC)	0.5 mg/L	0.5 mg/L	0.5 mg/L
Nickel (Ni)	0.0039 mg/L	0.005 mg/L	0.006 mg/L
Zinc (Zn)	0.0039 mg/L	0.0025 mg/L	0.012 mg/L
Nickel speciation	%	%	%
Ni^{2+}	80.2	68.5	19.1
$NiOH^+$	0.09	0.39	1.72
NiF^+	0.01	0.01	-
$NiCl^+$	0.04	0.04	0.02
$Ni(OH)_2$ (aq)	-	0.07	5.09
$Ni(OH)_3^-$	-	-	0.11
$NiSO_4$ (aq)	8.61	7.51	2.10
$NiCO_3$ (aq)	2.57	14.11	68.7
$NiHCO_3^+$	7.99	8.77	2.71
(6)Ni+2D(aq)	0.11	0.10	0.03
FA1-Ni(6)(aq)	0.39	0.48	0.39
FA2-Ni(6)(aq)	-		-
Zinc speciation	%	%	%
Zn^{2+}	82.4	60.2	2.4

$ZnOH^+$	0.72	2.60	1.62
$Zn(OH)_2$ (aq)	0.45	8.20	80.6
$Zn(OH)_3$-	-	-	0.57
ZnF^+	0.01	-	-
$ZnCl^+$	0.29	0.25	0.02
$ZnSO_4$ (aq)	9.66	7.20	0.29
$Zn(SO_4)_2^{2-}$	0.12	0.09	
ZnCO3 (aq)	4.10	19.2	13.4
$ZnHCO_3^+$	2.11	1.98	0.09
$Zn(CO_3)_2$2-	-	0.08	1.03
(6)Zn+2D(aq)	0.11	0.08	-
FA1-Zn(6)(aq)	0.02	0.03	-

Note: All data retrieved from historic monitoring site data Table 5-7. Nickel and zinc concentrations are reflective of data at SW01.(Refer to data inputs from Appendix B. Table B.3.)

Table 31 gives the Visual MinTEQ summary of downstream Goulburn River site SW01 monitoring data (Max, 80th percentile and Mean) speciation with increasing hardness, alkalinity (HCO_3^-), EC/salinity (all higher than SW12) and DOC and increasing nickel (0.0039, 0.005 and 0.006 mg/L) and zinc (0.0039, 0.0025, 0.012 mg/L) concentrations. Nickel in solution, Ni^{2+} decreases dramatically (80.2%, 68.5% and 19.1%) at higher hardness and alkalinity concentrations by forming nickel carbonate, $NiCO_3$ (aq) (2.57%, 14.11% and 68.7%), while nickel sulfate, $NiSO_4$ (aq) (8.61%, 7.51% and 2.10%), and nickel bicarbonate, $NiHCO_3^+$ (7.99%, 8.77%% and 2.71%) are almost constant. Nickel binding to fulvic acids from DOC, FA1Ni(6) (aq) was insignificant. Free zinc, Zn^{2+} decreases dramatically (82.4%, 60.2% and 2.4%) at higher hardness and alkalinity concentrations by forming zinc hydroxide, $Zn(OH)_2$ (aq) (0.45%, 8.20% and 80.6%). Zinc sulfate, $ZnSO_4$ (aq) (9.66%, 7.20% and 0.29%), zinc carbonate, $ZnCO_3$ (aq) (4.10%, 19.2% and 13.4%) show minor changes. Any zinc binding to fulvic acid, FA1-Zn (6) (aq) is minor.

The geochemical speciation modelling of nickel and zinc using Visual MINTEQ 3.0 (Gustaffson, 2013) shows the following based on varying Goulburn River and WTP Filtrate water quality conditions:

8.3.1 Nickel (Ni)

In UCML SW01 / SW12, at a fixed nickel concentration, decreasing alkalinity with marginal changes in hardness gives a large change in free nickel concentrations (Ni^{2+}), in solution; accompanied by a reduction in nickel carbonate, $NiCO_3$ (aq). This shows that hardness on its own does not completely control Ni^{2+} concentration. Compounding effects of alkalinity and hardness drive the complexation and ultimately solubility of nickel. Furthermore, at moderate to hard water conditions, alkalinity seems to govern in the proportion of free nickel available. Decreasing alkalinity with marginal change in hardness gives a large change in Ni^{2+} and accompanied by a reduction in $NiCO_3$ (aq). An inverse relationship between alkalinity and free nickel is noted at hard water conditions. As alkalinity increases, free nickel decreases and further complexation occurs in which nickel speciation is at a precursory step to soluble forms. When DOC decreases, FA1-Ni(g) (aq) is only slightly changed.

Changing concentrations of nickel do not sway percentage of free nickel concentrations as greatly as changing alkalinity and hardness variation does. As one would expect, any changes in free nickel concentrations, although slight, are reflective of changing nickel concentration inputted into the geochemical model.

Waters with low nickel to high hardness and alkalinity concentrations are more affected by changes of hardness and alkalinity.

8.3.2 Zinc (Zn)

In UCML SW01 / SW12 decreasing alkalinity with marginal change in hardness gives a large change in Zn^{2+} increases dramatically following a similar trend with Ni^{2+}. Also $Zn(OH)_2$(aq) and $ZnCO_3$ (aq) decrease. FAI-Zn (6) (aq) is minor. Constant DOC and constant filtered zinc concentrations also increases Zn^{2+} following a similar trend with $Ni^{2+}$with $Zn(OH)_2$(aq) (and $ZnCO_3$ (aq) decreasing.

Zn^{2+} is almost constant. $ZnSO_4$ (aq) (6.41%, 4.93% and 3.14%) is constant but minor. FA1-Zn (6) (aq) is minor but declines. Increasing zinc Zn^{2+} show similar concentrations. The remaining comprised of $ZnOH^+$ (aq) showing small increase, $Zn(OH)_2$ (aq) show greater increase, $ZnSO_4$ (aq) shows a decrease and $ZnCO_3$(aq) shows an increase, and collectively show slight variations.

WTP Filtrate with change in hardness, alkalinity (HCO_3^-) and EC/salinity and fixed zinc and DOC concentrations. Zn^{2+} show concentrations showing slight decrease. Zn^{2+} show similar concentrations. The remaining 43-45% is comprised of $ZnOH^+$ (aq) showing small increase, $Zn(OH)_2$ (aq) showing greater increase, $ZnSO_4$ (aq) showing a decrease and $ZnCO_3$(aq) collectively show slight variations. Thus the WTP Filtrate shows limited changes in nickel and zinc speciation with the same concentrations.

WTP Filtrate (Mean of 24 June 2021 Triplicate, 80% percentile and Maximum) with change in zinc (0.02, 0.5 and 5 mg/L) concentration and fixed hardness, alkalinity (HCO_{3-}), EC/salinity and DOC show Zn^{2+}has a small decrease of free ion. The remaining Zn is comprised of $ZnOH^+$ increasing, $Zn(OH)_2$ (aq) increasing, $ZnSO_4$ (aq) decreasing and $ZnCO_3$ (aq) increasing. Thus the WTP Filtrate shows limited changes in nickel and zinc speciation with the same concentrations.

The downstream Goulburn River site SW01 monitoring data (Maximum, 80th percentile and Mean) speciation with increasing hardness, alkalinity (HCO_3^-), EC/salinity and DOC and increasing zinc (0.0039, 0.0025 and 0.012 mg/L) concentrations shows that Zn^{2+}) decreases dramatically at higher hardness and alkalinity by forming $Zn(OH)_2$ (aq). $ZnSO_4$ (aq), $ZnCO_3$ (aq) show changes. FA1-Zn (6) (aq) is minor.

The calculated SSGVs for Zn may be controlled by the moderate level of hardness and alkalinity in the UCML SW01 / SW12 control water WTP and Goulburn River SW01.

Whilst there is no speciation modelling for sulfate and EC, the following details are relevant to consider with ecotoxicity data of sulfate and its relationship with salinity.

8.3.3 Sulfate (SO_4)

The analyte compound sodium sulfate ($NaSO_4$) is very soluble, including at pH 8.2 and the hardness of 82 mg/L $CaCO_3$. The chemical form of sulfate does not change under oxidising conditions. The measured concentrations did not change substantially throughout the course of the experiments, indicating that insoluble calcium sulfate, with a solubility of 2.1 g/L at 20 °C was not forming in the test solutions.

8.3.4 Electrical Conductivity (EC)

The measurement of EC in the different treatment test solutions are based on laboratory-calibration of the EC meter (Section 3.4; this report). This includes calibration of Ecotox Services Australia EC meters with a certified reference material. A comparison of the laboratory EC measurements provided by Envirolab and Ecotox Services Australia for the same mine water treatments (T=0) is shown in Table 16. The data from the two laboratories show acceptable results with less than 1% variation in measured mean EC for the CRM (Appendix D). The comparison in Table 16 provides confidence in the EC data from Envirolab used to develop the calibration curve and equation for conversion of EC to salinity (Section 8.5).

In addition, the direct application of EC measurement of WTP Filtrate as the indication salinity enables an assessment of the toxicity of salinity to the various test species. Because EC is measured in a fundamental unit, it is more generally applicable to any kind of test water from the MCO site.

Salinity is the measure in g/L or g/kg (grams of salt per litre/kilogram of water. Salinity in rivers, lakes, and the ocean is conceptually simple, but technically challenging to define and measure precisely. Conceptually the salinity is the quantity of dissolved salt content of the water. Salts are compounds like sodium chloride, magnesium sulfate, potassium nitrate, and sodium bicarbonate which dissolve into ions.

In water EC measures the conductivity and equates with the dissolved salts or ‘total dissolved salts’ (Section 7.8). Salinity is normally understood to be from the major anions and cations that conduct an electric current but the presence of soluble trace elements such as metals will also contribute to the EC even though the proportion of conductivity from specific ions may be small.

Different properties of salt anion and cations give different measurement properties. Salinity can be calculated directly from EC by using the appropriately calibrated factor (Equation 1). Different origins of salt give variations in the fact. E.g. marine salinity with the fingerprint of seawater may be difference from salinity of a geological origin like coal where the proportions of ions may be difference to those in seawater (IOC, SCOR and IAPSO, 2010).

8.3.5 Characterising Natural River and Treatment Water Salinity

Measured total dissolved solids (mg/L) throughout the Goulburn River provide a picture of the variation of salinity expected for sites upstream and downstream of the WTP release (Sections 5.1 and 7.8). As expected for the Hunter River catchments salinity is elevated towards the 300 mg/L range, with mean TDS values downstream (SW01, SW02) ranging between 397-430 mg/L (Table 32). Historic data from UCML SW01 / SW12 has a mean concentration of 331 mg/L. Upstream sites, influenced by natural mineralisation from Illawarra Coal measures have elevated salinity with SW05 at 436 mg/L and SW08 at 1977 mg/L. SW06 is characteristically different from other tributary inputs into the Goulburn River, with lower mean salinity values of 206 mg/L compared to TDS concentration at other monitoring sites. Similar characteristics are visible in the EC data shown in Table 33, with the following EC values for each surface water monitoring site: SW01 (mean: 632 µS/cm), SW02 (mean: 673 µS/cm), UCML SW01 / SW12 (mean: 446 µS/cm), SW05 (mean: 673 µS/cm), SW06 (mean: 206 µS/cm), SW08 (mean: 3161 µS/cm). Varying salinity and EC values demonstrate that despite being downstream of SW08, SW12 has reduced salinity, likely a feature of lower salinity inputs from Ryans Creek (SW06) and Sportsmans Hollow Creek. Sportsmans Hollow catchment does not have any monitoring data available, however due to the characteristically similar geology to that of Ryans Creek it is anticipated that similar lower salinity inputs from Sportsmans Hollow into the Goulburn River would be expected.

Salinity is made up of major dissolved anions and cations (bicarbonate, calcium, chloride, magnesium, potassium, sodium, and sulfate), which can vary in ratio depending on the source of dissolved salts. Depicting the ratio of cations and anions within the water gives us a better depiction of the type of salinity that is contributed both naturally (upstream Goulburn River sites, SW12, SW05, SW06, SW08) and WTP Filtrate and WTP Discharge (Chart 3 and 4). Only WTP Discharge is released to the Goulburn River. The Piper Diagram (Figure 9) shows a clear distinction between the salinity sourced from Goulburn River waters (SW01, SW02, SW12, SW05), which are composed of higher sulfate to sodium ratio downstream and lower sulfate to sodium ratio upstream. Treatment waters (WTP Filtrate) are located towards the top end of the Piper Diagram and also have a higher sulfate content compared to that of sodium and chloride (Chart 3), yet their proportions of sulfate are much larger compared to that of the Goulburn River (Chart 4). This is indicative of the source of WTP Filtrate which is most likely associated with coal mineralisation from underground workings and/or coal water storage operations. SW06 has a different ionic composition in the Piper Diagram, showing lower salinity values and lower contribution of sulfate to the dissolved ion ratios. SW06 demonstrates a cleaner water or reduced salinity input, whereas SW08 is similar to natural coal mineralisation upstream and has higher sulfate, sodium and chloride content and ultimately higher salinity values. SW08 is distinctly different compared to any of the other sites displayed on the Piper Diagram, yet has a slightly similar ionic proportions in its water chemistry to other Goulburn River sites (similar range), demonstrating its origin as a river water sample.

Table 32: Summary of total dissolved solids (TDS) in mg/L measured at upstream (UCML SW01 / SW12, SW05, SW06, SW08) and downstream (SW01, SW02) Goulburn River monitoring sites. Site measurements were collected between 2005 and 2021.

Summary	SW01	SW02	UCML SW01 / SW12	SW05	SW06*	SW08
n	269	274	271	278	65	249
n <LOR	0	0	0	0	0	0
Min	143	152	76	118	131	116
10%ile	256	300	222	284	160	1092
20%ile	312	351	256	320	170	1460
80%ile	479	498	400	543	220	2600
90%ile	508	536	460	621	248	2862
Max	808	1120	628	965	536	4200
Mean	397	430	331	436	206	1977
Std. Dev	205	214	226	266	67	1167
Std. Error	13	13	14	16	8.3	74

*SW06 monitoring data from 2005-2010, Includes Stream Health (SH) 012 data 2011-2021

Table 33: Summary of electrical conductivity (EC) (µS/cm) measured at upstream (UCML SW01 / SW12, SW05, SW06, SW08) and downstream (SW01, SW02) Goulburn River monitoring sites. Site measurements were collected between 2005 and 2021.

Summary	SW01	SW02	UCML SW01 / SW12	SW05	SW06*	SW08
n	211	225	234	241	59	192
n <LOR	0	0	0	0	0	0
Min	95	95	45	6	40	195
10%ile	281	379	112	340	208	1853
20%ile	465	518	207	468	236	2332
80%ile	795	830	619	890	364	4126
90%ile	831	866	767	1020	412	4569
Max	1180	1800	971	1570	630	5990
Mean	632	673	446	673	307	3161
Std. Dev	106	113	96	144	107	716
Std. Error	7	8	6	9	14	52

*SW06 monitoring data from 2005-2010

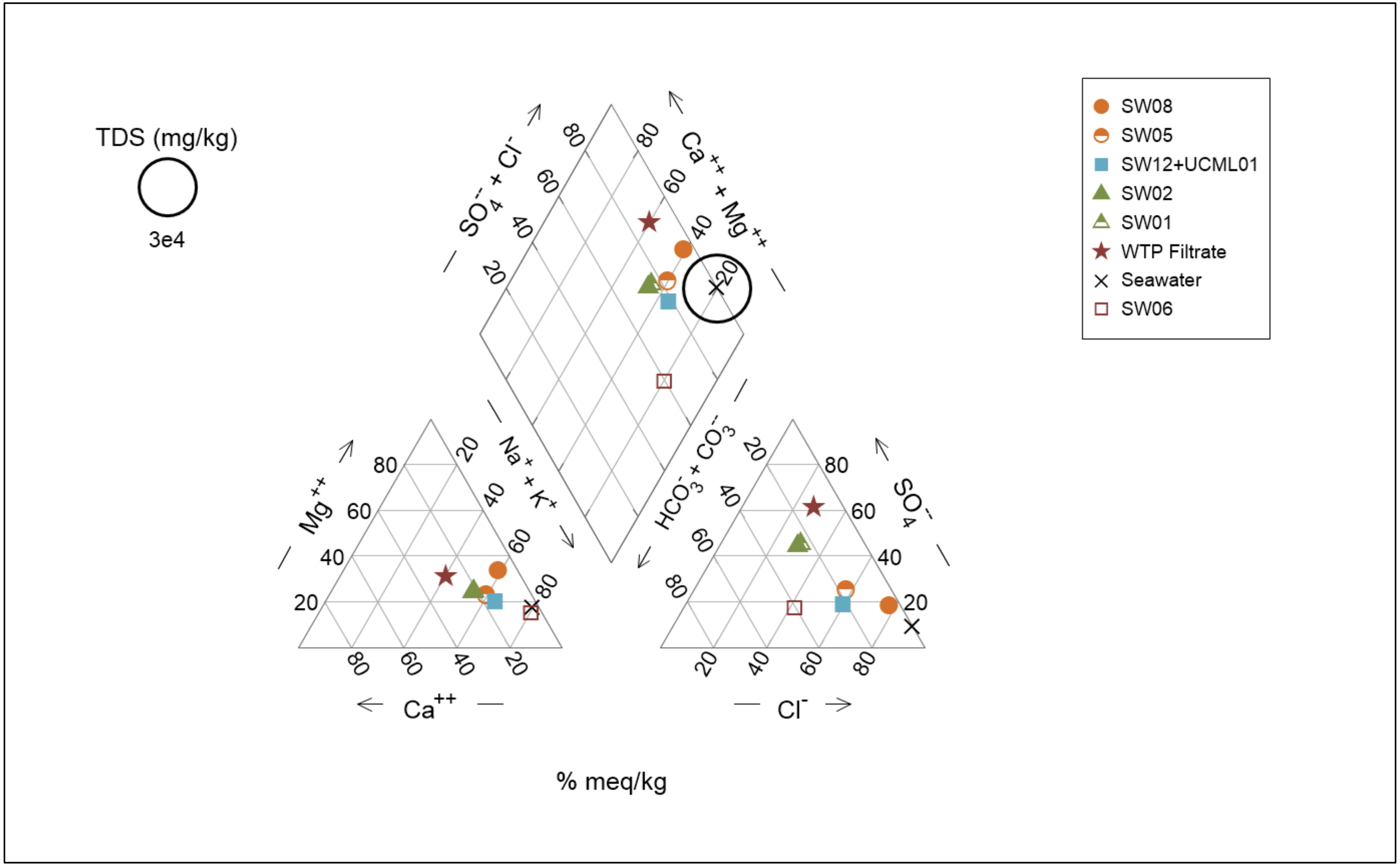


Figure 9: Piper diagram displaying distinct facies of Goulburn River surface waters (SW), WTP Filtrate and seawater reference (IOC, SCOR and IAPSO, 2010) based on key dissolved ions (calcium, chloride, bicarbonate, magnesium, potassium, sodium and sulfate) in water

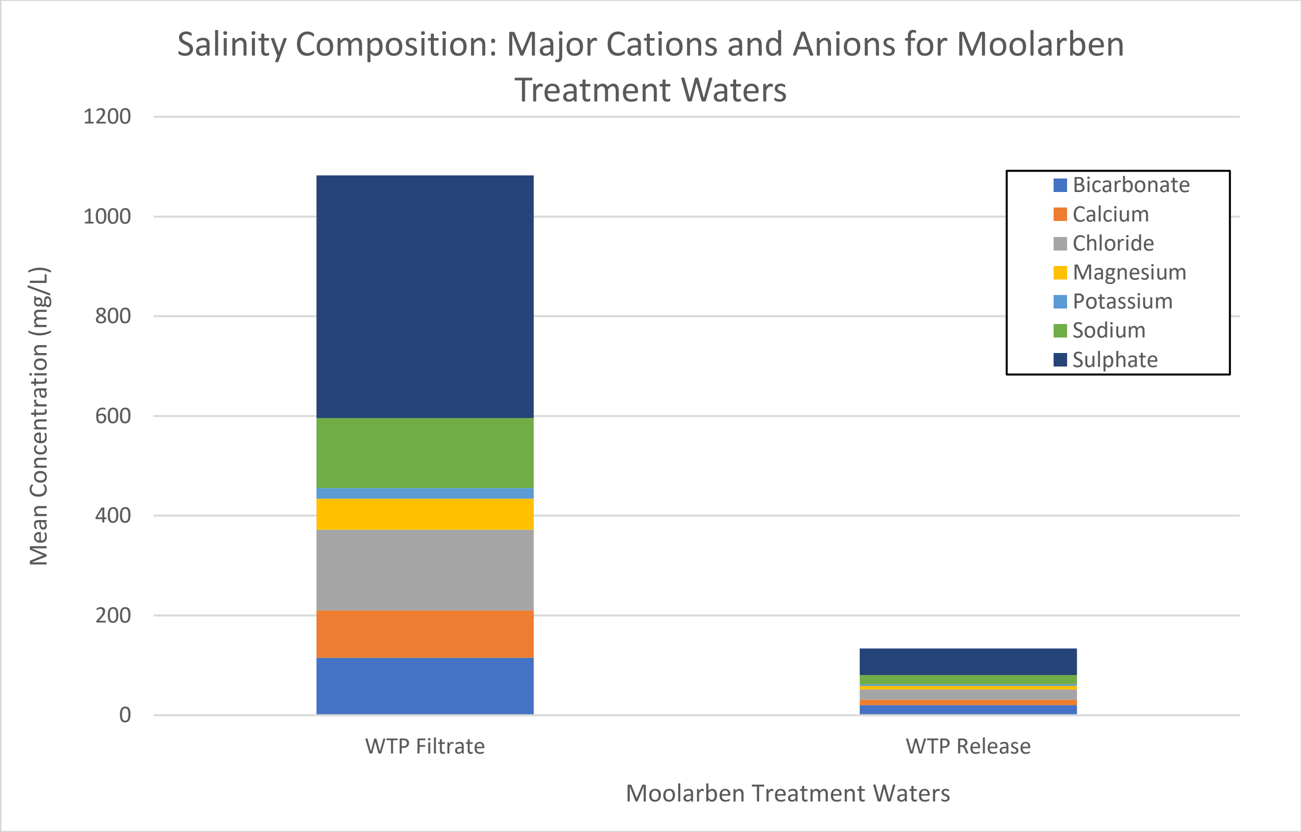


Chart 3: Proportions of dissolved ions in solution which contribute to the characterisation of different salinities in Moolarben treatment waters.

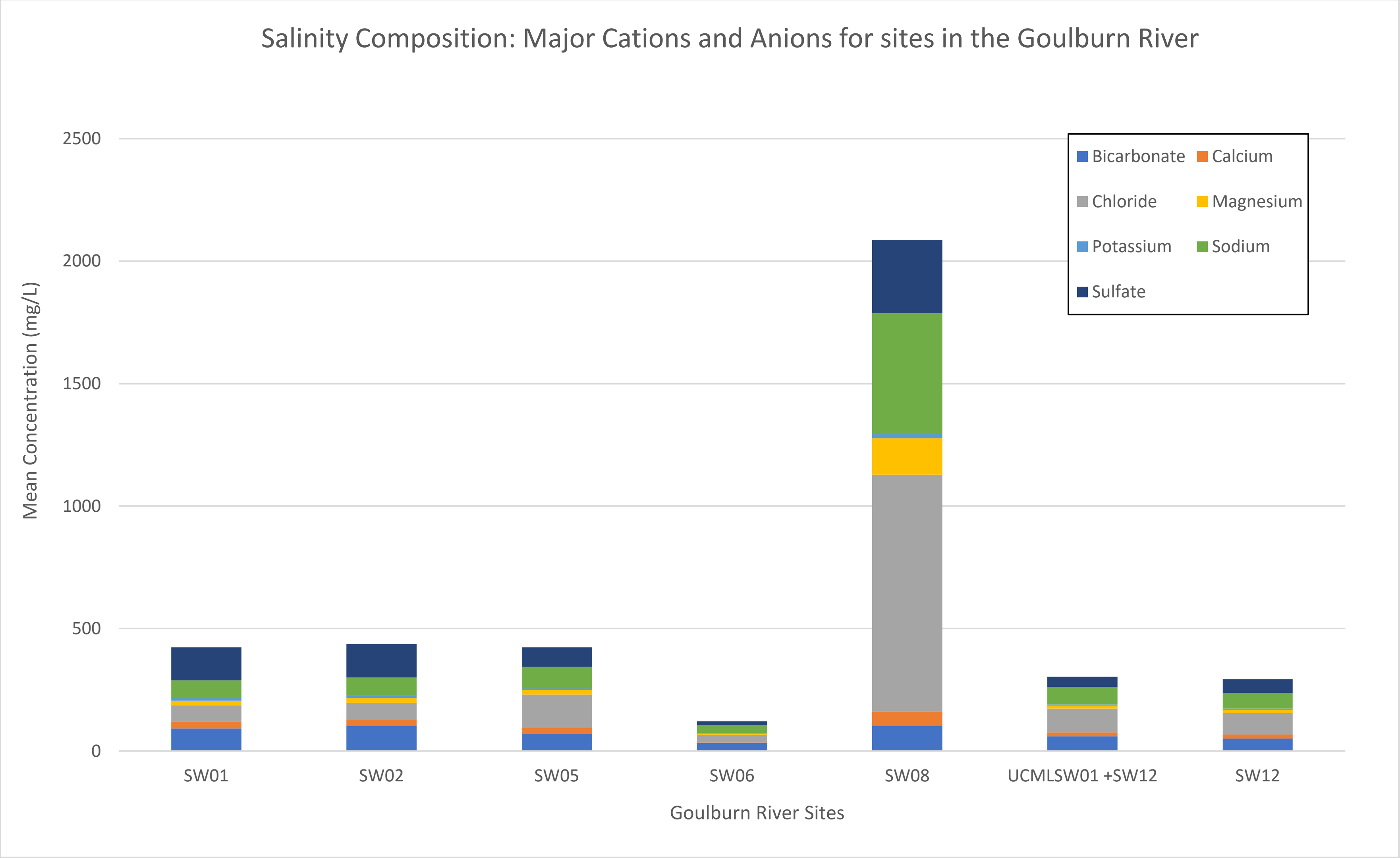


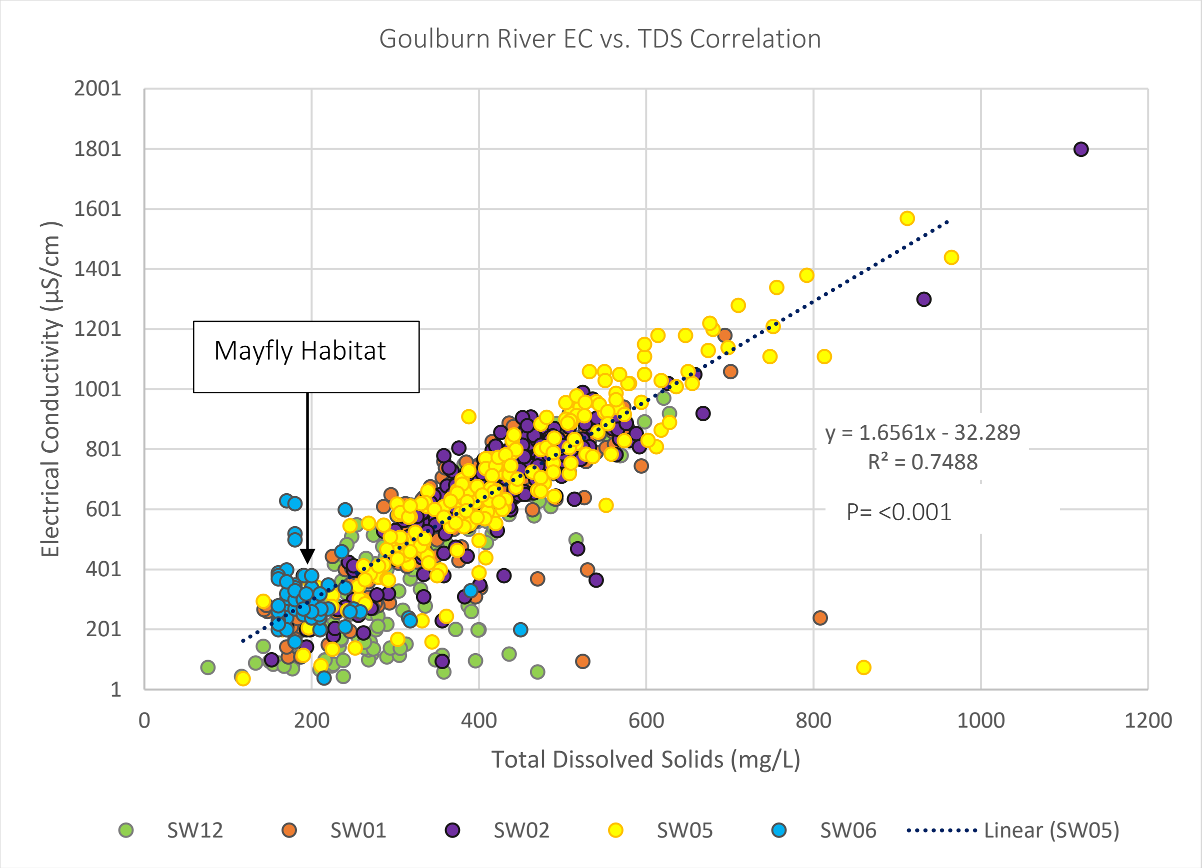
Chart 4: Proportions of dissolved ions in solution which contribute to the characterisation of different salinities in the Goulburn River.

8.3.6 Goulburn River Salinity Factors

It is established that TDS and EC have a strong correlation in surface waters (Section 7.8). This relationship is described as the salinity factor k (TDS/EC ratio). A salinity factor (k) is useful when only EC is measured reliably and can be used to determine TDS values from EC measurements. These values can only be used however if there is a significant linear correlation between TDS and EC. A clear relationship is shown for all sites in the Goulburn River using historical TDS to EC values (2005-2021), which is depicted in Chart 5 and Chart 6 (p <0.001).

Goulburn River waters salinity factors calculated using Equation (1) (refer to Section 7.8 Salinity) can vary between 0.6-1.03 on average (Table 34 and Chart 7). Such variation in salinity factor (k) has been observed at several locations, including for surface waters in Queensland (McNeil and Cox, 2000). Upstream Goulburn River sites (SW05, SW08) and downstream sites (SW02, and SW01) have salinity factors that are less varied, falling on average between 0.6 and 0.75 (Chart 7). SW12 however has a characteristically larger variation of salinity (Chart 7). These variations in the magnitude of salinity factor are an indicator of mixing sources of major ions from different types of water (Section 7.8). Site SW12 is influenced both by Ryans and Sportsmans Hollow Creeks, which have lower salinity (larger TDS/EC ratio) as shown in Table 35, and are reflective of low EC values to TDS and by Moolarben Creek which has a higher salinity (Section 1).

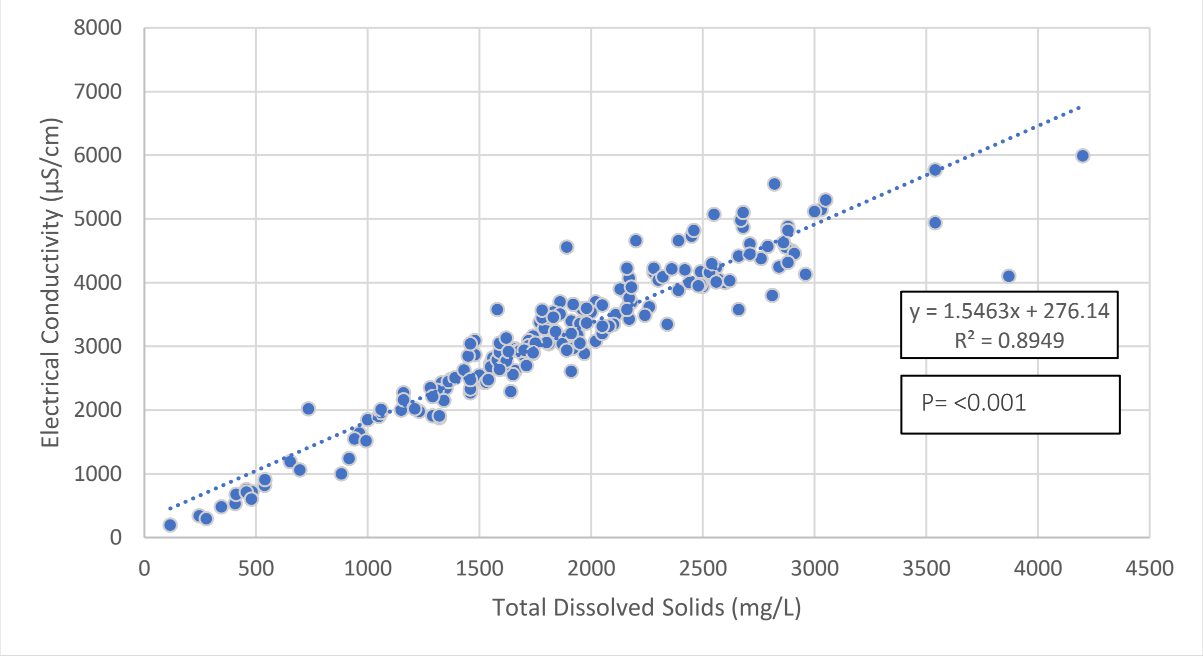
Table 36 gives total dissolved solids, electrical conductivity, and salinity factor k (TDS/EC ratio) values for WTP Filtrate that have similar ranges of k with SW01, SW02 and SW08, but different from SW12.



Note: SW08 excluded from this graphical display due to differences in water chemical facies

SW12 excludes combined data from UCMLSW01, to demonstrate appropriate graphical relationships of background site SW12 and co-located site UCMLSW01.

Chart 5. Electrical conductivity (µS/cm) and total dissolved solids (mg/L) correlation in Goulburn River surface water (SW) monitoring sites.

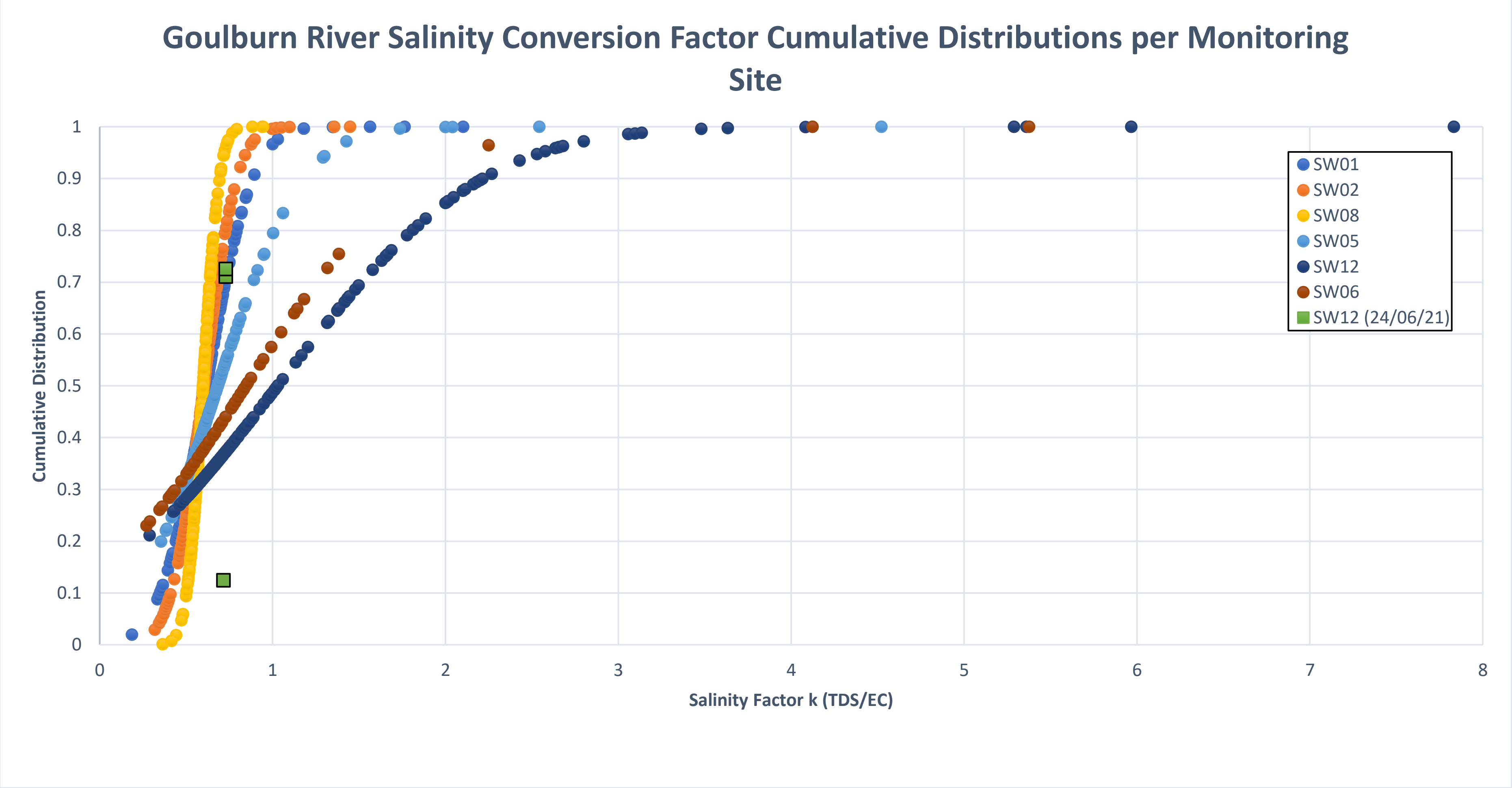


Note: Other Goulburn River monitoring sites are excluded due to the differences in water chemical facies

Chart 6. Electrical conductivity (µS/cm) and total dissolved solids (mg/L) correlation in Goulburn River surface water monitoring site SW08.

Table 34. Salinity factor k (TDS/EC ratio) for Goulburn River surface water monitoring sites including percentile and mean values. UCML SW01 / SW12 collected on 24/06/2021 represents data collected from the Diluent control water for ecotoxicity testing

Summary	SW08	SW05	SW06	UCML SW01 / SW12	UCML SW01 / SW12 24/06/21	SW02	SW01
n	192	181	64	248	3	178	188
min	0.36	0.35	0.27	0.29	0.72	0.32	0.19
20%	0.55	0.52	0.52	0.58	0.72	0.51	0.51
50%	0.59	0.61	0.68	0.69	0.73	0.58	0.58
80%	0.64	0.70	0.86	1.19	0.73	0.66	0.68
90%	0.68	0.80	1.14	2.06	0.73	0.72	0.75
95%	0.73	1.00	1.37	2.66	0.73	0.85	0.85
99%	0.94	2.14	4.59	5.33	0.73	1.16	1.59
max	0.94	4.52	5.38	7.83	0.73	1.45	2.10
mean	0.60	0.68	0.85	1.03	0.73	0.60	0.62
std dev	0.07	0.39	0.78	0.93	0.01	0.15	0.21
std error	0.01	0.03	0.10	0.06	0.00	0.01	0.02



Note: UCML SW01 / SW12 collected on 24/06/2021 represents data obtained from the Diluent control sample used in ecotoxicity testing

Chart 7. Salinity factor, k (TDS/EC ratio) variation for each of the surface water monitoring sites in the Goulburn River.

Table 35. Mean total dissolved solids, electrical conductivity, and salinity factor k (TDS/EC ratio) values (with 95% confidence intervals) for each of the Goulburn River monitoring sites

Goulburn River Monitoring Sites	TDS (mg/L) (95% CI)	EC (µS/cm) (95% CI)	Salinity Factor, k (TDS/EC)
SW01	397 (384-409)	632 (604-659)	0.62 (0.59-0.65)
SW02	430 (417-444)	673 (645-701)	0.60 (0.58-0.62)
UCML SW01 / SW12	331 (320-343)	446 (417-475)	1.03 (0.91-1.15)
SW05	436 (419-453)	673 (639 -706)	0.68 (0.62-0.74)
SW06*	206 (189-222)	307 (364-630)	0.85 (0.66-1.04)
SW08	1977 (1889-2066)	3161 (2999-4309)	0.61 (0.60-0.62)

Note: *SW06 monitoring data from 2005-2010 including field EC readings

Table 36. Total dissolved solids, electrical conductivity, and salinity factor k (TDS/EC ratio) values for WTP Filtrate

	WTP Filtrate		
	TDS (mg/L)	EC (µS/cm)	K (TDS/EC)
n	44	19	n.a.
min	788	1380	0.57
10%	910	1430	0.64
20%	957	1480	0.65
80%	1192	1740	0.69
90%	1247	1762	0.71
Max	1310	1880	0.70
Mean	1086	1648	0.66

8.3.7 Aluminium, Free Silica and Dissolved Organic Carbon

Both aluminium and sulfate concentrations in Moolarben Creek waters are associated with increased EC compared with Ryans and Sportsmans Hollow Creek tributaries of the Upper Goulburn River. The presence of aluminium and sulfate concentrations in Moolarben Creek waters is attributed to seepage from naturally occurring coal mineralisation (Section 1). The measured concentrations of dissolved aluminium in Moolarben Creek waters appear to exceed the ANZECC water quality guidelines for protection of aquatic species in freshwaters. However, the ecotoxicity studies summarised in Table 25 show that both UCML SW01 / SW12 control water and WTP Filtrate water collected 24 June 2021 showed no effects from 100% of each test water on the aquatic test species. In fact aluminium concentrations in the 100% test waters for UCML 01/SW12 exceed the ANZECC water quality guidelines for aluminium in freshwaters (Appendix B, Table B.1). It is clear that there are other processes which can result in reduced toxicity of dissolved aluminium to aquatic species, when aluminium concentrations remain high.

Studies have shown that the toxicity of filtered aluminium in river water to aquatic species is minimised by the presence of either free silica or dissolved organic carbon in water (Camilleri et al., 2003). Comparison of historical data for filtered aluminium, free silica and dissolved organic carbon concentrations in Goulburn River waters shows the presence of levels of free silica and dissolved organic carbon far exceed the dissolved aluminium (Table 37). This data suggests that the presence of sufficiently high free silica and dissolved organic carbon concentrations in the Goulburn River waters, and particularly at UCML SW01 / SW12, may offer protection to aquatic species against toxicity from dissolved aluminium. The toxicity of filtered aluminium in river water is minimised by free silica and dissolved organic carbon (Table 37).

Table 37. Historic data on aluminium, free silica and dissolved organic carbon concentrations in Goulburn River water

Goulburn River Monitoring Sites	Aluminium Filtered (mg/L)				Free Silica as SiO2 (mg/L)				Total Organic Carbon (mg/L)			
	2013-2021				2013-2021				2019-2021			
	n	Mean	80%	Max	n	Mean	80%	Max	n	Mean	80%	Max
SW01	16	0.03	0.06	0.10	-	-	-	-	-	-	-	-
SW02	20	0.02	0.02	0.15	-	-	-	-	-	-	-	-
UCML SW01 / SW12	73	0.08	0.13	0.46	34	20	26	37	23	14.13	18	31
SW05	41	0.04	0.06	0.25	21	19	24	32	32	14	18	25
SW06*	2	0.04	-	0.05	1	31	31	31	1	6	6	6
SW08	16	0.01	0.01	0.03	-	-	-	-	-	-	-	-

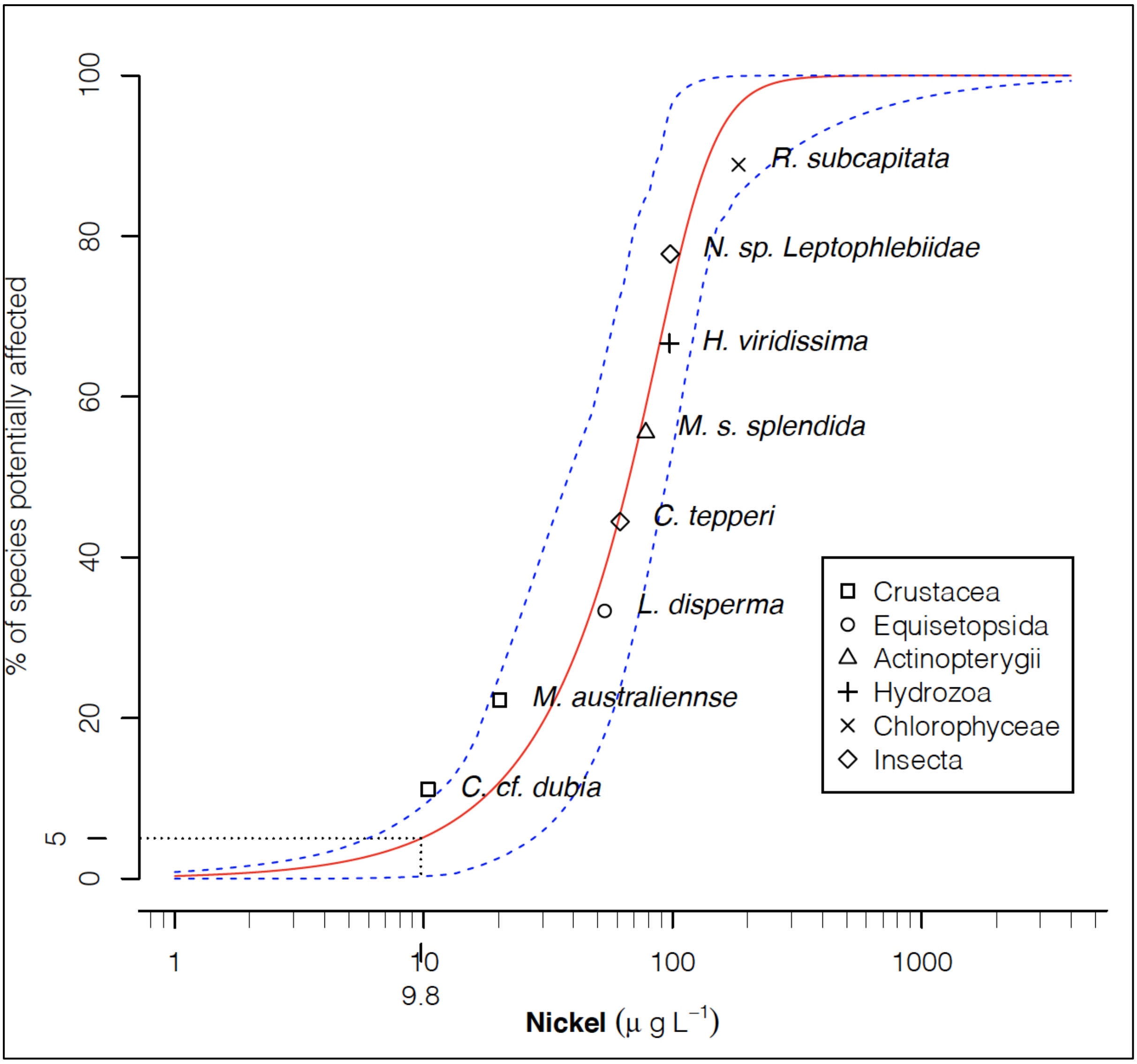
Note: ANZG (2018) 95% protection level for aquatic species in freshwater (pH >6.5) is 0.055 mg/L.

8.4 SSD Derivation and SSGV

This section provides data used to generate SSD (Table 38) and the SSD plots with labelled 95% protection level SSGVs (Charts 8 to 10). Table 38 details chronic EC/IC10 endpoints used as inputs in the BurrliOZ Type III software (CSIRO, 2015) for each of the eight test species and toxicants (Nickel, Zinc and Sulfate) for which an SSD plot was generated. The SSD plots that give the SSGVs at a level of 95% species protection are shown in Charts 8 to 10 and Appendix E. Table 39 provides a detailed summary of SSGVs assigned at different levels of aquatic ecosystem protection (%) for Nickel (µg/L), Zinc (µg/L) and Sulfate (mg/L) (with 95% confidence intervals).

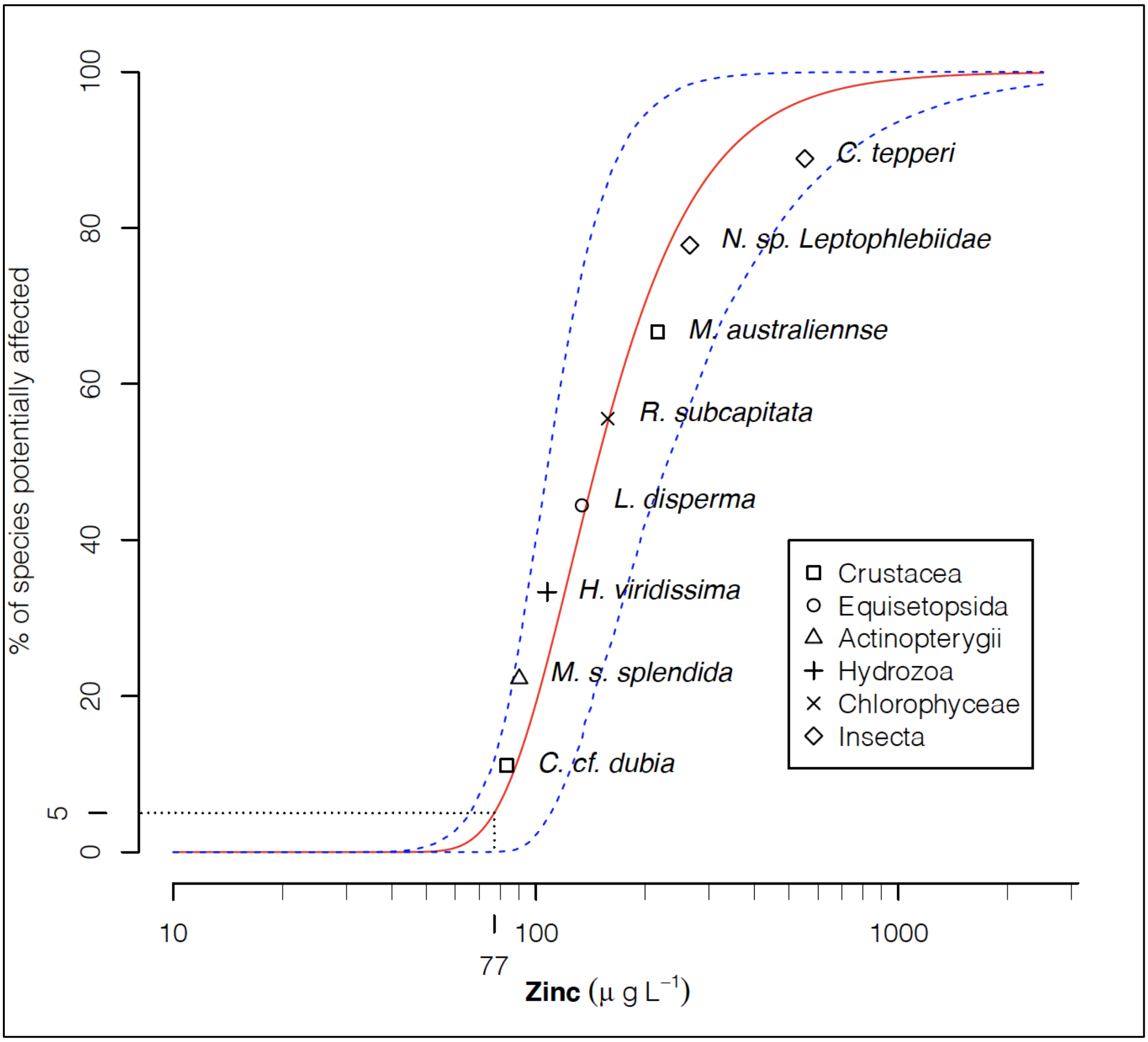
Table 38. Chronic EC/IC10 inputs used in the BurrliOZ 2.0 Type III software to generate SSD plots and SSGVs

Test Species	Exposure	Endpoint	Nickel (µg/L)	Zinc (µg/L)	Sulfate (mg/L)
			EC/IC10	EC/IC10	EC/IC10
Freshwater Cladoceran *Ceriodaphnia dubia*	Acute Chronic	Survival Reproduction	33.6 11	70.0 87.7	1293.4 1270.7
Freshwater Aquatic Duckweed, *Lemna disperma*	Chronic	Growth Rate	53.3	134	3032.9
Eastern Rainbowfish, Melanotaenia splendida splendida	Acute Chronic	Fish Imbalance Reproduction	87.5 78.0	161.1 90	2873.3 1222.3
Freshwater Hydra, *Hydra viridissima* (Pilbara isolate)	Chronic	Growth Rate	97.1	107.5	1518.1
Green Alga, Raphidocelis subcapitata	Chronic	Growth Rate	183.9	157.9	794.5
Freshwater Chironomid, *Chironomus tepperi*	Acute	Survival	61.5	552.2	3022.3
Freshwater Prawn Macrobrachium australiense	Acute	Survival	20.2	216.6	4625
Mayfly Larval, Nousia sp.AV1 (Leptophlebiidae)	Acute	Survival	97.8	266	1465.9



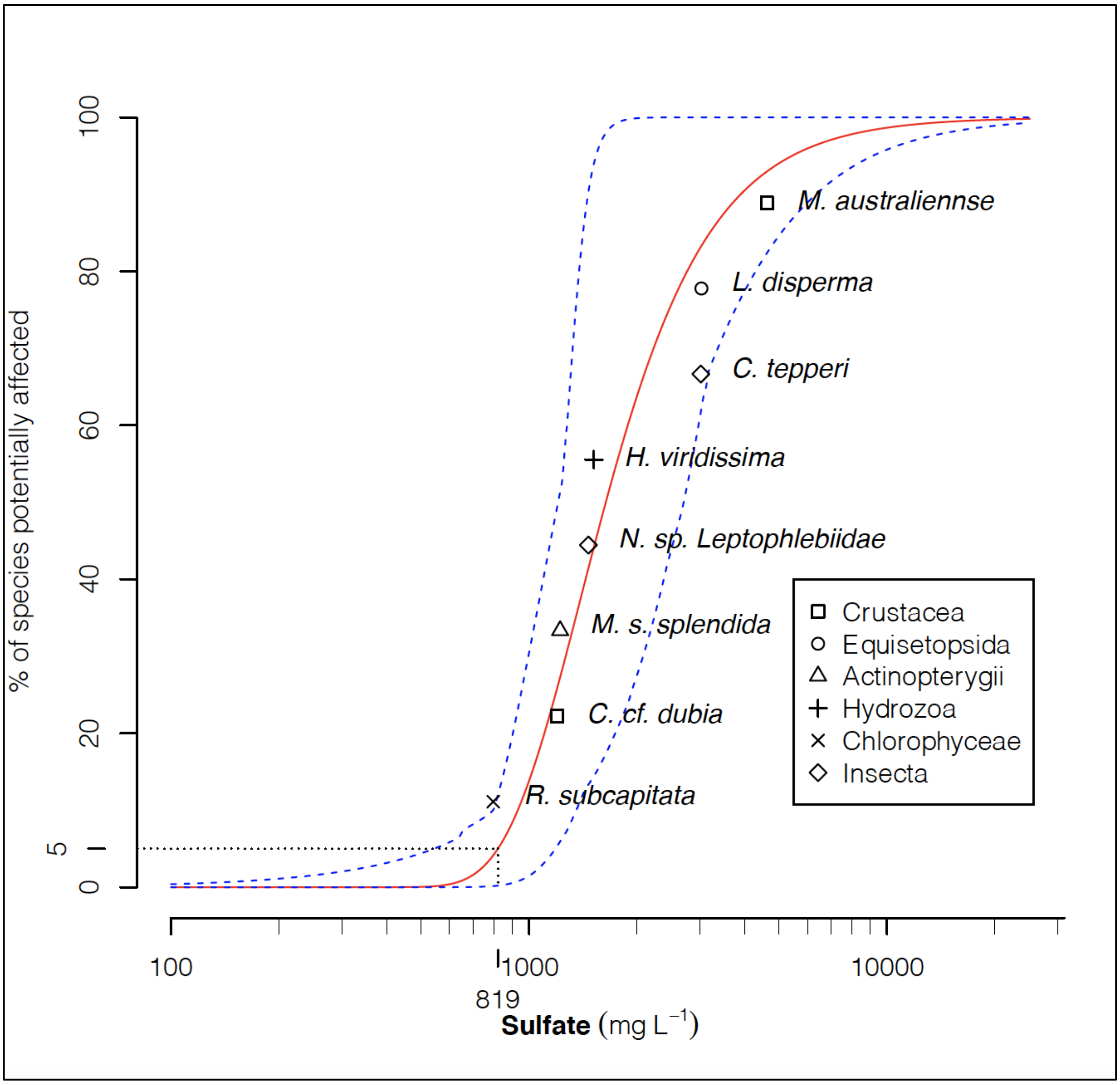
Note: C.tepperi, M. australiennse, N. sp. leptophlebiidae are acute to chronic converted endpoints.

Chart 8. Species sensitivity distributions (SSDs) for Nickel (µg/L) with derived site-specific guideline value for the Goulburn River at a level of 95% aquatic species protection.



Note: C.tepperi, M. australiennse, N. sp. leptophlebiidae are acute to chronic converted endpoints.

Chart 9. Species sensitivity distributions (SSDs) for Zinc (µg/L) with derived site-specific guideline value for the Goulburn River at a level of 95% aquatic species protection.



Note: C.tepperi, M. australiennse, N. sp. leptophlebiidae are acute to chronic converted endpoints.

Chart 10. Species sensitivity distributions (SSDs) for Sulfate (mg/L) with derived site-specific guideline value for the Goulburn River at a level of 95% aquatic species protection.

Table 39. Site specific guideline values (SSGVs) for Nickel, Zinc (µg/L) and Sulfate (mg/L) (with 95% confidence intervals) assigned at different levels of aquatic ecosystem protection (%).

Protection	Nickel (µg/L)	Zinc (µg/L)	Sulfate (mg/L)
99%	2.6 (0.8 - 17.3)	63 (53 - 95)	661 (188 - 956)
95%	9.8 (5.9 - 25.8)	77 (65 - 112)	819 (544 - 1314)
90%	17 (9.7 - 36.5)	86 (74 - 126)	931 (818 - 1539)
80%	3 (17.0 – 57.0)	101 (85 – 148)	1105 (971 – 1779)

Under MCO's Surface Water Management Plan, the current ANZG (2018) aquatic toxicant guideline for freshwater species (for 95% level of protection) discharge limit for zinc (8.0 µg/L) is well below the derived value, while the current discharge limit for nickel (11.0 µg/L) is within the range of the derived value. Due to the complexity of conversion between sulfate and EC, the SSGV for EC is described further in the following sections.

In the current study the ecotoxicity of river water has different components with wide variation in toxicities. The SSGVs for nickel, zinc and sulfate show large a large difference between sulfate and the two metals. Sulfate as its analyte chemical of sodium sulfate is dissolved in water to give the sulfate solution. The weight of sodium sulfate that is dissolved can be used to calculate the dissolved salt as mg/L in the solution and compared with the measured EC of that solution. Thus it is possible to convert EC measurements to salinity in units of mg/L.

The direct application of EC measurement as the indication of salinity enables an assessment of the toxicity of salinity to the various test species. Because EC is measured in a fundamental unit, it is more generally applicable to any kind of test water from the MCO site. However, it should be noted that the SSGVs have been derived specifically for the water in the Goulburn River – any limit that is applied to WTP Discharge water (an "end-of-pipe" limit) will not account for mitigating factors such as dilution due to Goulburn River flow. The application of these and other potential factors could be considered in any future works that explore the applicability of an "end-of-pipe" limit that varies based on flowrate and/or concentrations in the Goulburn River.

Comparison with the WTP Filtrate , discharge but not permeate of EC converted to salinity (mg/L) showed no response to aquatic species, even though sulfate, nickel and zinc concentrations were higher that the SSGVs for respective analyte.

Based on historic and current readings of sulfate in the UCML SW01 / SW12 sample (background water quality sample) taken for ecotoxicity testing, sulfate is naturally elevated in the Goulburn River (73 mg/L 24/06/2021).

8.5 Sodium Sulfate Conversion and Utilizing Salinity Factor k

A SSGV for EC based on WTP filtrate was not able to be developed as the WTP filtrate testing demonstrated no statistically significant toxicity to aquatic test species. Additional evidence considered includes comparing to other studies and conversion of sulfate utilizing salinity factors.

Sulfate concentrations are attributed to the natural mineralisation emphasised by the local geology of exposed, unmined coal measures and/or groundwater aquifer inputs from Moolarben Creek, a tributary of the Goulburn River. WTP Filtrate samples (mine water samples) also have high sulfate concentrations (mean: 531 mg/L), which are greatly reduced in WTP discharge samples. These levels suggest that salinity inputs to the Goulburn River are governed by sodium sulfate ($NaSO_4$). This supports the decision to investigate the ecotoxicity of sulfate in SW12 waters with prescribed dosages of sulfate in Diluent control water (SW12 24 June 2021) to determine the response of test species. Based on the nature of salinity in the Goulburn River, sulfate dose response ecotoxicity tests also provides a means of calculating a sulfate SSGV to be used as an analogue for salinity. Salinity SSGVs are estimated by applying the g/mol weight factor of $SO_4/NaSO_4$ of 0.6762 (Table 15) to the SSGV derived for sulfate for ecotoxicity tests using 8 aquatic species. Table 40 provides a summary of acute toxicity data for the mayfly from its ecotoxicity test (Appendix G).

Table 40. Comparison of Mayfly toxicity response to salinity in Dowse et al. (2017) using marine waters to natural salinity from coal measures (Moolarben)

Nousia sp.AV1 (*Leptophlebiidae*)	96h IC10	CI[a] (95% confidence interval)	96h EC50	CI
Sulfate (mg/L)	2356	1653.7 - 2806.8	3423.7	2894.18 - 3999.32
$NaSO_4$ as Salinity (mg/L)	3485	2445.6 - 4150.8	5063.1	1957.04 - 2704.34
Austrophlebioides pusillus (Dowse et al. 2017)	96h LC10	CI	96h EC50	CI
Salinity (mg/L)	2400	1400 - 3100	4800	4200 - 5500

Note: a CI = 95% Confidence Interval

The Dowse et al., (2017) study shows that the osmoregulatory response of Mayfly nymph species, *Austrophlebioides pusillus (Ephemeroptera: Leptophlebiidae)* to marine salinity increases and is able to identify that mortality rises in association with salinity rise are related factors other than osmotic pressure. Dowse identifies that at 96h the limiting concentration for LC10 is 2400 mg/L, whereas in Moolarben it is 3485 mg/L. Comparison of the studies on Mayfly toxicity with Dowse et al. (2017) show that the CIs overlap for both sets of data for different Mayfly species (Table 31). In addition the SSGV for sulfate develop with 6 aquatic species for the Fitzroy River Basin, Queensland (Dunlop et al., 2016) gave 545 mg/L sulfate with 95% confidence limits of 380-1103 mg/L which overlaps with the MCO value for sulfate in Table 39.

Thus the SSGV for sulfate (Table 41) can be expressed as salinity mg/L based on the weight of $NaSO_4$ in solution for toxicity testing. Due to the natural input of salinity from the coal measures present in the underlying geology of Moolarben Creek, which is different from the salinity of from marine waters, it is expected that the ratio of ions that are the components of salinity to be different. Based on the conditions of salinity in the Goulburn River, sulfate represents a significant component of the ions that make up the saline conditions. Using sodium sulfate as an analogue, an SSGV value for salinity for 95% protection of aquatic species in the Goulburn River is predicted to be 1211 mg/L, well above the limits outlined by the EPA. This demonstrates that aquatic species are less sensitive to salinity (as sodium sulfate) compared with nickel or zinc. Electrical Conductivity may be used to express salinity in µS/cm as it equates with total dissolved salts (mg/L). The current EPL release limit for MCO is the 100th percentile for EC at 685 µS/cm, which is equivalent to 493 mg/L as salinity. A converted value from SSGV of salinity 1211 mg/L to EC is 1670 us/cm.

Table 41. SSGV Sulfate (mg/L) to Salinity $NaSO_4$ (mg/L) .

SSGV Sulfate (mg/L)	Salinity $NaSO_4$ (mg/L)[a]
819 (544-1314)	1211 (804.5-1943.2)

Note a Conversion of sulfate to sodium sulfate salinity (mg/L) NaSO4 =819/0.6762 (from Table 15)

Compared with the rest of salinity factors generated for surface water monitoring sites for the Goulburn River (Table 42), salinity factors k vary (0.6-1.03). Salinity factor, 0.73, generated for SW12 samples taken on June 24, 2021 (sample taken for ecotoxicity testing) are well within the range of expected values in the Goulburn River. Table 42 provides the expected EC values generated from the range of salinity factors generated for the Goulburn River, with 95% confidence intervals.

Table 42. Variability of SSGV based on mean salinity factor (TDS/EC) values for surface water monitoring site in the Goulburn River (with 95% confidence intervals).

Goulburn River Monitoring Sites	SSGV Sulfate	Calc. TDS (mg/L)	Salinity Factor, ***k*** (TDS/EC)	Calc. EC (µS/cm)
SW08	819 (544-1314)	1211 (804.5-1943.2)	0.61	1985 (1319-3186)
SW05	819 (544-1314)	1211 (804.5-1943.2)	0.68	1781 (1183-2858)
SW06	819 (544-1314)	1211 (804.5-1943.2)	0.85	1425 (946-2286)
UCML SW01 / SW12	819 (544-1314)	1211 (804.5-1943.2)	1.03	1176 (781-1887)
SW12 24/06/21	819 (544-1314)	1211 (804.5-1943.2)	0.73	1670 (1110-2680)
SW02	819 (544-1314)	1211 (804.5-1943.2)	0.6	2018 (1341-3239)
SW01	819 (544-1314)	1211 (804.5-1943.2)	0.62	1953 (1298-3134)

Both electrical conductivity and sulfate concentration was measured directly in each toxicity test solution during the dosed sulfate toxicity tests in SW12 diluent control sample waters for aquatic species responses (Appendix G) to develop the sulfate SSGV (Section 8.4). From these direct measurements the correlation between measured sulfate and electrical conductivity can be derived (Chart 11). The complete data set used to give the linear regression is given in Appendix F.

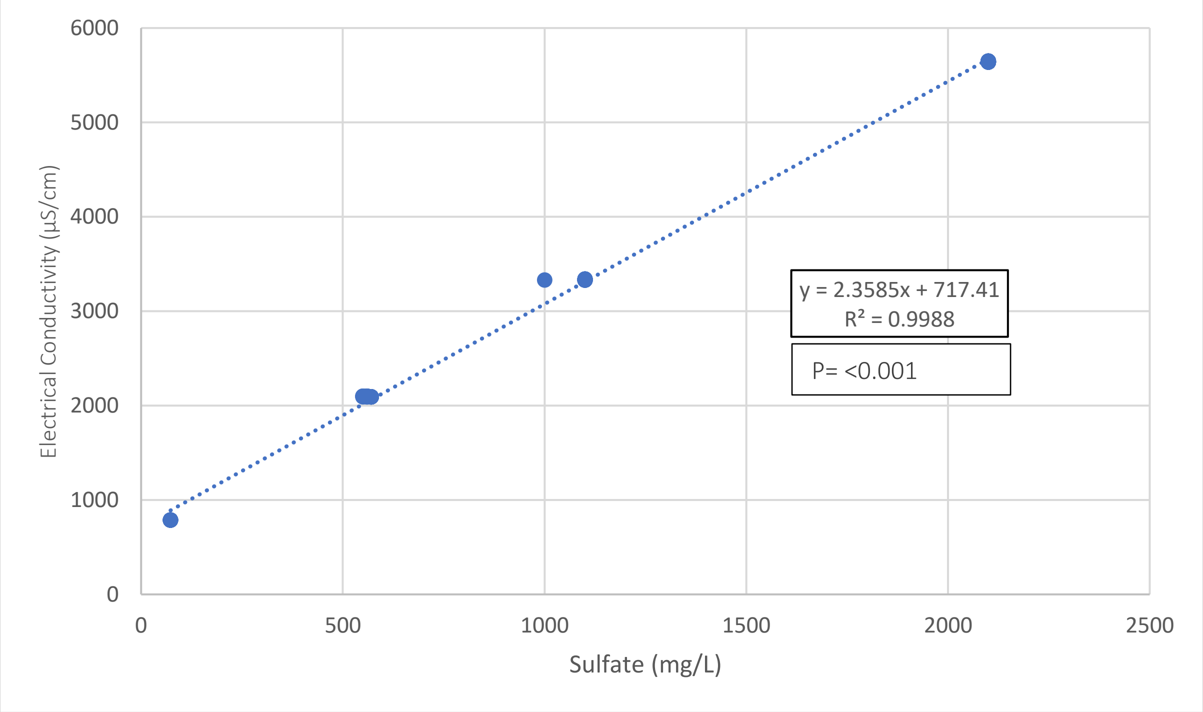


Chart 11. Linear regression between sulfate concentration (mg/L) and electrical conductivity (µS/cm) measurement from dosed sulfate ecotoxicity tests in SW12 diluent control water.

The linear regression between measured sulfate dosage (mg/L) and electrical conductivity (µS/cm) with R^2 0.9988 and $P<0.001$ is used to derive Equation 5. Rationale for using a lower sulfate concentration range is explained in more detail in Appendix F. At a SSGV value of 819 mg/L (equal to 'x') , an SSGV value for electrical conductivity (equal to 'y') can be derived from Equation 5.

$$y = 2.3585x + 717.41 \tag{5}$$

The SSGV for EC (with 95% confidence interval), based on the above derived relationship is equal to 2649 (2000-3816) µS/cm.

The validity of this calculation is supported by the measured dosed response values of sulfate in SW12 (control diluent water) and associated EC values measured directly on the ecotoxicity test solutions (Appendix G). Measured EC values directly corresponding to measured sulfate concentrations (Appendix F, Table F.1, measured sulfate: 570 mg/L) ranging in approximation to the SSGV value of 819 mg/L are read at an EC value of 2090 µS/cm (08 July 2021) and 2100 µS/cm (02 July 2021). The R^2 value represents the 'goodness of fit', and this R^2 value (0.9988) demonstrates an improved fit of the EC calculation by utilizing a linear regression with a higher R^2 value (compared to that of R^2 0.9938).

8.6 SSGV Suitability: Reality Checking Guideline Values

The suitability of SSGVs derived for Nickel, Zinc and Sulfate are evaluated by comparing the SSGVs to raw toxicity data that the guideline values were derived from. This approach, outlined by Warne et al. (2018), determines whether any species (for which toxicity data are available) are affected if exposed to a derived guideline value concentration. SSGVs are considered to provide inadequate protection if any of the following conditions are met:

A. If a guideline value is greater than the geometric mean of experimental chronic IC10/EC10/EC10/NEC or NOEC data for any important species (that is species that are important on the basis of commerce, rarity or ecological significance) (Table 43).
B. If there is a discrepancy between the theoretical level of protection that should be provided and that indicated as being offered, based on experimental toxicity data. For example, if more than 5% of the experimental data are below the 95% Protection Level (Table 44).

In cases where protection levels are deemed inadequate, it is recommended by Warne et al. (2018) that the SSGV level of protection is increased. For example, if 95% protection level fails suitability checks, it could become 99% protection level. If an increase in the protection level still fails suitability checks than additional toxicity data is required.

Converted acute data, which was derived through ACRs, is excluded from the SSGV suitability check.

Table 43 summarizes the geometric mean suitability check (condition A) as advised in Warne et al. (2018). Table 43 indicates that there were no SSGVs (at 95% protection level) greater than the geometric mean of the raw chronic IC10/EC10/EC10 experimental data.

Table 43. Comparison of SSGV (at 95% protection level) with geometric mean of Raw chronic IC10/EC10/EC10 experimental data.

Analyte	Geometric mean of raw chronic IC10/EC10/EC10 data	95% Protection Level SSGV (CI)	SSGV > geometric mean of raw chronic IC10/EC10 data
Nickel (µg/L)	60	9.8 (5.9 - 25.8)	No
Zinc (µg/L)	111	77 (65 - 112)	No
Sulfate (mg/L)	1399	819 (544 - 1314)	No

Table 44 summarizes the discrepancy suitability check (condition B) which compares SSGV (at 95% protection level) with both acute and raw chronic IC10/EC10/EC10 experimental data. There was a total of 10 tests for all acute and chronic responses. All raw experimental data for Nickel showed no response below the 95% protection level, whereas Zinc and Sulfate showed a response in 1% of test species (*R. subcapitata* [acute] for Zinc and *C. cf. dubia* [chronic] for Sulfate). According to Warne et al. (2018), if less than 5% of the experimental data showed a response below the 95% protection level, SSGVs are still deemed suitable. SSGVs derived for Zinc and Sulfate are therefore adequate protection levels for all test species.

Table 44. Discrepancy between SSGV (95%) and experimental Raw chronic IC10/EC10/EC10 data.

Analyte	95% Protection Level SSGV	SSGV > raw acute and raw chronic IC/EC10 data
Nickel (µg/L)	9.8 (5.9 - 25.8)	No
Zinc (µg/L)	77 (65 - 112)	No
Sulfate (mg/L)	819 (544 - 1314)	No

The above suitability checks suggest that SSGVs derived for Nickel, Zinc and Sulfate provide acceptable levels of ecosystem protection for Upper Goulburn River waters.

8.7 Comparison of SSGVs and ANZG (2018) Default Guidelines with current MCO Controlled Water Release Criteria

SW12 (UCML SW01/SW12) 80th percentile values, which reflect mostly filtered metal concentrations (natural concentrations) together with sulfate/salinity/EC concentrations in the upper Goulburn River. Typically metal values remain below standard default metal guideline values (DGVs) in ANZG (2018). Filtered aluminium however remains naturally elevated with 80th percentile values for UCML SW01/SW12 80th percentile value at 126 µg/L (DGV: 55 µg/L). The potential effect of aluminium toxicity on aquatic species is offset by the relatively high concurrent concentrations of free silica and dissolved organic carbon (Section 8.3.7). Water hardness can reduce the toxicity of metals (Cd, Ni, Pb, Zn) to aquatic species and demonstrates greater allowances of metal concentrations in an aquatic ecosystem arising from a reduction in labile (bioavailable) concentration (Figure 6; Table 45). Hardness corrected trigger values are based on the Warne et al. (2018) algorithm that follows Markich et al (2001). Note that ANZG (2018) described in Warne et al. (2018) disallows hardness correction for filtered copper concentration, showing that filtered copper concentration has no buffer from hardness in freshwaters. SSGVs developed in this report, are derived from toxicity dosed test responses of three analytes (Ni, Zn, SO_4) for eight aquatic species in UCML SW01 / SW12 diluent control water and provide the most relevant and accurate boundary to which aquatic species (95% level of protection to aquatic species) may begin to experience toxic effects (EC/IC10) in upper Goulburn River waters. Although water hardness is elevated in the Goulburn River, the SSGV aquatic toxicity tests demonstrate that aquatic species in the upper Goulburn are likely to remain sensitive to nickel at a SSGV of 9.8 µg/L compared to an ANZG (2018) DGV of 11 µg/L and hardness corrected value of 40 µg/L. Note that the confidence interval of the SSGV for nickel (5.9-25.8 µg/L) overlaps the ANZG (2018) DGV guideline value of 11 µg/L. Toxicity tests also show however, that aquatic species in the upper Goulburn River are considerably less sensitive to Zinc with an SSGV 77 (65 - 112) µg/L compared to an ANZG (2018) DGV of 8 µg/L and a hardness corrected value of 29 µg/L. SSGV results based on direct toxicity measurements emphasize that site specific evaluation is always more accurate.

Electrical conductivity (µS/cm) SSGVs are based on a regression model of measured conductivity values during sulfate dosed toxicity tests (Section 8.5). Salinity values were calculated by utilising a salinity factor, k (Equation 1; TDS/EC ratio) determined for UCML SW01 / SW12 waters used in ecotoxicity testing, from estimated SSGV for electrical conductivity . Toxicity values for salinity 2061 (1748-2624) mg/L (and/or electrical conductivity values, 2649 [2000-3816] µS/cm) from the linear regression using Equation 5 in Section 8.5 are much higher than limits set by NSW EPA 685 µS/cm (493 mg/L). Toxicity values are also much higher than background concentration 619 µS/cm and 400 mg/L.

In Table 45, the MCO metal criteria in controlled release waters show the current WTP limits for metals that Moolarben has set to be removed from WTP Filtrate and for WTP Discharge waters. For nickel, zinc, and EC values these are set to ANZG (2018) and NSW EPA guideline values (nickel 11 µg/L, zinc 8 µg/L and electrical conductivity 685 µS/cm). Comparison with the SSGVs shows that the MCO metal criteria is conservative for filtered zinc and sulfate concentrations in Goulburn River, but restricted for filtered nickel concentration.

Table 45: Comparison of UCML SW01 / SW12 80th percentile values for heavy metals and sulfate/salinity/EC in upper Goulburn River, Guideline Default Guideline Trigger Value (DGV) with and without Hardness Modified Trigger Values and SSGV with 95% level of protection for aquatic species (95% CI) with MCO (2017) Metal Design Criteria in Controlled Release Water.

Parameter	Units	n	UCML SW01/SW12 80th percentile Value (2005/2013-2021)	ANZG (2018) Default Guideline Trigger Value (DGV)	ANZG (2018) Guideline Hardness Modified Trigger Values[a] (HMDGV)	SSGV with 95% level of protection for aquatic species (95% CI)	MCO (2017) Metal Criteria in Controlled Release Water
Aluminium (Filtered)	µg/L	73	126	55	-	-	140
Manganese (Filtered)	mg/L	75	0.4	1.9	-	-	1.9
Nickel (Filtered)	µg/L	75	1	11	40.3	9.8 (5.9 - 25.8)	11
Zinc (Filtered)	µg/L	75	6	8	29.3	77 (65 - 112)	8
Cadmium (Total)	µg/L	58	0.05	0.2	0.78	-	0.2
Copper (Filtered)	µg/L	75	0.5	1.4	-	-	1.4
Arsenic (Total)	µg/L	58	0.8	13	-	-	13
Lead (Filtered)	µg/L	75	0.5	3.4	23.6	-	3.4
Sulfate	mg/L	72	68	-	-	819 (544 - 1314)	-
Electrical Conductivity	µS/cm	234	619	685[b]	-	2649 (2000-3816)	685
Salinity (TDS)	mg/L	271	400	493	-	1920 (1450-2766)	-

Note: a Hardness modified trigger values are based on Warne et al. (2018) hardness-dependent algorithm and follows Markich et al (2001). Hardness modified DGVs are corrected to UCML 01 and SW12, 80th percentile hardness value of 138 mg/L $CaCO_3$.
b NSW EPA discharge limit value (EPL 1)

9 Conclusions

Conditions 32A(b) and 32A(c) of Project Approval (05_0117) specify monitoring locations to be used to determine background salinity levels and heavy metals are to be within the "Goulburn River" and "upstream of the project site" (Moolarben Discharge Point). It was determined that the only section of the Goulburn River that is indicative of background heavy metal and salinity levels and satisfies the constraints of Condition 32A Project Approval (05_0117) is the location represented by UCML SW01 (and other co-located sites).

The site UCML SW01 / SW12 can be considered appropriate for background concentrations of the upstream Goulburn River, however, would not be directly representative of background concentrations (that is, concentrations with no influence from mine discharge) within the Goulburn River due to contributions such as the diversion and other tributaries.

UCML SW01 / SW12 was endorsed by the New South Wales Environment Protection Authority (NSW EPA) via correspondence received 22 May 2020 as required by Condition 32A(b).

In order to determine the recommended ecotoxicity limit, a "multiple lines of evidence" approach was undertaken as per ANZG (2018). A key line of evidence explored included direct ecotoxicity testing of eight aquatic species across ten tests to measure ecotoxicity responses to dosed analytes in diluent control water sampled at the NSW EPA-endorsed monitoring location UCML SW01 / SW12. Plant treatment waters (WTP Filtrate, WTP Permeate, and WTP Discharge) were included as additional controls in the ecotoxicity testing. Ecotoxicity testing was undertaken by Ecotox Services Australia who supplied seven of the relevant aquatic test species. The eighth test species, Mayfly (*Nousia sp.* AV1. [*Leptophlebiidae*]) was locally sourced from Ryans Creek and transported to Ecotox Services Australia for testing, as recommended by the NSW EPA in correspondence dated 22 May 2020.

UCML SW01 / SW12, WTP Filtrate and WTP Discharge samples (100% of each) were also tested, but demonstrated no statistically significant toxicity on any aquatic test species. WTP Filtrate is WTP Ultrafiltration output and WTP Discharge is WTP output water at MCO. WTP Filtrate water quality was above historical 80 percentile values, demonstrating a 'worst-case' input concentrations into the RO process. It was anticipated that test species would show a response to WTP Filtrate waters and enable derivation of a SSGV for EC based on WTP filtrate, however upon further investigation, high concentrations of hardness and alkalinity in WTP input waters (WTP Filtrate) has influenced metal speciation to an extent which reduces metal toxicity (Section 8.1 - 8.3). The complexation that occurs in the selected analytes was confirmed using aquatic speciation modelling software Visual MINTEQ 3.0.

In accordance with ANZG (2018), the SSGVs derived for selected analytes nickel, zinc and sulfate are specific to the aquatic ecosystem of the Goulburn River and ultimately reflect what species can tolerate based on local river water chemistry (Table 39 and Section 8.4). Selected analytes were examined due to being identified as the most relevant analytes in WTP Filtrate treatment water and for their significance to appropriately protect the aquatic ecosystem.

Aquatic species in the Goulburn River are likely to be sensitive to the fluctuations in nickel concentrations, if the SSGV value of 9.8 µg/L and its confidence interval (5.9-25.8 µg/L) for 95% protection level of aquatic species is exceeded. The confidence interval (5.9-25.8 µg/L) further demonstrates the statistical range of concentrations that are acceptable for nickel. The hardness and alkalinity effect in the local river water (UCML SW01 / SW12- control water) influences the toxicity of analytes to test species. The complexation that occurs with zinc in UCML SW01 / SW12 reduces the toxicity to test species, allowing for an SSGV of 77 µg/L with a confidence interval of (65-112 µg/L). SSGV derived for sulfate is 819 mg/L (544-1314) demonstrating reduced sensitivity of this analyte in comparison to heavy metals (nickel and zinc), to aquatic species in the Goulburn River. SSGVs demonstrate that the analytes which are metals (nickel and zinc) are more toxic to aquatic species than sulfate (as a component of salinity) in the Goulburn River.

As a salinity SSGV was not able to be developed based on responses to WTP filtrate sulfate as sodium sulfate was selected as an analogue for salinity. Electrical conductivity can be used to express salinity in units of µS/cm as it equates with total dissolved salts (mg/L). The current EPA approved EPL release criteria for MCO is the 100th percentile for EC at 685 µS/cm, which is equivalent to 493 mg/L as salinity (using a salinity factor k of 0.73). Sulfate not only represents a component of salinity, but based on the natural conditions of the Goulburn River and WTP Discharge salinity characteristics (Section 7.8), sulfate is in fact a significant component in these river and treatment waters. Using sodium sulfate as an analogue, an SSGV value for salinity is predicted to be 1211 mg/L. A salinity factor k of 0.73 was calculated from TDS/EC ratios obtained from ALS data analysis of UCML SW01 / SW12 diluent control water sample utilized for ecotoxicity testing. This salinity factor overlaps with salinity factor ranges obtained for the Goulburn River (mean range for SW01, SW02, SW05, SW06, SW08 and SW12, [k 0.6 – 1.03], Section 6.3.6). Using a salinity factor value of 0.73, a conductivity value of 1670 µs/cm can be calculated from a TDS value of 1211 mg/L (Section 8.5). This demonstrate that aquatic species are less sensitive to salinity (as sodium sulfate) compared with nickel and zinc.

As an additional line of evidence, a more robust, direct method of converting salinity to EC was also examined using linear regression. Measured EC (µS/cm) data (y) were plotted against sulfate concentration (x) retrieved as direct measurements from ecotoxicity testing of dosed sulfate tests in SW12 diluent control waters. A linear regression equation (5) was derived as:

$$y = 2.3585x + 717.41$$

with $R^2 = 0.9988$ and $P<0.001$.

Using the derived equation, the sulfate SSGV generated from ecotoxicity testing (Section 8.4, Chart 10) can be estimated as an EC (with 95% confidence interval) SSGV of 2649 (2000-3816) µS/cm with a TDS calculated equivalent of 1920 (95% confidence interval: 1450-2766) mg/L).

Utilising the basic salinity factor method described above, 80th percentile values for background EC were determined for a number of monitoring sites on the Goulburn River and nearby tributaries, expressed with 95% CI: 619 (606-631) to 890 (872-908) µS/cm (excluding SW08 and SW06 outliers). As expected, background EC values measured in the Goulburn River sites are much lower than the toxicity threshold estimated for salinity values. Although the direct ecotoxicity testing calculated an SSGV for EC for 95% aquatic species protection of 2649 µS/cm, background concentrations were also considered with the proposed recommendations detailed below, following the guidance per ANZG (2018).

9.1 Background Salinity And Heavy Metals

Condition 32A(c) – *The study must determine appropriate background salinity and heavy metal levels for the Goulburn River upstream of the project site.*

The background concentrations (retrieved from upstream reference site UCML SW01 / SW12) were determined to be:

Salinity: 400 mg/L (equivalent to 619 µS/cm)
Aluminium: 126 µg/L
Manganese: 397 µg/L
Nickel: 1.0 µg/L
Zinc: 6.0 µg/L
Cadmium: 0.05 µg/L
Copper: 0.5 µg/L
Arsenic: 0.5 µg/L
Lead: 0.5 µg/L

10 Recommendations

10.1 EC Limit For Discharge

Condition 32A(d) – *The study must recommend an electrical conductivity limit for treated water discharges to the Goulburn River from the Moolarben Coal Complex based on the process outlined in the ANZECC Guidelines.*

Based on multiple lines of evidence (including site-specific ecotoxicity studies, background concentrations, no apparent toxicity response to treatment waters and detailed salinity conversion factor considerations), the current NSW EPA limit (EPL Site 1) for EC of 685 µS/cm is considered acceptable, and given the lack of toxicity responses to salinity, a limit that reflects the historical EC limit at the MCC, as well as the current EC limit at Ulan, of 900 µS/cm could also be considered acceptable.

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12 Appendices

12.1 Appendix A

Summary of test species suitability.

Freshwater Cladoceran, *Ceriodaphnia dubia*

The *Ceriodaphnia* cf. *dubia* freshwater cladoceran (water flea) 48 h acute survival test is a very common species used tests to assess the potential effects of a toxicant to freshwater aquatic ecosystems. The test (ESA 2016a, 2017) is based on, and modified from, a USEPA protocol (USEPA, 2002a) and has been used routinely in Australia for assessing the toxicity of mine waters (Bailey et al., 2000a). Cladocerans have an important ecological niche as a primary consumer. C. cf. dubia has a very broad global distribution, including records from Australia, Brazil, South Africa, Norway, Kenya, Indonesia and New Zealand (Sharma, 2014). The taxonomy of C. cf. dubia has been unclear with more likely species than are currently recognised, and past identifications and distribution records outdated (Greenwood et al., 1991).

The test organisms were cultured at Ecotox Services Australia laboratory at Lane Cove, NSW.

Table A.1. Strengths and weaknesses of the cladoceran (C. cf. dubia) toxicity test.

Strengths	Weaknesses	Opportunities
Found in a variety of aquatic habitats.	Very broad distribution indicates broad tolerances.	Typical for lacustrine microcrustacea species, such as cladocera which are often excluded from macroinvertebrate survey analyses .
Chronic test available.	Unclear taxonomy.	
Provides a test for a primary consumer trophic level species.		
Well-established toxicity-testing species.	Relevant to summer and winter seasons.	
Provides a test for a primary consumer trophic level species	Cladocera are excluded from AusRivAS counts so it is unclear of their presence in the river (Smith et al., 1999).	May be present in the Goulburn River catchment.

Method – Immobilisation test

The test is based on, and modified from, a USEPA protocol (Bailey et al., 2000a; USEPA, 2002a). Laboratory cultured juvenile C. cf. dubia were exposed to a dilution series of the test toxicant for 48 h. At the end of the exposure, the number of surviving C. cf. dubia was counted. Statistical analyses were then applied to determine the concentration that caused a percentage of mortality to the test population (e.g., EC_{50}). The C. cf. dubia acute toxicity test has been demonstrated to be sensitive to heavy metals, organics and pesticides and has been used routinely in Australia for assessing the toxicity of sewage effluents, mine tailings and pulp/paper mill effluents e.g., Bailey et al. (2000b).

Method – Reproduction impairment test

This method measures chronic toxicity to C. cf. dubia, using less than 24 h old neonates during a three-brood (seven-day), static renewal test. The test began with asexually reproducing female freshwater C. cf. dubia that were less than six hours old i.e., neonates. These neonate females were exposed to a dilution series of the test substance, an effluent or reference toxicant under 'static-renewal' conditions. These females were transferred daily to fresh solutions of the same concentration. Each day, observations were made on the survival of each female, the number of neonates produced and neonate survival. Each female was accounted for as alive, dead or missing, rather than assuming missing animals were dead. The test was terminated when three broods were produced by each surviving control female (normally over a 5–7 day period). The method is based on the Ceriodaphnia Survival and Reproduction Test developed by the USEPA (USEPA, 2002b).

Freshwater Aquatic Duckweed, *Lemna disperma*

The proposed macrophyte species is a small floating endemic *Lemna disperma* Hegelm. (*Bot. Jahrb.* **21**: 290 (1896)) is known as Common Duckweed (Conn, B.J., 1994).

Lemna disperma Hegelm (Spathiflorae, Lemnaceae) is described as a Root single Thallus floating on water surface, elliptic to rounded or broadly obovate, 1–4 mm long, slightly swollen, green above, if red underneath then reddening beginning from margin, opaque; air spaces in 2 or 3 layers spread throughout thallus, with largest ones mostly longer than 0.3 mm long; veins 3–5, emerging from same point at node; base with stalk indistinct; apex rounded. Colony made up of generations at angles less than 90° to the previous one. (vicflora.rbg.vic.gov.au). This macrophyte has a broad distribution throughout temperate regions of Australia and propagates both by seed and vegetatively by frond production and eventual separation (Allison, H. E. and Holdway, D. A. (1989a), Mannion, 1990; Mazzeo et al., 1998; Sainty and Jacobs, 2003). However, asexual reproduction is the prevalent mode of reproduction for many aquatic macrophyte taxa (Taraldsen & Norberg-King, 1990) and they often formats on the surface of still waters (Sainty and Jacobs, 2003). Both the Organisation for Economic Co-operation and Development (OECD) (OECD, 2006) and United States Environmental Protection Agency (USEPA and OCSPP, 2012) have published guidelines describing toxicity testing using con-specifics of L. gibba or L. minor as test organisms. Asexual reproduction is the prevalent mode of reproduction for many aquatic macrophyte taxa (Taraldsen and Norberg-King, 1990) and the small size and rapid reproduction of this simple plant makes it an ideal laboratory macrophyte test species (Riethmuller et al., 2003b).

Table A.2. Strengths and weaknesses of the duckweed (*L. disperma*) toxicity test.

Strengths	Weaknesses	Opportunities
Source of food for water fowl, fish and small invertebrates.	Very broad distribution indicates broad tolerances.	Field surveys usually show widespread presence and allow distribution of this species.
Small size and easily measured and rapid reproduction.	Is ecologically relevant test organisms to the Goulburn River.	Opportunity to complete field survey to determine presence of this particular genus and species in the Goulburn River.
Other sub-lethal effects, such as loss of motility and flaccidity, can also be observed as a sub-lethal test response (McCullough, 2006).		
Chronic test available.		

The 7-day Growth inhibition of the freshwater aquatic duckweed Lemna Disperma follows the ESA Laboratory culture Test Protocol: ESA SOP 112 (ESA 2016b) and is based on ASTM (2012)

Method – Reproduction impairment test

Based upon an OECD method (OECD, 2006), a standard number of vegetatively reproducing Lemna plants were exposed to dilution series of the test toxicant over 96 h under controlled conditions. The number of fronds is a measure of clonal reproduction rate was counted at the end of the test and from this, the degree of plant growth was calculated and compared with an appropriate control to determine the percentage inhibition of growth for each treatment.

Eastern Rainbowfish, *Melanotaenia splendida splendida*

Melanotaenia splendida inornata (Castelnau) Eastern rainbowfish (*Melanotaenia splendida inornata*) are of a diverse family with two other con-generics that inhabit a range of conditions from rapidly flowing streams to lakes and swamps across drainages of northern Australia Territory and Queensland draining into the Gulf of Carpentaria (Allen, 1980). These fish are often very abundant in both flowing and pool conditions of rivers and creek systems and form an important part of the diet of aquatic predators; including piscivorous fishes, mammals, reptiles and waterfowl (Allen et al., 2002). *Melanotaenia splendida* spp. are not identified to sub-species in survey reporting from the Goulburn River. However, Melanotaenia splendida spp. are the most commonly found fish in Australian catchments draining to the Pacific Ocean. The rainbowfish used in this test used Rainbowfish embryo hatching test using *Melanotaenia splendida splendida* were cultured in the laboratory until required for testing.

The 96-hr fish imbalance toxicity test using the eastern rainbowfish *Melanotaenia splendida splendida* follows the ESA Test Protocol: ESA SOP 117 (ESA 2016c), based on USEPA (2002a), but adapted for use with native rainbowfish.

Table A.3 Strengths and weaknesses of the eastern rainbowfish (M. s. splendida) toxicity test.

Strengths	Weaknesses	Opportunities
Found in a variety of aquatic habitats in northern Australia.	Sub-species may not be specifically from the Goulburn River catchment.	May be able to identify specimens from the Goulburn River to subspecies level.
Found in a variety of aquatic habitats in Australia.	Is ecologically relevant test organisms to the Goulburn River.	Opportunity to complete field survey to determine presence of this particular genus and species in the Goulburn River.
Chronic test available		

Method – Imbalance test

The acute fish imbalance toxicity test involves exposing M. s. splendida larvae to the test material for 96 h. At the end of the exposure period, the number of balanced and the number of un-balanced fish larvae are recorded. Method – Embryo hatch survival test The chronic toxicity test using M. s. splendida was developed to fulfil a need to provide chronic ecotoxicity test data for use with SSDs under the ANZECC/ARMCANZ Water Quality Guidelines (2000). In the embryo hatch survival test, the number of embryos successfully emerged at around Day 6 and the number of surviving larval fish at Day 12 are recorded.

Freshwater Hydra, Hydra viridissima (Pilbara isolate)

The green hydra (*Hydra viridissima*) is a passive carnivore that contains a symbiotic green alga in the gastrodermal cells of the animal which helps supply nutrient requirements (Pardy and White, 1977).

Otherwise, they are strictly carnivorous, feeding mainly on small crustaceans, including Cladocera and Copepoda, insects and annelids. When hydras are exposed to toxic substances, they may undergo graded changes in their body structure, which can be used as indicative of the degree of toxicity to be assayed at lethal or sublethal concentrations. Moreover, these organisms are present in a variety of freshwater environments, are easy to culture and maintain in the laboratory, reproduce rapidly and can be used in simple toxicity tests (Trottier et al., 1997). Two species of Hydra (Cnidaria, Hydrozoa) occur ubiquitously in the surface waters of the Northern Territory; the cosmopolitan green Hydra ("Hydra" H. viridissima syn. H. littoralis) (Brereton, 1956; Williams, 1980) with its symbiotic green alga, and the pink Hydra (Hydra vulgaris) (Bale, 1919). Although both species have been used in ecotoxicity testing of mine waters H. viridissima has been found to be more sensitive to mine waters (Allison and Holdway, 1989b; McCullough, 2006; McCullough et al., 2008). Hydra viridissima was first collected from Bowerbird Billabong in Kakadu National Park (Riethmuller et al., 2003a) and has been used for bioassays on the Ranger Uranium Mine since 1987 (McBride et al., 1988; Allison and Holdway, 1989b).

The 96-hr acute toxicity test was conducted using the freshwater hydra *Hydra viridissima* (Pilbara isolate) ESA Laboratory culture using the Test Protocol: ESA SOP 125 (ESA, 2016d), based on Riethmuller et al. (2003b).

Table A.4. Strengths and weaknesses of the hydra (H. viridissima) toxicity test.

Strengths	Weaknesses	Opportunities
Hydra are considered important predators in aquatic systems although the overall effect of these small invertebrates in aquatic systems has yet to be thoroughly studied (Elliott et al., 1997).	Hydras, the most representative freshwater Cnidaria, are of common occurrence in bodies of water in every continent except Antarctica.	No survey data from the Goulburn River, although this is typical for poorly preserved Cnidaria. Opportunity to complete field survey to determine presence in the Goulburn River.
Found in a variety of aquatic habitats in Australia. Chronic test available. Chronic test available.	Cnidaria may be important to Goulburn River aquatic ecosystems.	Opportunity to complete field survey to determine presence of this particular genus and species in the Goulburn River.
Other sub-lethal effects, such as loss of motility and flaccidity, can also be observed as a sub-lethal test response (McCullough, 2006).		Chronic test available

Method – Reproduction impairment test

Asexually reproducing (budding) test H. viridissima were exposed to a dilution series of the test toxicant for 96 h. Although below the definition threshold of ≥7 d for temperate species, the 96 h test is at tropical temperatures and therefore represents a relatively greater life-cycle exposure period than the recommended 7 d (Batley et al., 2018). Observations of any changes to the hydra population (i.e. changes in the number of intact hydroids, where one hydroid equals one animal plus any attached buds) were recorded at 24 h intervals. The method is based on the hydra population growth test described by Hyne et al. (1996) and Riethmuller et al. (2003b).

Green Alga, *Raphidocelis subcapitata*

Unicellular algae were first used in toxicity tests in eutrophication assessment (USEPA, 1971). Being broadly sensitive to many toxicants and with a short generation time, unicellular algal tests readily conform to the requirements of short-term chronic tests and are typically highly reproducible thus forming a fundamental part of a bioassay analysis suite (Stauber, 1995).

72-hr microalgal growth inhibition test using the green alga *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum) using the* **Test Protocol:** ESA SOP 103 (ESA 2016e), based on USEPA (2002b).

The test organisms cultured at Ecotox services Australia laboratory at Lane Cove, NSW were originally sourced from CSIRO Microalgal Supply Service, Hobart, Tasmania.

Table A.5. Strengths and weaknesses of the green alga (*Raphidocelis subcapitata*) toxicity test.

Strengths	Weaknesses	Opportunities
Found in a variety of aquatic habitats.	Very broad distribution indicates broad tolerances.	May be present in the Goulburn River catchment.
Chronic test available.		
Well-established toxicity-testing species.		
Provides a test for a primary consumer trophic level species.		
Well-established toxicity-testing species.		
Provides a test for a primary consumer trophic level species		

Method –Reproduction impairment test

Exponentially growing cells of *Raphidocelis subcapitata* were exposed to dilution series of the test toxicant over several generations over 72 h with cell counts undertaken at both 48 and 72 h. From these counts, cell division rates were calculated. A test substance was considered toxic when a statistically significant ($P \leq 0.05$) concentration-dependent inhibition of algal growth occurred (Franklin et al., 1998).

Freshwater Chironomid, *Chironomus tepperi*

Chironomus tepperi (Skuse) Chironomidae in freshwater ecotoxicology Chironomids, or “non-biting midges” (Diptera: Chironomidae), are a species-rich aquatic insect family. They inhabit almost every type of freshwater habitat on earth and are ecologically important, particularly as prey items (Armitage et al., 1995) with species varying in their tolerance to pollution (Pettigrove and Hoffmann, 2005). C. tepperi is distributed across subtropical and tropical regions of both hemispheres of the whole world, including through inadvertent human dispersion though rice cultivation (Mehler et al., 2017). They are found across mainland Australia including South Australia and NSW in temporary ponds (Stevens et al., 2006). Chironomus tepperi is frequently used in Australia as part of a TIE for evaluating sediment toxicity. Endpoints commonly described in the literature for tests of this species include mortality, immobilisation, emergence (from pupal to adult stage) and growth. However, survival in sediment toxicity tests has been found to be an insensitive endpoint with concerns that they may not be environmentally relevant when tested for in this manner (Gagliardi, 2017).

The ESA Laboratory culture used 5 days old test organisms following Bailey et al., (2000a), ESA (2013), OECD (2011) and USEPA (2000a).

Table A.6 . Strengths and weaknesses of the freshwater midge (C. tepperi) toxicity test.

Strengths	Weaknesses	Opportunities
Aquatic insects are fundamental to the ecology of seasonally flowing tropical Australian rivers (Paltridge et al., 1997).	Not particularly sensitive to toxicants.	No survey data from the Goulburn River, although genus very common and species possible to occur.
Genus found in a variety of aquatic habitats in Australia.		Opportunity to complete field survey to determine presence of this particular genus and species in the Goulburn River.
Provides a test for a primary consumer that is not an obligate herbivore.		New chronic test now available

Method – Pupal survival and emergence test

The test protocol is an ESA custom test based upon OECD (2011) and Bailey et al. (2000a). Test volumes were 200 mL in 250 mL squat-form borosilicate glass beakers. Five newly hatched larvae (24–48 h post hatch) were introduced into each replicate beaker. Test solutions were renewed three times per week over the 14 d exposure period. Larvae were fed at each renewal by adding approximately 200 mg per beaker of powdered Sera Vipan fish-food flake. At day 10, approximately 1 g of boiled paper tissue was introduced to each beaker ahead of pupation. At 14 d, the test was terminated by counting the number of pupa and emerged midges.

48-hr acute toxicity test using the freshwater chironomid Chironomus Tepperi were undertaken by following the Test Protocol: ESA SOP 121 (ESA 2013), based on OECD (2011), USEPA (2002) and Bailey et al. (2000a).

Freshwater Prawn, *Macrobrachium australiense*

Macrobrachium australiense (Holthuis) is one of 13 species of Macrobrachium currently recognised within Australian waterways, five of which are considered endemic (Short, 2004). Macrobrachium spinipes (cf. M. rosenbergii) is the name currently assigned to the local Cherabin, which is a poorly understood but not endangered freshwater prawn (De Grave et al., 2013). Macrobrachium spp. have been recorded l at monitoring sites in the Goulburn River. As an omnivore, Macrobrachium sp are likely to represent a variety of trophic levels through feeding preference changes over its life-history in the Upper Goulburn River and its tributaries. Equally, Macrobrachium spp. are a favoured dietary component of many fish species recorded within the Goulburn River catchment.

Table A.7. Strengths and weaknesses of the freshwater prawn (M. australiense) toxicity test.

Strengths	Weaknesses	Opportunities
Ecologically relevant test species for the Goulburn River. Omnivore.	Broad distribution indicates broad tolerances.	Potential to collect and use locally sourced stock. Chronic test available.

Strengths	Weaknesses	Opportunities
		Provides a test for a top-trophic feeding species.
Few other secondary consumer tests available.	Is ecologically relevant test organisms to the Goulburn River.	Opportunity to complete field survey to determine presence of this particular genus and species in the Goulburn River.

Method – Juvenile growth and survival

The test protocol is an ESA custom test (ESA, 2016f) SOP 123 –Acute Toxicity Test Using Freshwater Shrimp based on USEPA (2002a). Test volumes were 80 mL in 100 mL squat-form borosilicate glass beakers. Five juvenile (2–4mm) were introduced into each replicate beaker. Test solutions were renewed three times per week over the 14 d exposure period. Juvenile shrimp were fed each day by adding one pellet of Sera Shrimps Natural Sinking Granules per beaker. At 14 d, the test was terminated by counting the number surviving juveniles and measuring length.

Mayfly Larval, *Nousia sp.* AV1 *(Leptophlebiidae)*

The phylogeny of Australian Leptophlebiidae has not been studied in detail although previous research has indicated close relationships with the Neotropical fauna. Phylogenetic relationships of Australian Leptophlebiidae were examined using a cladistic analysis of 34 morphological characters and 21 genera. Character polarity was assessed by out-group comparison and then analysed with NONA (version 2). The three most parsimonious trees produced were used to construct a strict consensus tree. Relationships among the Australian fauna specify some monophyletic groups and unresolved terminals. This study elucidates some previously unknown relationships among the Australian fauna and indicates that the currently recognised genera of Leptophlebiidae in Australia require further definition. Comparison with hypotheses previously proposed for Australian Leptophlebiidae demonstrate partial agreement, recognizing 'Meridialaris' and 'Atalonella' clades (sensu Pescador and Peters 1980a) and support for a sister relationship between the burrowing genera Jappa and Ulmerophlebia. Cladistic characters are tabulated and discussed with illustrations. Examined taxa, materials and comprehensive bibliographic sources are provided.

Parnong and Campbell (1997) identified two new species of Austrophlebioides Campbell and Suter, A. marchanti sp.nov. from Victoria, and A. booloumbi sp.no\. from southeastern Queensland, a necessitating a slight modification to the generic definition. A. decipiens (Harker) comb.n. is transferred to Austrophlebioides from Deleatidium. *Austrophlebioides* Campbell and Suter was described to include the species A, pusillus (Harker) and *A. unguicularis* (Ulmer). The literature showed that Deleatidium decipiens Harker should also be included in the genus Austrophlebioides based on the distinctive morphology of the genitalia, and was formally transferred. The description of A, marchanti in the genus required some modifications of the generic diagnosis. The series of fine ventral spines present on the penes, noted as a generic characteristic in the original description of Austrophlebioides is absent in A. marchanti, and was considered to be a specific rather than a generic characteristic. In addition, the fore wings of some specimens of both A, marchanti and A, booloumbi have vein ICu detached from the crossvein which attaches CuA to CuP in contrast to the original generic diagnosis.

Austrophlebioides Campbell and Suter was described to include the species A. pusillus (Harker) and A. unguicularis (Ulmer). It is clear from the literature that Deleatidium decipiens Harker should also be included in the genus Austrophlebioides based on the distinctive morphology of the genitalia, and we thus formally transfer it. The description of A. marchanti in the genus requires some modifications of the generic diagnosis. The series of fine ventral spines present on the penes, noted as a generic characteristics in the original description of Austrophlebioides is absent in A marchanti, and we consider it to be a specific rather than a generic characteristic. In addition the fore wings of some specimens of both A marchanti and A. booloumbi have vein ICu detached from the crossvein which attaches CuA to CuP in contrast to the original generic diagnosis.

Ecology: Instream habitat: Leptophlebiid nymphs generally occur in fast flowing upland streams but also live in meandering lowland rivers, lakes and reservoirs and artificial dams in semi-arid areas. Nymphs are found in log crevices, amongst macrophytes and debris, also on or under cobbles and bedrock. Only Atalophlebia australasica is common in small, temporary streams, some of which are completely dry for more than half of the year.

Feeding ecology: Leptophlebiidae species are detritivores or herbivores. Most nymphs scrape the surface of gravel, rocks and logs feeding on algae and detritus. However, Atalomicria has very long maxillary palps with brushes for collecting detritus and nymphs of Neboissophlebia and Ulmerophlebia shred leaf accumulations.

Habit: Nymphs appear to be mostly nocturnal. Flattened bodies enable the nymphs to cling to substrata and resist the force of fast flowing waters. Deleatidium and Kirrara nymphs also have broad gills that form a suction cup around the abdomen to help cling to rocks. Jappa and Ulmerophlebia are burrowing nymphs but only Jappa has a pair of horns projecting forwards from its head to aid in burrowing. Atalophlebia has feathery gills that increase the available surface area to extract oxygen from slow flowing waters.

Life history: In North America, the female dips her abdomen into the water, releasing a few eggs at a time. Oviposition is usually completed within five minutes, during daylight. In Britain, eggs can be produced parthogenetically. The length of the egg phase is temperature dependant. Smaller mature larvae have fewer instars than larger mature larvae and the number of instars is not constant, ranging from ten to fifty. Over wintering can take place in either the egg phase or larval phase. For Australian species, final instar nymphs crawl out of the water before ecdysis. There are two adult stages; a sub-imago, which has incompletely developed reproductive structures, that usually lasts for 24 hours, and then an imago with developed reproductive structures. Adults only live for a few days. Leptophlebiidae species are univoltine or bivoltine but may have multiple cohorts present at one time. Adults of some species emerge intermittently throughout the year with a poorly synchronised life cycle while for other species adult emergence is confined to a period of 3 months, usually in summer.

Information Sources: Dean 1999a, Dean 2011, Peters and Campbell 1991, Gooderham and Tsyrlin 2002, Dean 2000a, Dean and Suter 1996, 2004, Merritt and Cummins 1996, Elliott et al. 1988, Christidis and Dean 2005

https://www.mdfrc.org.au/bugguide/display.asp?type=6&class=17&subclass=&Order=6&family=45&genus=127&couplet=0

Dowse et al., (2017) showed that salinity causes mortality in the Mayfly *A. pusillus*, but a breakdown in osmoregulation did not precede . Furthermore, mortality of 99% of the population occurred at an external salinity which was considerably less than the haemolymph osmolality. The research demonstrated that a diversity of osmoregulatory responses to increasing salinity.

Austrophlebioides pusillus nymphs were collected by Dowse et al., (2017) from the Hunter River at Moonan Flat, New South Wales (NSW), Australia (S 31°55′529′′ E 151°14′235′′). Mean salinity measured as electrical conductivity (EC) was 0.211 mS cm−1 (±0.085 s.d.), range 0.123–0.314 mS cm^{-1}, n = 5) at 25°C.

All tests in this study were undertaken using Ecotox Services Australia laboratory cultures as broodstock, except for Nousia sp.AV1 (Leptophlebiidae) (Appendix A) which used a test organism directly sourced from a sub-catchment of the Goulburn River catchment (described below). This followed the directive from NSW EPA that MCO needed to use Mayfly as a test aquatic species sourced from the Upper Goulburn River. Mayflies were collected from the wild at Ryans Creek, placed in trays which could be aerated during transfer to the test at Ecotox Services Laboratory and delivered for use within 24 h. The field collecting was undertaken 22-24 June 2021.
Historical data (Table 11) for Mayfly numbers in the upper catchment of the Goulburn River and sub-catchments have been identified as one of the following three species (Section 7.5; Figure 9): Baetidae, Caenidae or Leptophlebiidae (MCO, 2018).

Mayfly Larval, *Nousia sp.*AV1 *(Leptophlebiidae)* which used a test organism directly sourced from a sub-catchment of the Goulburn River catchment . Mayflies have to be collected from the wild, placed in trays which can be aerated during transfer to the test in Laboratory and delivered for use within 24 hours. The field collecting was 22-24 June 2021. 96-hr acute toxicity test using the larval Mayfly Nousia sp. AV1 (Leptophlebiidae) Test Protocol: ESA SOP 123 (ESA 2016f) Field collected using dipnets from Ryans Creek, a tributary of Moolarben Creek. The specimens of the Mayfly larvae collected from Ryans Creek were identified by Jamie Devine, Sydney Water 14 July 2021 as Leptophlebiidae Nousia sp.AV1.

Formal recognition of Nousia sp.AV1 has so far not been undertaken so it is currently still named by its Australian Voucher number until further taxonomic investigation is completed, valid species may actually be being lumped together and separation is currently not possible. Further, in reference to sp.AV1, Dean (1999) states, ‘Sixteen Nousia species have been recognised, but it is probable that some are actually species complexes. In particular, the voucher species AV1 is a variable and widespread taxa which requires further investigation’.

Table A.8. Strengths and weaknesses of the Mayfly Larval, *Nousia sp.*AV1 *(Leptophlebiidae)* toxicity test.

Strengths	Weaknesses	Opportunities
Ecologically relevant test species for the Goulburn River. Source from sub-catchment of the Goulburn River	Broad distribution indicates broad tolerances.	Able to collect and use locally sourced stock. Provides a test for a lower-trophic feeding species.
Other species may be present.	Is ecologically relevant test organisms to the Goulburn River.	Field surveys have been undertaken to determine presence of this particular genus and species in the Goulburn River.

(18) (PDF) Phylogenetic Relationships of the Australian Leptophlebiidae. Available from: https://www.researchgate.net/publication/229151213_Phylogenetic_Relationships_of_the_Australian_Leptophlebiidae [accessed Oct 04 2021].

12.2 Appendix B

Analysis of bulk test waters for ecotoxicity testing

Samples of the bulk test waters for ecotoxicity testing were sent to ALS Environmental Laboratories for water quality analysis prior to dispatch to Ecotox Services Australia, Lane Cove NSW (see Appendices B.1 and B.2).

Table B.1. UCML SW01 / SW12 and WTP Filtrate grab samples collected June 24 2021 and analysed by ALS Environmental Laboratories .

Analyte	Units	Guideline (95%)	UCML SW01 / SW12 collected 24/6/2021				WTP Filtrate collected 24/6/2021			
			n	Mean	SD	SE	n	Mean	SD	SE
Ammonia as N (NH_3)	mg/L	0.9	3	0.03	<0.01	<0.01	3	0.22	0.03	0.02
Nitrite+ Nitrate as N (NOx)	mg/L	-	3	0.22	0.02	0.01	3	0.67	0.01	0.00
Total Kjeldahl Nitrogen as N (TN)	mg/L	-	3	0.63	0.06	0.03	3	0.30	0.00	0.00
Total Nitrogen as N (TN)	mg/L	-	3	0.83	0.06	0.03	3	1.00	0.00	0.00
Total Phosphorus as P (FTP)	mg/L	-	3	0.04	0.02	0.01	3	<0.01	<0.01	<0.01
Filtered Reactive Phosphorus as P (FTP)	mg/L	-	3	<0.01	<0.01	<0.01	3	<0.01	<0.01	<0.01
Dissolved organic carbon (DOC)	mg/L	-	3	9.00	1.00	0.58	3	<1	<1	<1
Hardness	mg/L ($CaCO_3$)	-	3	152.00	3.61	2.08	3	621.67	1.15	0.67
Electrical conductivity (EC)	µS/cm	-	3	783.00	4.36	2.52	3	2066.67	5.77	3.33
pH	pH unit	-	3	7.07	0.06	0.03	3	8.03	0.06	0.03
Colour (True)	PCU	-	3	46.67	2.89	1.67	3	<1	<1	<1
Total alkalinity	mg/L ($CaCO_3$)	-	3	46.33	1.15	0.67	3	153.33	11.59	6.69
$HCO3^-$	mg/L	-	3	46.33	1.15	0.67	3	153.33	11.59	6.69
CO_3^{2-}	mg/L	-	3	<1	<1	<1	3	<1	<1	<1
OH^-	mg/L	-	3	<1	<1	<1	3	<1	<1	<1

Analyte	Units	Guideline (95%)	UCML SW01 / SW12 collected 24/6/2021				WTP Filtrate collected 24/6/2021			
			n	Mean	SD	SE	n	Mean	SD	SE
Turbidity	NTO	-	3	21.63	0.35	0.20	3	<0.1	<0.1	<0.1
TSS	mg/L	-	3	6.00	1.00	0.58	3	<1	<1	<1
TDS	mg/L	-	3	567.67	3.21	1.86	3	1560.00	34.64	20.00
Calcium (filtered)	mg/L	-	3	25.67	0.58	0.33	3	125.33	0.58	0.33
Magnesium (filtered)	mg/L	-	3	21.33	0.58	0.33	3	75.00	0.00	0.00
Potassium (filtered)	mg/L	-	3	6.00	0.00	0.00	3	25.33	0.58	0.33
Sodium (filtered)	mg/L	-	3	87.00	1.00	0.58	3	202.00	1.00	0.58
Bromide	mg/L	-	3	0.40	0.00	0.00	3	0.30	0.00	0.00
Fluoride	mg/L	-	3	0.20	0.00	0.00	3	1.00	0.00	0.00
Iodide	mg/L	-	3	<0.05	<0.05	<0.05	3	<0.1	<0.1	<0.1
Chloride	mg/L	-	3	174.33	2.08	1.20	3	207.00	1.00	0.58
Sulfate	mg/L	-	3	78.33	0.58	0.33	3	662.00	6.56	3.79
Aluminium (total)	µg/L	55	3	373.33	404.15	233.33	3	<10	<10	<10
Aluminium (filtered)	µg/L		3	13.00	2.00	1.15	3	<5	<5	<5
Arsenic (total)	µg/L	24	3	<1	<1	<1	3	<1	<1	<1
Arsenic (filtered)	µg/L		3	0.43	0.06	0.03	3	0.30	0.00	0.00
Boron (total)	µg/L	940	3	<50	<50	<50	3	<50	<50	<50
Boron (filtered)	µg/L		3	11.33	0.58	0.33	3	31.67	1.15	0.67
Strontium (total)	µg/L	-	3	206.67	11.15	6.44	3	644.67	8.62	4.98
Strontium (filtered)	µg/L		3	216.67	2.52	1.45	3	621.67	2.08	1.20
Barium(total)	µg/L	-	3	26.33	2.52	1.45	3	69.67	4.51	2.60
Barium (filtered)	µg/L		3	25.00	0.40	0.23	3	65.87	0.40	0.23

Analyte	Units	Guideline (95%)	UCML SW01 / SW12 collected 24/6/2021				WTP Filtrate collected 24/6/2021			
			n	Mean	SD	SE	n	Mean	SD	SE
Cadmium(total)	µg/L	0.2	3	<0.1	<0.1	<0.1	3	<0.1	<0.1	<0.1
Cadmium (filtered)	µg/L		3	<0.05	<0.05	<0.05	3	<0.05	<0.05	<0.05
Cobalt (total)	µg/L	1.4	3	<1	<1	<1	3	10.67	0.58	0.33
Cobalt (filtered)	µg/L		3	0.20	0.00	0.00	3	11.87	0.21	0.12
Chromium(total)	µg/L	3.3	3	<1	<1	<1	3	<1	<1	<1
Chromium (filtered)	µg/L		3	0.23	0.06	0.03	3	0.30	0.14	0.08
Copper (total)	µg/L	1.4	3	<1	<1	<1	3	<1	<1	<1
Copper (filtered)	µg/L		3	<0.5	<0.5	<0.5	3	0.77	0.12	0.07
Iron (total)	µg/L	-	3	943.33	256.97	148.36	3	<50	<50	<50
Iron (filtered)	µg/L		3	118.67	4.93	2.85	3	3.00	1.00	0.58
Lithium(total)	µg/L	-	3	3.67	0.58	0.33	3	382.00	8.00	4.62
Lithium (filtered)	µg/L		3	3.13	0.23	0.13	3	359.00	9.00	5.20
Manganese (total)	µg/L	1900	3	59.00	1.00	0.58	3	1066.67	30.55	17.64
Manganese (filtered)	µg/L		3	42.27	0.15	0.09	3	980.67	17.62	10.17
Molybdenum (total)	µg/L	34	3	<1	<1	<1	3	4.00	0.00	0.00
Molybdenum (filtered)	µg/L		3	0.30	0.00	0.00	3	3.67	0.06	0.03
Nickel (total)	µg/L	11	3	1.00	<1	<1	3	47.33	1.15	0.67
Nickel (filtered)	µg/L		3	1.00	0.00	0.00	3	54.53	0.50	0.29
Lead (total)	µg/L	3.4	3	<1	<1	<1	3	<1	<1	<1
Lead (filtered)	µg/L		3	<0.1	<0.1	<0.1	3	<0.1	<0.1	<0.1
Zinc (total)	µg/L	8	3	<5	<5	<5	3	27.00	6.08	3.51
Zinc (filtered)	µg/L		3	1.33	0.58	0.33	3	25.67	0.58	0.33

Conversion of Electrical Conductivity to Salinity using conversion factors.

Determining the salinity (total dissolved solids or TDS in mg/L) for river and plant treatment waters from electrical conductivity (EC in µS/cm) requires deriving a conversion factor (F) for each water type. Equation (B1) demonstrates the relationship between salinity and electrical conductivity.

$$TDS = EC \times F$$

(B1)

Conversion Factors (F) were derived from grab sample data for UCML SW01 / SW12 (river water) and WTP Filtrate (plant treatment water) analysed by ALS Environmental in 24/06/2021. Table B.2 demonstrates the Conversion Factors for UCML SW01 / SW12 and WTP Filtrate, which are applied to other river waters and treatment waters, where appropriate.

Table B.2. Derived conversion factor (F) values for total dissolved solids (TDS) and electrical conductivity (EC) in bulk UCML SW01 / SW12 and WTP Filtrate samples collected 24 June 2021 and analysed by ALS Environmental. Laboratories.

ALS grab Sample 24/06/2021	TDS	EC	F
UCML SW01 / SW12	568	783	0.7249
WTP Filtrate	1560	2067	0.7548

Visual MinTEQ 3.0 software is a geochemical modelling program (Gustaffson, 2013) utilised in this report to define the speciation of the metal analytes nickel and zinc, which are examined in the ecotoxicity testing process (see Section 8.3).

The details in this part of the Appendix B describe the inputs used for each geochemical model run (mean, 80th percentile and maximum) (Table B.3.). Electrical conductivity and total dissolved solids are represented by individual cation and anion concentration inputs. Hardness is calculated in the program run from input of calcium, magnesium, and bicarbonate (from total alkalinity) concentration data. The rest of the parameters are direct inputs into the MinTEQ 3.0 software are listed in Table B.3. Goulburn River upstream background site (UCML 01/SW12), downstream Goulburn River site (SW01) and treatment water (WTP Filtrate) are examined. The different water quality characteristics of each influence the complexation and speciation of nickel and zinc, and provide insights into toxicity response of different water types or effects (e.g. from increasing concentrations of analytes in the same water quality to varying concentrations of alkalinity and hardness).

Table B.3. Parameters and input values in concentration units, pH and Eh (mV) for SW12, WTP Filtrate and SW01 geochemical modelling MinTEQ 3.0 program runs.

Component Name	SW12			WTP Filtrate			SW01		
	Mean (24/06/2021)	80th Percentile	Maximum	Mean (24/06/2021)	80th Percentile	Maximum	Mean	80th Percentile	Maximum
Al+3	0.37	0.126	0.46	0.005	0.008	0.024	0.026	0.026	0.1
Ba+2	0.03	0.037	0.112	0.07	0.0684	0.0977	0.023	0.027	0.029
Ca+2	25.67	22	36	125	121	126	26.8	28.4	30
Cl-1	174.3	134.4	229	207	206	208	67.5	82	122
DOC (NICA-Donnan)	9	9	9	0.5	0.5	0.5	0.5	0.5	0.5
F-1	0.2	0.4	0.5	1	1	1	0.28	0.28	0.3
Fe+3	0.94	0.57	1.3	0.025	0.006	0.025	1.118	2.062	5.22
K+1	6	6	12	25	25	27	9.4	10.2	11
Mg+2	21.33	16.8	27	75	55	75	18.8	20	20
Na+1	87	87.8	125	202	196.4	203	73.4	73.4	73.4
Zn+2	0.001	0.001	0.001	0.02	0.02	0.02	0.0039	0.0025	0.012
Ni+2	0.001	0.001	0.001	0.04	0.04	0.04	0.0039	0.0050	0.006
Mn+2	0.06	0.3972	4.05	1.07	0.981	1.39	0.0281	0.0358	0.06
N(NO3-)	0.22	0.19	3.984	0.64	1.75	2.1	3.98	3.98	3.98
SO4-2	78.3	68	196	662	641	669	135	142	147
Alkalinity (as HCO3-)	46	95	122	153	139	161	92	122	180
Eh (mV)	120	120	120	120	120	120	320	320	320
pH	7.07	7.5	8.7	8.5	8.13	8.31	7.4	8.1	9.3
Temperature	15	15	15	25	25	25	15	15	15

12.3 Appendix C

Nominal and measured dissolved nickel concentrations (Ni µg/L), zinc (Zn µg/L) and sulfate (mg/L) for ecotoxicity tests commenced on 2 July 2021 and 8 July 2021.

Table C.1 Diluent control UCML SW01 / SW12 was utilized to prepare dilution series of stock nickel solutions. Test data obtained from Ecotox Services Australia and follow Envirolab Certificate of Analysis 276720 (N. Leptophlebiidae), 276721, and 276731 (all other test species).

Test Species & Test	Time	Dilution of Stock Solution* Nominal Concentration (Ni µg/L)	Measured dissolved (Ni µg/L)	Change Measured Dissolved from Normal Conc. µg/L (%)
All	t = 0h	0	<1	- (-)
		4.7	10	5.3 (113)
		9.4	11	1.6 (17)
		18.8	18	0.8 (4)
		37.5	35	2.5 (7)
		75	68	7.0 (9)
		150	140	10 (7)
		300	300	0.0 (0)
Ceriodaphnia dubia (acute) *Chironomus tepperi*	t = 48h	0	<1	- (-)
		4.7	10	5.3 (113)
		9.4	11	1.6 (17)
		18.8	18	0.8 (4)
		37.5	35	2.5 (7)
		75	73	2.0 (3)
		150	140	10 (7)
		300	290	10 (3)
Raphidocelis subcapitata	t = 72h	0	<1	- (-)
		4.7	11	6.3 (134)
		9.4	12	2.6 (28)
		18.8	19	0.2 (1)
		37.5	37	0.5 (1)
		75	71	4.0 (5)
		150	140	10 (7)
		300	290	10 (3)
Hydra viridissima	t = 96h	0	<1	- (-)

Test Species & Test	Time	Dilution of Stock Solution* Nominal Concentration (Ni µg/L)	Measured dissolved (Ni µg/L)	Change Measured Dissolved from Normal Conc. µg/L (%)
Macrobrachium australiense *Melanotaenia splendida sp.* (acute)		4.7	10	5.3 (113)
		9.4	11	1.6 (17)
		18.8	18	0.8 (4)
		37.5	35	2.5 (7)
		75	69	6.0 (8)
		150	140	10 (7)
		300	300	0.0 (0)
Ceriodaphnia dubia (chronic) *Lemna disperma*	t = 7d	0	<1	- (-)
		4.7	10	5.3 (113)
		9.4	11	1.6 (17)
		18.8	18	0.8 (4)
		37.5	35	2.5 (7)
		75	72	3.0 (4)
		150	140	10 (7)
		300	300	0 (0)
Melanotaenia splendida sp. (chronic)	t = 12d	0	<1	- (-)
		4.7	9	4.3 (91)
		9.4	11	1.6 (17)
		18.8	18	0.8 (4)
		37.5	35	2.5 (7)
		75	71	4.0 (5)
		150	140	10 (7)
		300	300	0 (0)
Nousia Leptophlebiidae [1]	t = 0h	0	<1	- (-)
		4.7	8	3.3 (70)
		9.4	10	0.6 (6)
		18.8	17	1.8 (10)
		37.5	32	5.5 (15)
		75	64	11 (15)

Test Species & Test	Time	Dilution of Stock Solution* Nominal Concentration (Ni µg/L)	Measured dissolved (Ni µg/L)	Change Measured Dissolved from Normal Conc. µg/L (%)
		150	120	30 (20)
		300	260	40 (13)
Nousia Leptophlebiidae [1]	t = 96h	0	<1	- (-)
		4.7	8	3.3 (70)
		9.4	10	0.6 (6)
		18.8	17	1.8 (10)
		37.5	35	2.5 (7)
		75	66	9.0 (12)
		150	130	20 (13)
		300	270	30 (10)

* Diluent control - SW12 was utilized to prepare dilution series of nickel stock solutions

[1] *N. Leptophlebiidae* tests commenced on 8 July 2021 and follow Envirolab Certificate of Analysis 276720

Table C.2. Details of nominal and measured dissolved Zinc concentrations (Zinc µg/L) for ecotoxicity tests commenced on 2 July 2021 and 8 July 2021. Diluent control SW12 was utilized to prepare dilution series of stock nickel solutions. Test data obtained from Ecotox Services Australia and follow Envirolab Certificate of Analysis 276720 (Mayflys), 276721, and 276731 (all other test species).

Test Species & Test	Time	Dilution of Stock Solution Nominal Concentration (Zn µg/L)	Measured dissolved (Zn µg/L)	Change Measured Dissolved from Normal Conc. µg/L (%)
All	t = 0h	0	5	5(0)
		38	46	8 (21)
		75	78	3 (4)
		125	130	5 (4)
		250	250	0 (0)
		500	480	20 (4)
		1000	980	20 (2)
Ceriodaphnia dubia (acute) *Chironomus tepperi*	t = 48h	0	3	3(0)
		38	46	8 (21)
		75	80	5 (7)
		125	130	5 (4)
		250	260	10 (4)
		500	490	10 (2)

Test Species & Test	Time	Dilution of Stock Solution Nominal Concentration (Zn µg/L)	Measured dissolved (Zn µg/L)	Change Measured Dissolved from Normal Conc. µg/L (%)
		1000	970	30 (3)
Raphidocelis subcapitata	t = 72h	0	3	3 (0)
		38	44	6 (16)
		75	78	3 (4)
		125	130	5 (4)
		250	260	10 (4)
		500	500	0 (0)
		1000	980	20 (2)
Hydra viridissima	t = 96h	0	4	4 (0)
Macrobrachium australiense		38	47	9 (24)
Melanotaenia splendida sp. (acute)		75	82	7 (9)
		125	130	5 (4)
		250	260	10 (4)
		500	480	20 (4)
		1000	980	20 (2)
Ceriodaphnia dubia (chronic)	t = 7d	0	4	4 (0)
Lemna disperma		38	45	7 (18)
		75	77	2 (3)
		125	130	5 (4)
		250	260	10 (4)
		500	490	10 (2)
		1000	940	60 (6)
Melanotaenia splendida sp. (chronic)	t = 12d	0	4	4 (0)
		38	43	5 (13)
		75	79	4 (5)
		125	120	5 (4)
		250	240	10 (4)
		500	490	10 (2)
		1000	940	60 (6)

Test Species & Test	Time	Dilution of Stock Solution Nominal Concentration (Zn µg/L)	Measured dissolved (Zn µg/L)	Change Measured Dissolved from Normal Conc. µg/L (%)
Nousia Leptophlebiidae[1]	t = 0h	0	5	5(0)
		38	45	7 (18)
		75	73	2 (3)
		125	120	5 (4)
		250	250	0 (0)
		500	440	60 (12)
		1000	850	150 (15)
Nousia Leptophlebiidae[1]	t = 96h	0	7	7(0)
		38	44	6 (16)
		75	75	0 (0)
		125	130	5 (4)
		250	240	10 (4)
		500	460	40 (8)
		1000	920	80 (8)

* Diluent control - SW12 was utilized to prepare dilution series of nickel stock solutions

[1] *N. Leptophlebiidae* tests commenced on 8 July 2021 and follow Envirolab Certificate of Analysis 276720

Table C.3. Details of nominal and measured dissolved Sulfate concentrations (SO_4 µg/L) for ecotoxicity tests commenced on 2 July 2021 and 8 July 2021. Diluent control SW12 was utilized to prepare dilution series of stock nickel solutions. Test data obtained from Ecotox Services Australia and follow Envirolab Certificate of Analysis 276720 (Mayfly), 276721, and 276731 (all other test species).

Test Species & Test	Time	Dilution of Stock Solution Nominal Concentration (SO_4 mg/L)	Measured dissolved (SO_4 mg/L)	Change Measured Dissolved from Normal Conc. mg/L (%)
All	t = 0h	0.00	73	73 (0)
		750.00	570	180 (24)
		1250.00	1100	150 (12)
		2500.00	2100	400 (16)
		5000.00	4200	800 (16)
		10000.00	7800	2200 (22)
		20000.00	17000	3000(15)

Test Species & Test	Time	Dilution of Stock Solution Nominal Concentration (SO_4 mg/L)	Measured dissolved (SO_4 mg/L)	Change Measured Dissolved from Normal Conc. mg/L (%)
Ceriodaphnia dubia (acute) *Chironomus tepperi*	t = 48h	0.00	nd	- (-)
		750.00	560	190 (25)
		1250.00	1100	150 (12)
		2500.00	2100	400 (16)
		5000.00	3800	1200 (24)
		10000.00	7700	2300 (23)
		20000.00	17000	3000 (15)
Raphidocelis subcapitata	t = 72h	0.00	nd	- (-)
		750.00	550	200(27)
		1250.00	1000	250 (20)
		2500.00	2100	400 (16)
		5000.00	3900	1100 (22)
		10000.00	7900	2100(21)
		20000.00	17000	3000 (15)
Hydra viridissima *Macrobrachium australiense* *Melanotaenia splendida sp.* (acute) *Nousia Leptophlebiidae* [1]	t = 96h	0.00	nd	- (-)
		750.00	560	190 (25)
		1250.00	1100	150 (12)
		2500.00	2100	400 (16)
		5000.00	3900	1100 (22)
		10000.00	7700	2300 (23)
		20000.00	17000	3000 (15)
Ceriodaphnia dubia (chronic) *Lemna disperma*	t = 7d	0.00	nd	- (-)
		750.00	550	200 (27)
		1250.00	1100	150 (12)
		2500.00	2100	400 (16)
		5000.00	3800	1200 (24)
		10000.00	8200	1800 (18)
		20000.00	17000	3000 (15)

Test Species & Test	Time	Dilution of Stock Solution Nominal Concentration (SO_4 mg/L)	Measured dissolved (SO_4 mg/L)	Change Measured Dissolved from Normal Conc. mg/L (%)
Melanotaenia splendida sp. (chronic)	t = 12d	0.00	nd	- (-)
		750.00	560	190 (25)
		1250.00	1100	150 (12)
		2500.00	2100	400 (16)
		5000.00	4000	1000 (20)
		10000.00	8000	2000 (20)
		20000.00	18000	2000 (10)

12.4 Appendix D

QA/QC data and certificates Envirolabs Services Australia Pty Ltd Chatswood NSW and Ecotox Services Australia, Lane Cove NSW (see Appendices D.1 to D.6).

From Section 5.6.1.2

Envirolab advise that 'In circumstances where no duplicate and/or sample spike has been reported at 1 in 10 and/or 1 in 20 samples respectively, the sample volume submitted was insufficient in order to satisfy laboratory QA/QC protocols' and that 'Measurement Uncertainty estimates are available for most tests upon request'. Thus, the Quality Control details for each analyte summarised by codes in the respective Envirolab Certificates of Analysis are not included directly, but actual details of measurement uncertainties with a single result for each Spike recovery % may be supported by '*Measurement Uncertainty estimates available for most tests upon request*'. These single numbers are the only outputs given in the Certificate of Analysis of quality control (generally in the range 70-130% for inorganics/metals) for each analytical batch. These quality control samples are indicative of the trueness of the measured concentrations of analytes.

The Envirolab Certificate of Analysis in the Appendices of the Ecotox Services Australia Laboratory toxicity test reports' display a variety of LCS (LCS-W1, LCS-W2, LCS-W3, LCS-W4, LCS-W5, L LCS-W6,) and 'surrogate spikes' (276720-1, 276720-15, 276720-3, 276720-23, 276721-13, 276721-33, 276723-3, 276721-33, 276723-2, 276730-2, 276730-22, 276731-11, 276731-31,) with the comment that a single result for each Spike recovery % may be supported by a '*Measurement Uncertainty estimates available for most tests upon request*'. See Appendix G

The Ecotox Services Australia Laboratory report (Appendix G includes details of Envirolab Certificates of Analysis 276879, 276720, 276721, 276723,276730 and 276731 are listed together with measurement uncertainties (MU% at 95% confidence) for all analytes.

Certificate of Analysis 276879
Electrical Conductivity LCS-W1 EC 106 µS/cm

Certificate of Analysis 276720
Spike recoveries
Calcium dissolved LCS-W1 108% ; 276720-15 99%
Magnesium dissolved LCS-W1 99% % ; 276720-15 96%
Nickel dissolved LCS-W1 94% ; 276720-3 94%
Zinc dissolved LCS-W1 99% ;
Nickel dissolved LCS-W2 93% ;
Zinc dissolved LCS-W2 97% ; 276720-23 106%
Certificate of Analysis 276721
Spike recoveries
Nickel dissolved LCS-W2 93% ; 276721-13 96%
Nickel dissolved LCS-W3 98% ; 276721-33 97%
Nickel dissolved LCS-W4 99% ;

Certificate of Analysis 276723
Spike recoveries
pH LCS-W1 100% ;
Electrical Conductivity LCS-W1 96% ;
Total Alkalinity as $CaCO_3$ LCS-W1 106% ;
Sulphate SO_4 LCS-W1 96% ;

Dissolved Organic Carbon LCS-W1 95% ;

pH LCS-W1 100% ;
Electrical Conductivity LCS-W1 96% ;

Calcium dissolved LCS-W1 108% ; 276720-15 99%
Magnesium dissolved LCS-W1 99% % ; 276720-15 96%
Nickel dissolved LCS-W1 94% ; 276720-3 94%
Zinc dissolved LCS-W1 99% ;
Nickel dissolved LCS-W2 93% ;
Zinc dissolved LCS-W2 97% ; 276720-23 106%
Calcium dissolved LCS-W1 108% ; 276723-2 104%
Magnesium dissolved LCS-W1 99% % ; 276723-2 100%

Certificate of Analysis 276730
Spike recoveries
pH LCS-W1 100% ;
Electrical Conductivity LCS-W1 95% ;
Sulphate SO4 LCS-W1 95% ;
pH LCS-W2 100% ;
Electrical Conductivity LCS-W2 95% ;
Sulphate SO4 LCS-W2 99% ;

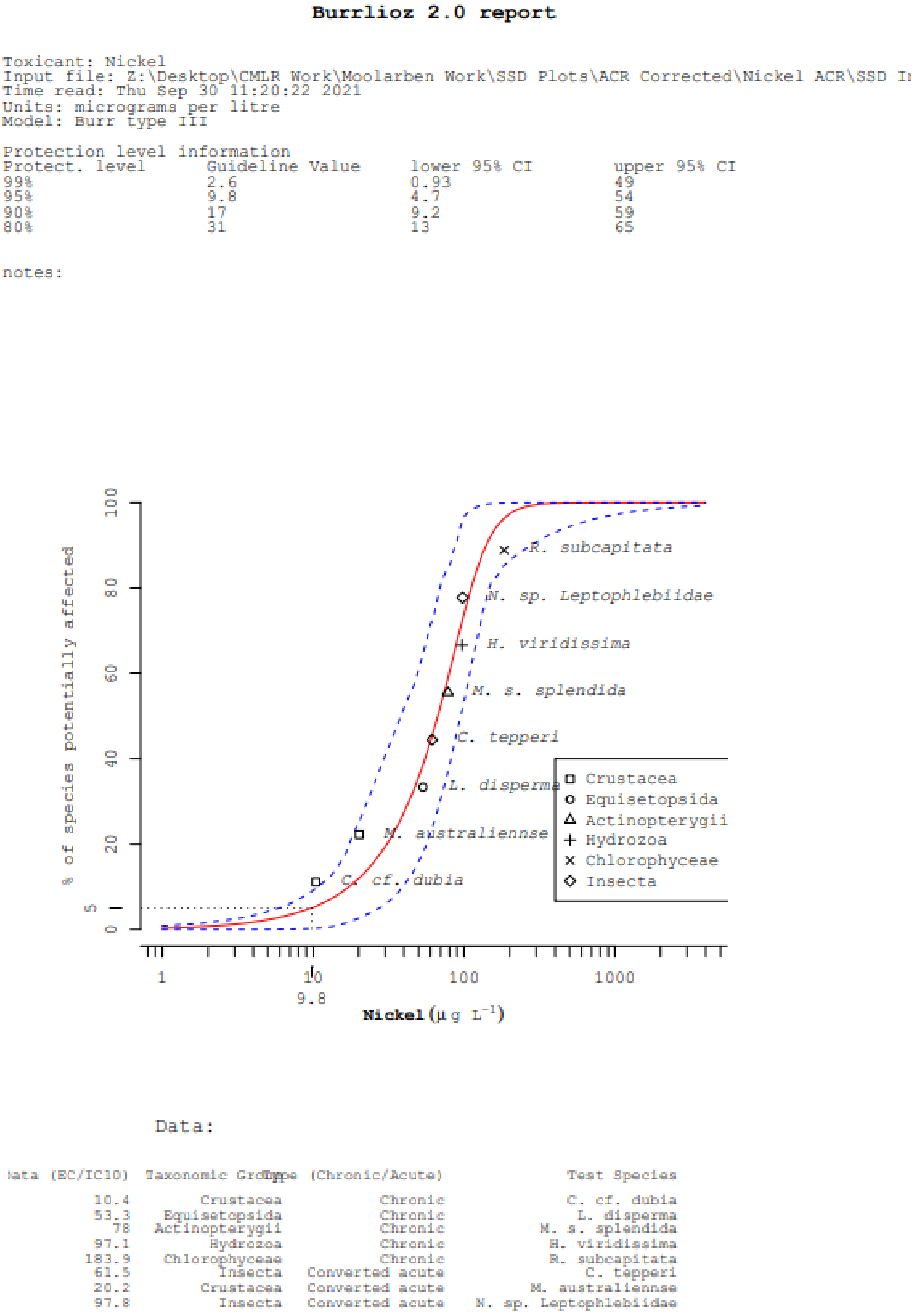
Certificate of Analysis 2767321
Spike recoveries
Nickel dissolved LCS-W4 99% ;
Zinc dissolved LCS-W4 99% ; 276721-33 101%
Nickel dissolved LCS-W5 97% ;
Zinc dissolved LCS-W5 100% ; 276721-33 106%
Nickel dissolved LCS-W6 102% ;
Zinc dissolved LCS-W6 103% ;

Test certificates for certified reference materials are details that underpin the ‘trueness’ of the concentration results for test solution analyses from the ecotoxicity testing which are then used as data input for the assessment of the decision on the SSD model fitting that gives the SSGV values of the analyte. The test certificates for standards with date of expiry for each analyte should be retained by the laboratory as part of their NATA accreditation process following ISO/IEC 17025 (ISO/IEC, 2017). The test certificate details can be included in the test report but the laboratory may not want to provide them, and are not described in the scope of the NATA accreditation listing.

Envirolab have provided test certificates for certified reference materials for nickel, sulfate and zinc (see Appendix G, except for EC where the Ecotox Services Australia certified reference materials could be used to provide comparative).

12.5 Appendix E

The BurrliOZ software print out summaries for respective analyte SSD and fitted data.

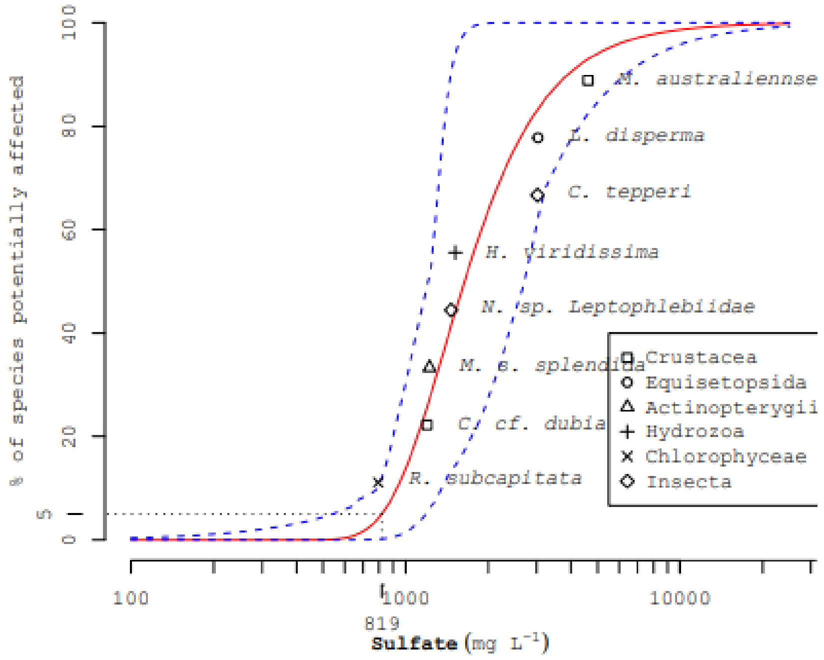


Burrlioz 2.0 report

```
Toxicant: Nickel
Input file: Z:\Desktop\CMLR Work\Moolarben Work\SSD Plots\ACR Corrected\Nickel ACR\SSD I
Time read: Thu Sep 30 11:20:22 2021
Units: micrograms per litre
Model: Burr type III

Protection level information
Protect. level      Guideline Value     lower 95% CI        upper 95% CI
99%                 2.6                 0.93                49
95%                 9.8                 4.7                 54
90%                 17                  9.2                 59
80%                 31                  13                  65


notes:
```

```
Data:

ata (EC/IC10)  Taxonomic GroType (Chronic/Acute)            Test Species

        10.4        Crustacea            Chronic             C. cf. dubia
        53.3    Equisetopsida            Chronic              L. disperma
          78   Actinopterygii            Chronic         M. s. splendida
        97.1         Hydrozoa            Chronic          H. viridissima
       183.9    Chlorophyceae            Chronic          R. subcapitata
        61.5          Insecta    Converted acute               C. tepperi
        20.2        Crustacea    Converted acute         M. australiennse
        97.8          Insecta    Converted acute   N. sp. Leptophlebiidae
```

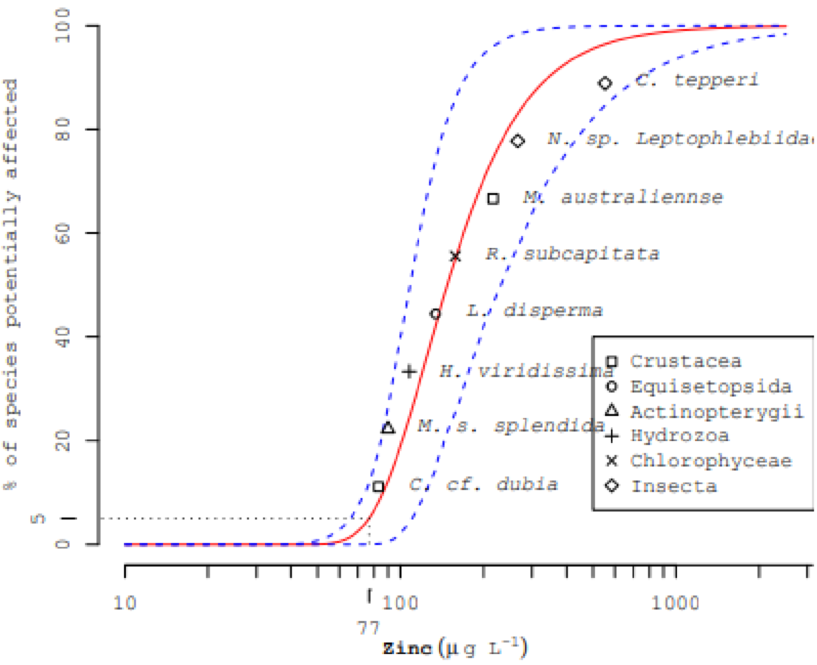
```
                    Burrlioz 2.0 report

Toxicant: Sulfate
Input file: Z:\Desktop\CMLR Work\Moolarben Work\SSD Plots\ACR Corrected\Sulfate ACR\SSD
Time read: Thu Sep 30 10:50:54 2021
Units: milligrams per litre
Model: Burr type III

Protection level information
Protect. level      Guideline Value     lower 95% CI        upper 95% CI
99%                 661                 224                 1073
95%                 819                 599                 1259
90%                 931                 748                 1453
80%                 1105                868                 1731


notes:
```



```
          Data:

ata (EC/IC10)  Taxonomic GroType (Chronic/Acute)              Test Species

       1197.2        Crustacea             Chronic           C. cf. dubia
       3032.9    Equisetopsida             Chronic            L. disperma
       1222.3   Actinopterygii             Chronic        M. s. splendida
       1518.1         Hydrozoa             Chronic         H. viridissima
        794.5   Chlorophyceae              Chronic         R. subcapitata
       3022.3          Insecta     Converted acute             C. tepperi
         4625        Crustacea     Converted acute       M. australiennse
       1465.9          Insecta     Converted acute N. sp. Leptophlebiidae
```

Burrlioz 2.0 report

```
Toxicant: Zinc
Input file: Z:\Desktop\CMLR Work\Moolarben Work\SSD Plots\ACR Corrected\Zinc ACR\SSD Inp
Time read: Thu Sep 30 11:28:26 2021
Units: micrograms per litre
Model: inverse.weibull

Protection level information
Protect. level      Guideline Value     lower 95% CI        upper 95% CI
99%                 63                  52                  99
95%                 77                  65                  114
90%                 86                  74                  129
80%                 101                 85                  149


notes:
```



Data:

```
ata (EC/IC10)  Taxonomic GroType  (Chronic/Acute)              Test Species

        83.1         Crustacea            Chronic             C. cf. dubia
         134     Equisetopsida            Chronic              L. disperma
          90    Actinopterygii            Chronic          M. s. splendida
       107.5          Hydrozoa            Chronic           H. viridissima
       157.9     Chlorophyceae            Chronic           R. subcapitata
       552.2           Insecta    Converted acute               C. tepperi
       216.6         Crustacea    Converted acute         M. australiennse
         266           Insecta    Converted acute   N. sp. Leptophlebiidae
```

12.6 Appendix F

Appendix F.

The following appendix describes the relationship between measured electrical conductivity (EC) associated with dosed sulfate concentrations in diluent control water SW12 undertaken by Ecotox Services Australia (Appendix G). Figure F.1. The electrical conductivity (EC) was measured in each ecotoxicity test solution at Ecotox Services Australia while the sulfate concentrations in each test solution were measured at Envirolab Services Pty Ltd. Table F.1 lists all electrical conductivity readings sulfate concentrations measured in the test water solutions.

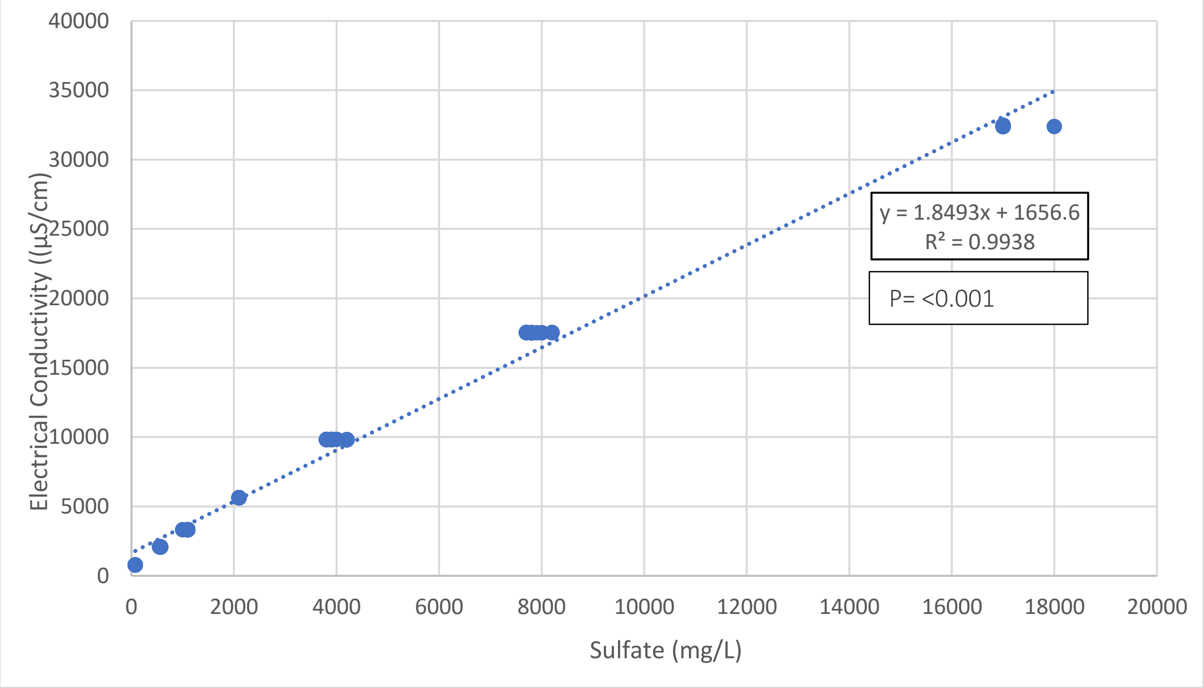


Figure F.1. Linear regression of measured sulfate (mg/L) dosages in SW12 diluent control waters with measured electrical conductivity measurements (µS/cm).

Figure F.1 shows the linear regression of measured EC values versus sulfate concentrations based on measurements given in Table F.1. Measurements during the ecotoxicity testing can be used to derive an EC discharge limit (with 95% confidence intervals) by generating a linear regression of EC versus measured sulfate concentrations Utilising the SSGV value for sulfate (819 [544-1314] mg/L) generated from SSDs (Section 8.4) in the linear regression equation enables the derivation of an EC value that is equivalent to the sulfate SSGV with a 95% confidence interval.

SSGV Sulfate (mg/L) = 819 (544-1314) (Section 8.4) from Equation F.1

$$y = 1.8493X + 1656.6$$

(F.1)

X (mg/L) = 819
X (mg/L) = 544
X (mg/L) = 1314

Equation F.1 utilising the whole data set in Table F.1, retrieved from ecotoxicity testing data (Appendix G)can be used as a line of evidence, following guidance of ANZG (2018), to derive an EC discharge limit of: **3171 (2663-4087) µS/cm.**

Sulfate concentrations at the upper end of the range correspond to levels that show toxicity effects on aquatic test species (Appendix G). As a result, it was observed that high sulfate concentrations showed some bias in the fitting when compared with the linear regression plot in Figure F.2 that restricted the upper sulfate concentration to 2500 mg/L compared with 18,000 mg/L in Figure F.1. The R^2 value for Figure F.2 for Equation F.2 was 0.9988 which indicates a better fit than an R^2 value of 0.9938 for Equation F.1. Therefore, Equation F.2 should be used for Moolarben conditions of release where sulfate concentrations do not exceed 2100 µS/cm (Figure F.2).

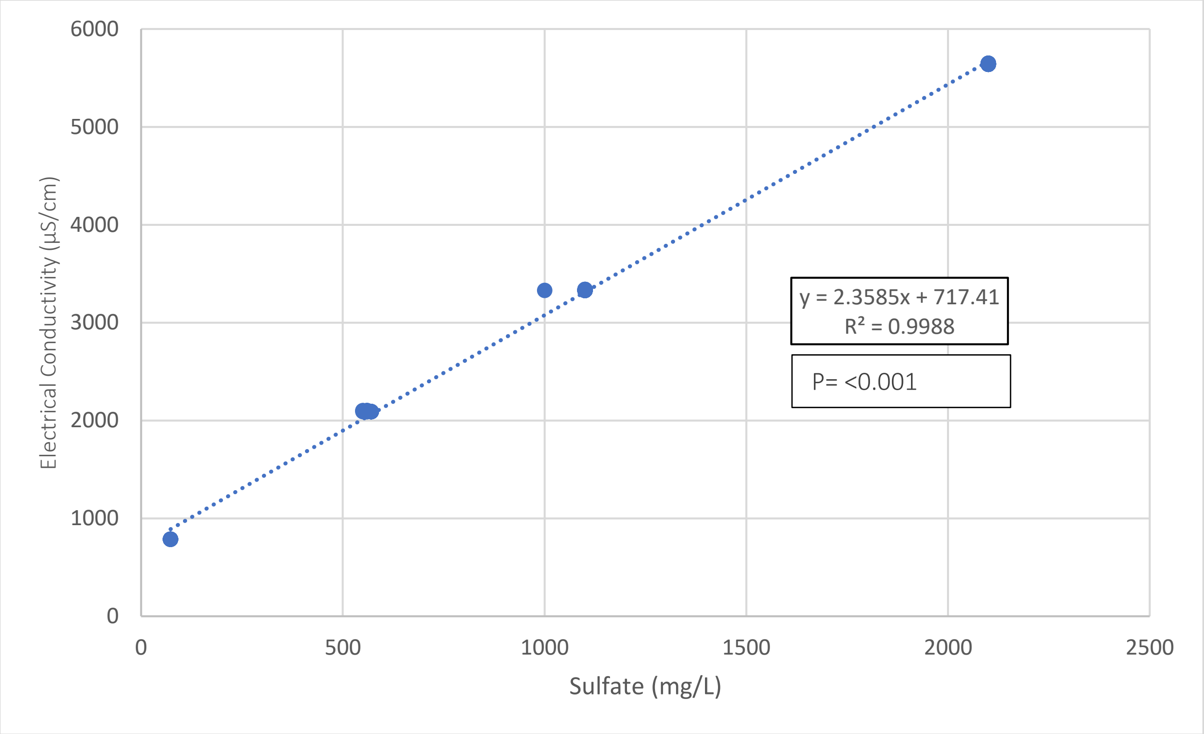


Figure F.2. Linear Regression of dosed sulfate concentrations (mg/L) in SW12 diluent water with electrical conductivity concentrations (µS/cm), excluding high dosed concentrations of sulfate and associated electrical conductivity measurements.

SSGV Sulfate (mg/L) = 819 (544-1314)

$$y = 2.3585X + 717.41$$
(F2)

X (mg/L) = 819
X (mg/L) = 544
X (mg/L) = 1314

The linear regression equation (F.2) developed from measured sulfate concentration and EC measured data from sulfate dosing tests in SW12 diluent control water (X2) can be used as a line of evidence, following guidance from ANZG (2018), to derive an appropriate EC toxicity limit of: **2649 (2000-3816) µS/cm**, showing a direct statistical relationship with the sulfate SSGV.

The data that was used to obtain regression line results (for Appendix F) is listed in Table F.1

Table F.1 Ecotoxicity sulfate and electrical conductivity measurements in sulfate dosage tests in SW12 diluent waters for each species.

Ceriodaphnia dubia (acute)		
t=0h		
Sulfate (g/L) (Nominal)	Sulfate (mg/L) Measured	Electrical Conductivity (µS/cm)
DMW Control	DMW Control	176
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=48h		
DMW Control	DMW Control	173
Surface Water Diluent Control	nd	790
0.75	560	2090
1.25	1100	3330
2.5	2100	5650
5	3800	9830
10	7700	17550
20	17000	32400
Ceriodaphnia dubia (chronic)		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
DMW Control	DMW Control	176
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=7d		
SO4 g/L	SO4 mg/L	Conductivity (us/cm)
DMW Control	DMW Control	181
Surface Water Diluent Control	nd	793
0.75	550	2100
1.25	1100	3330
2.5	2100	5640
5	3800	9840
10	8200	17550
20	17000	32400

Lemna Disperma		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
SIS Control	DMW Control	291
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=7d		
SO4 g/L	SO4 mg/L	Conductivity (us/cm)
SIS Control	DMW Control	299
Surface Water Diluent Control	nd	794
0.75	550	2100
1.25	1100	3340
2.5	2100	5650
5	3800	9830
10	8200	17540
20	17000	32400
Melanotaenia splendida splendida (acute)		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
DMW Control	DMW Control	176
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=96h		
SO4 g/L	SO4 mg/L	Conductivity (us/cm)
DMW Control	DMW Control	180
Surface Water Diluent Control	nd	790
0.75	560	2100
1.25	1100	3330
2.5	2100	5650
5	3900	9840
10	7700	17540
20	17000	32400
Melanotaenia splendida splendida (chronic)		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)

DMW Control	DMW Control	176
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=12d		
DMW Control	DMW Control	181
Surface Water Diluent Control	nd	791
0.75	560	2100
1.25	1100	3330
2.5	2100	5650
5	4000	9850
10	8000	17540
20	18000	32400
Hydra viridissima		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
WW-DMW Control	DMW Control	785
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=96h		
WW-DMW Control	DMW Control	794
Surface Water Diluent Control	nd	794
0.75	560	2100
1.25	1100	3330
2.5	2100	5650
5	3900	9840
10	7700	17560
20	17000	32500
Raphidocelis subcapitata		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
USEPA Control	USEPA Control	125
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830

10	7800	17540
20	17000	32400
t=72h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
USEPA Control	USEPA Control	125
Surface Water Diluent Control	nd	786
0.75	550	2090
1.25	1000	3330
2.5	2100	5640
5	3900	9830
10	7900	17540
20	17000	32400
Chironomus tepperi		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
DMW Control	DMW Control	176
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=48h		
DMW Control	DMW Control	184
Surface Water Diluent Control	nd	795
0.75	560	2090
1.25	1100	3340
2.5	2100	5640
5	3800	9840
10	7700	17550
20	17000	32400
Macrobrachium australiense		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
DMW Control	DMW Control	176
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=96h		
SO4 g/L	SO4 mg/L	Conductivity (us/cm)

DMW Control	DMW Control	182
Surface Water Diluent Control	nd	790
0.75	560	2100
1.25	1100	3340
2.5	2100	5650
5	3900	9840
10	7700	17540
20	17000	32500
Mayfly Nousia		
t=0h		
Sulfate (g/L)	Sulfate (mg/L)	Electrical Conductivity (µS/cm)
Surface Water Diluent Control	73	786
0.75	570	2090
1.25	1100	3330
2.5	2100	5640
5	4200	9830
10	7800	17540
20	17000	32400
t=96h		
Surface Water Diluent Control	nd	799
0.75	560	2090
1.25	1100	3340
2.5	2100	5650
5	3900	9830
10	7700	17550
20	17000	32400

12.7 Appendix G

Toxicity Assessment of WTP Filtrate, WTP Permeate, WTP Discharge, Sulphate, Nickel and Zinc - Moolarben Coal Operations Test Report by Ecotox Services Australia Pty Ltd and appendices (Appendices G.1 to G.8).