Appendix F - Sampling Methods

1. Appendix F: Ecological risk assessment sampling methodologies

1.1. Soil sampling

The soil assessment was completed with reference to the guidance within the following documents:

- Standards Australia 2005, AS4482.1 – 2005 Guide to the investigation and sampling of sites with potentially contaminated soil Part 1: Non-volatile and semi-volatile compounds (Standards Australia AS4482.1-2005);
- Standards Australia 1999, 4482.2-1999 Guide to the Sampling and Investigation of Potentially Contaminated Soil. Part 2: Volatile Substances (Standards Australia AS4482.2-1999);
- The relevant schedules of the National Environment Protection (Assessment of Site Contamination) Amendment Measure 2013, (National Environment Protection Council).

The soil assessment completed at the site was undertaken in accordance with the methodology detailed in Table 1-1.

Table 1-1: Soil sampling methodology

Activity	Detail
Strata logging	Soil and rock logging was generally in accordance with AS 4482.1 and the Unified soil classification system.
Sample collection	Soil samples were collected from soil bores using a combination of NDD methods, hand auger and crow bar. Near surface soil samples were collected using a trowel or by loosening the soil surface with a spade. Soil samples were collected at regular intervals from hand augers (by gloved hand) or air cuttings (from down-hole hammer air return by a gloved hand). Soil sampling locations were plotted using a hand-held GPS device.
Decontamination procedure	Decontamination of re-usable sampling equipment was completed using laboratory supplied and certified PFAS Free deionised water. Fresh disposable nitrile gloves were worn for each sample collection.
Disposal of soil cuttings	Soil cuttings from each location were backfilled into the borehole upon completion of the soil sampling. Soils were re-compacted to the extent practicable using the hand auger and a crowbar. Surplus soil cuttings from monitoring well installation activities were initially collected in 200L open head drums and transported to a designated lay-down area. From there, the drums were emptied into HDPE lined and covered skips for short-term storage. On completion of the drilling program the soils in the skips were transported to an area of the base (nominated by base staff) for disposal. These soils were placed onto the surface of the site near the former fire training area (NT0241) which is to be redeveloped with a pavement covering in the next 12 months.
Sample IDs	Soil sample nomenclature – 1302_location_depth_yymmdd

Activity	Detail
Analytical laboratories	Primary samples were submitted to Eurofins for analysis Inter-laboratory duplicate samples were submitted to ALS for analysis
Quality Control	Intra- and inter-laboratory duplicates were collected at a frequency of 1 in 20 primary samples Rinsate samples were collected each day when re-usable equipment was used for sample collection
Sample preservation	Samples were placed in laboratory supplied LDPE jars (with unlined lids). Samples were stored on ice, in an esky, and transported under chain-of-custody documentation while on-site and in transit to the laboratory.

1.2. Surface water

Surface water sampling was undertaken in accordance with guidance provided in the following documents:

- AS/NZS 5667.4:1998 'Water Quality – Sampling Part 4: Guidance on Sampling from Lakes, natural and man-made'.
- AS/NZS 5667.6:1998 'Water Quality – Sampling, Part 6: Guidance on sampling of rivers and streams'.
- AS/NZS 5667.9: 1998 'Water Quality – Sampling, Part 9: Guidance on sampling from marine waters'.

The surface water sampling completed at the site was undertaken in accordance with the methodology detailed in Table 1-2.

Table 1-2: Surface water sampling methodology

Activity	Detail
Sample collection	Surface water samples were collected using an aluminium pole sampler with a laboratory supplied sampling container fixed to the end. The pole sampler was lowered just beneath the water's surface with the open end facing down to limit surface films from entering the sampling bottle. Water sampling locations were selected to be within 1m of the edge of the water surface (where samples could be collected safely) and within flowing water. Once filled, the sampling bottle was removed from the sampler, sealed and placed into an esky on ice and transported to the laboratory under standard chain-of-custody procedures. Once sample was collected a water quality meter was placed in the water body and field parameters such as pH, DO, EC, ORP and water temperature were recorded. The depth of water at each sampling location was also measured at the time of sampling
Decontamination procedure	Decontamination of sampling equipment, where appropriate, was completed after each sample, by washing equipment in laboratory supplied and certified PFAS fee deionised water. The water quality meter did not come into contact with the sample, as the meter was used in the stream following sample collection.

Activity	Detail
Sample IDs	1302_SWxxx_yymmdd
Analytical laboratories	Primary samples were submitted to Eurofins for analysis Inter-laboratory duplicate samples were submitted to ALS for analysis
Quality control	Intra- and inter-laboratory duplicates were collected at a frequency of 1 in 20 primary samples Rinsate samples were collected each day when re-usable equipment was used for sample collection
Sample preservation	Samples were placed in laboratory supplied LDPE jars (with unlined lids). Samples were stored on ice, in an esky, and transported under chain-of-custody documentation while on-site and in transit to the laboratory.

1.3. Sediment sampling

The sediment sampling was undertaken in accordance with guidance provided in the following documents:

- Standards Australia 2005, AS4482.1 – 2005 Guide to the investigation and sampling of sites with potentially contaminated soil Part 1: Non-volatile and semi-volatile compounds (Standards Australia AS4482.1-2005);
- Standards Australia 1999, 4482.2-1999 Guide to the Sampling and Investigation of Potentially Contaminated Soil. Part 2: Volatile Substances (Standards Australia AS4482.2-1999);
- The relevant schedules of the National Environment Protection (Assessment of Site Contamination) Amendment Measure 2013 (National Environment Protection Council).

The sediment sampling completed at the site was undertaken in accordance with the methodology detailed in Table 1-3.

Table 1-3: Sediment sampling methodology

Activity	Detail
Sample collection	Sediment samples were collected using two methods depending on the presence or absence of surface water at the sampling locations. • Where water was present, sediment samples were collected using a stainless steel dredge sampler lowered to the bottom of the water body, and dragged along the bottom for approximately 1m to collect a sediment sample. The sampler was then removed from the water body and the sample placed (using gloves) into a laboratory supplied sampling container. New gloves were used between each sampling location. Where sediment sampling locations coincided with surface water sampling locations, the surface water sample was collected prior to any sub-surface sediment sample. • Where water was not present in drains or water bodies, a sample was collected directly from the base of the drain/stream bed using the sample jar or a decontaminated stainless steel trowel, and placed directly into laboratory supplied containers.

Activity	Detail
Decontamination procedure	Decontamination of sampling equipment, where appropriate, was completed after each sample, by washing equipment in laboratory supplied and certified PFAS fee deionised water.
Sample IDs	1302_SDxx_yymmdd
Analytical laboratories	Primary samples were submitted to Eurofins for analysis Inter-laboratory duplicate samples were submitted to ALS for analysis
Quality control	Intra- and inter-laboratory duplicates were collected at a frequency of 1 in 20 primary samples Rinsate samples were collected each day when re-usable equipment was used for sample collection
Sample preservation	Samples were placed in appropriate laboratory supplied bottles. Samples were stored on ice, in an esky, and transported under chain-of-custody documentation while on-site and in transit to the laboratory.

1.4. Fish, crustaceans and molluscs

Table 1-4: Fish, crustacean and molluscs sampling methodology

Activity	Detail
Sample collection	Aquatic Biota sampling was undertaken in the freshwater reaches of Ludmilla Creek and Rapid Creek using a mixture of gill netting, bait traps, backpack electro fishing and cast netting to collect fish and crustaceans. The sampling method used to capture the organisms was varied depending on the nature of the stream being sampled, and the logistical constraints present. An electrofishing pack was used to optimise sampling in freshwater reaches. This was completed by a certified operator, using methods that comply with the guidelines set out by the Australian Code of Electrofishing Practice and the ethics approval. Stunning used the minimum power necessary to attract and stun the fish effectively. Sampling was halted if any non-target animals or reptiles came within 15 m of fishing. Electrofishing occurred for 5 – 10 minutes per pool, or until adequate samples had been collected. All non-target species were released from electrical current immediately. A selection of species present in each water body was collected for analysis and was based on the observations of species types within each waterbody Selected fish were euthanised humanly according to our animals ethics permit conditions. Captured target fish were euthanised via a combination of ice slurry, Aqui-S solution anaesthesia, clubbing, pithing and/or cervical dislocation.
Permit Details	**Permit No.** S17/3409

Activity	Detail
	Permit holder: Ian Dixon of Eco Logical Australia Pty Ltd (ELA) **Valid Dates**: 30 March 2017 to 30 March 2022
Sample preparation	Equipment was rinsed with laboratory supplied PFAS free DI water. Samples were stored in large polyethylene bags or snaplock bags and packed in ice until preparation. Specimens were weighed, measured and the species and location caught was recorded. For target fish of sufficient size, the following sample preparation was conducted: • New opened bag used as cutting board cover • Knife/blade was cleaned with DI water or new disposable scalpel blade was used • Fillets and liver were separated into separate samples • Packaged and labelled separately as three samples • Where reusable equipment was used, a rinsate sample was collected after cleaning reusable equipment (run DI water over equipment and collect in PFAS sample bottle) Other samples (small fish, crustaceans, molluscs) were kept whole. Composite samples of whole organisms were created where required to ensure sufficient sample size for analysis. In relation to crustaceans the shell was generally removed and then the remaining whole organism kept for sample analysis. Where large enough mud crabs where separated into organs and flesh for sample analysis. All samples were double bagged and frozen.
Decontamination procedure	Decontamination of sampling equipment, where appropriate, was completed after each sample, by washing equipment in laboratory supplied and certified PFAS fee deionised water.
Sample IDs	Fish, crustacean and mollusc sample nomenclature - 1302_FHxxx_yymmdd
Analytical laboratories	Primary samples were submitted to Eurofins for analysis Inter-laboratory duplicate samples were submitted to NMI or ALS for analysis
Quality control	Where fish were large enough, left and right fillets were used as duplicate samples for quality control analysis. Alternatively, samples of similar description were used for general comparison (i.e. similar sized fish / biota of same species caught at same location). Where re-usable equipment was used for sampled preparation a rinsate sample was collected after cleaning the re-usable equipment. At least once rinsate sample was collected for each day for sample preparation.
Sample preservation	Frozen samples were packed in eskies with additional frozen water bottles and sealed, prior to overnight dispatch to Eurofins Brisbane.

Photo 1- Backpack Electrofishing

Photo 2 - Cast net

Photo 3 - Gill net

1.5. Vegetation

Table 1-5: Vegetation sampling methodology

Activity	Detail
Sample collection	For vegetation sampling, a knife or secateurs was used to collect the sample, if required. Where applicable, excess soil or sediment was removed from the sample. All vegetation samples were double bagged. Gloves were changed between samples and equipment rinsed with DI water. Site notes described the following: • Type of fruit, vegetable, plant. • Whether the sample is whole, partial (and if so what part) or composite • Sample condition • Sample size For the purpose of the ecological risk assessment the portion/s of the plants considered likely to be consumed by higher orders in the food chain were sampled. This was typically the 'edible' plant material and/or leafy vegetation.
Sample preparation	The following instructions were provided to the laboratory regarding the preparation and analysis of terrestrial biota samples: Ensure all equipment, gloves and other materials used in the preparation and analysis are PFAS free. Describe and weigh each sample prior to analysis. Specific preparation instructions for the different types of vegetation analysed are provided in the analytical results tables. Samples were homogenised by the laboratory and a 5 g specimen taken for extraction and analysis.
Decontamination procedure	Reusable sampling equipment (e.g. knife or secateurs) was decontaminated between sample locations by rinsing in laboratory supplied PFAS free DI water and using a scrubbing brush.
Sample IDs	Fruit, vegetable and plant sample nomenclature - 1302_VGxxx_yymmdd
Analytical laboratories	Primary samples were submitted to Eurofins for analysis Inter-laboratory duplicate samples were submitted to NMI or ALS for analysis
Quality control	Separate vegetative parts from the same plant/source were used to provide quasi duplicate samples. Where re-usable equipment was used for sampled preparation a rinsate sample was collected after cleaning the re-usable equipment. At least once rinsate sample was collected for each day for sample preparation.
Sample preservation	Frozen samples were packed in eskies with additional frozen water bottles and sealed, prior to overnight dispatch to Eurofins Brisbane.

Photo 4 - Vegetation collection

1.6. Terrestrial vertebrates (Amphibians, reptiles, small mammals)

Table 1-6: Terrestrial vertebrate sampling methodology

Activity	Detail
Sample collection	Animals were caught through a variety of methods including: • Pit fall traps • Baited traps • Donations from indigenous communities of animals caught for food • Carcass or serum samples from injured wildlife Site notes described the following: • Type of animal. • Whether the sample is whole, partial or composite • Animal size • Sample type (whole, portion, organs, flesh, etc) Where serum was collected, blood samples were collected in new PE or PP vials (no glass or Teflon) and subsequently separated to obtain 5 ml of serum. Samples were kept chilled and transferred to the NATA certified analysing laboratory.
Sample preparation	Animals were kept whole for analysis and are representative of the ecological food web. Larger animals and animals for human consumption were sub-sampled to collect specific tissue or organ samples prior to dispatch to the laboratory. In some instances larger animals were sampled by collecting a serum samples so as not to harm the animal. For small animals composite samples were created from individuals collected from the same location to obtain sufficient sample weight for analysis. Samples were stored in large PE bags or snaplock bags and packed in ice until preparation. Specimens were weighed, measured and the species and location caught was recorded. For animals of sufficient size, the following sample preparation was conducted: • New opened bag used as cutting board cover • Knife/blade was cleaned with DI water or new disposable scalpel blade was used • Carcass and organs were separated into separate samples • Where reusable equipment was used, a rinsate sample was collected after cleaning reusable equipment (run DI water over equipment and collect in PFAS sample bottle) All samples were double bagged and frozen.

Activity	Detail
	The following instructions were provided to the laboratory regarding the preparation and analysis of terrestrial vertebrates samples: • Ensure all equipment, gloves and other materials used in the preparation and analysis are PFAS free. Specific preparation instructions for the different samples analysed are provided in the analytical results tables. Samples were homogenised by the laboratory and a 5 g specimen taken for extraction and analysis. Where serum was collected, blood samples were collected in new PE or PP vials (no glass or Teflon) and subsequently separated to obtain 5 ml of serum. Samples were kept chilled and transferred to the NATA certified analysing laboratory.
Decontamination procedure	Reusable sampling equipment (e.g. knife or secateurs) was decontaminated between sample locations by rinsing in laboratory supplied PFAS free DI water and using a scrubbing brush. Where re-usable equipment was used for sampled preparation a rinsate sample was collected after cleaning the re-usable equipment. At least once rinsate sample was collected for each day for sample preparation.
Sample IDs	Terrestrial vertebrate sampling nomenclature - 1302_TVxxx_yymmdd TV – indicates terrestrial vertebrate SM – indicates small mammal C – indicates composite sample
Analytical laboratories	Primary samples were submitted to Eurofins for analysis Primary serum samples were submitted to Envirolab for analysis Inter-laboratory duplicate samples were submitted to NMI or ALS for analysis
Quality control	Similar samples (similar size of same species from same location) were used to provide quasi duplicate samples. Where re-usable equipment was used for sampled preparation a rinsate sample was collected after cleaning the re-usable equipment. At least once rinsate sample was collected for each day for sample preparation.
Sample preservation	Frozen samples were packed in eskies with additional frozen water bottles and sealed, prior to overnight dispatch to Eurofins Brisbane.

Photo 5 - Vertebrate cage trap

Photo 6 - Vertebrate Elliot trap

Photo 7 - Vertebrate funnel trap

Photo 8 - Vertebrate pitfall trap

1.7. Terrestrial invertebrates

Table 1-7: Terrestrial invertebrate sampling methodology

Activity	Detail
Sample collection	Animals were caught through a variety of methods including: • Pit fall traps • Digging in soil and termite mounds • Covering soil with plastic and collecting organisms under the plastic after 24-hours
Sample preparation	Composite samples of similar groups of invertebrate samples were collect at sampling locations to obtain sufficient sample weight for analysis. The total specimen composite sample was weighed and the species composition of the sample recorded along with the location caught. Samples were rinsed with PFAS free deionised water to remove soil prior to submission to the laboratory. The following instructions were provided to the laboratory regarding the preparation and analysis of terrestrial invertebrate samples: • Ensure all equipment, gloves and other materials used in the preparation and analysis are PFAS free. Specific preparation instructions for the different samples analysed are provided in the analytical results tables. Samples were homogenised by the laboratory and a 5 g specimen taken for extraction and analysis.
Decontamination procedure	No re-useable sampling equipment was used.
Sample IDs	Terrestrial Invertebrate nomenclature - 1302_IVxxx_yymmdd
Analytical laboratories	Primary samples were submitted to Eurofins for analysis Inter-laboratory duplicate samples were submitted to NMI or ALS for analysis
Quality control	Where possible similar samples (similar size of same species from same location) were used to provide quasi duplicate samples. Ethylene glycol was used as a preservative in the invertebrate trapping and sampling methodology. Two samples of ethylene glycol that has been used in the sampling, and had come in contact invertebrates during sample collection from a pitfall trap. This include one sample of ethylene glycol directly from the pitfall trap (unstrained) and another sample after it has been strained. This was undertaken to evaluate if the ethylene glycol was extracting any PFAS from the invertebrate samples. A fresh sample of ethylene glycol was also analysed by the laboratory to evaluate whether it contained any PFAS compounds.
Sample preservation	Frozen samples were packed in eskies with additional frozen water bottles and sealed, prior to overnight dispatch to Eurofins Brisbane.

Photo 9 - Invertebrate pitfall trap

Photo 10 - Invertebrate sweep net

Photo 11 - Invertebrate UV light trap