

Direct toxicity assessments

1 Purpose and scope

This document provides general advice on requirements and considerations for sample collection for direct toxicity assessments (DTA).

2 Associated documents

Physical and chemical assessment:

- *Manual collection of surface water samples (including field filtration)*
- *Collection and preservation of sediment*

Sampling design and preparation:

- *Permits and approvals*
- *Record keeping, including taking field photographs and videos*

3 Introduction

Direct toxicity assessments (DTA) involve the use of toxicity tests to determine the acute and/or chronic toxicity of waste water discharges or total pollutant loads in receiving waters. The use of DTA allows an assessment of the toxicity of mixtures of chemicals rather than individual chemicals, and is part of both the ANZECC and ARMCANZ (2000) (water) and Simpson et al. (2013) (sediment) decision frameworks for environmental protection.

The specialised nature of these bioassays (toxicity tests) requires specific expertise rarely available outside of dedicated testing facilities. Most DTA programs rely on the availability of off-the-shelf toxicity tests that utilise standard test species. These standard tests are often accredited for use by the National Association of Testing Authorities (NATA), which provides confidence in the test results. However, the use of site-specific species should be considered where standard species are deemed unrepresentative of the local ecosystem, and can be used provided standard toxicity testing procedures are adhered to. Advice on the use of local test species should be obtained from the testing laboratory.

DTA can be conducted on water, whole sediments or aqueous extracts (e.g. elutriate or pore-waters) from sediments. Wastewater and sediment samples destined for DTA often need to be collected by non-specialists. When collecting samples for DTA, there will generally be a requirement to collect:

- wastewater from a specific release point (or sediment from a downstream location)
- uncontaminated water to be used as a diluent in toxicity tests.

4 Preparation for sampling

The analysing laboratory should be contacted early on in the planning phase before sample collection to obtain detailed advice on the appropriate procedures for collecting water or sediment samples for DTA.

When collecting sediment, wastewater or bulk diluent water for the purpose of conducting a DTA, it is important to collect the sample according to the testing laboratory's advice, which should include:

- volume of wastewater, diluent or sediment required. The volume of each of these will depend upon the type

of testing (e.g. larger organisms will require more sample volume), and the number of tests to be conducted.

- type of sample bottle/container. These may be provided by the lab. If not, the lab will advise on the requirements for sample containers
- field storage requirements (i.e. immediately placed into esky with ice bricks)
- instruction on any additives for the purpose of preservation or stabilisation
- details on laboratory holding times for the samples
- instruction on sample security
- instructions for transport
- instructions for mandatory *in situ* water quality measurements.

5 Sampling considerations

5.1 Waters

Wastewater samples collected for the purpose of DTA should represent the variation in water quality as the concentration of contaminants in wastewaters can vary over time (i.e. minutes, hours or days), and space (i.e. across a channel profile). It is important to:

- define the sampling strategy that will sample the expected variability or
- select for worst-case water quality.

Where wastewater is well mixed and homogenous over time, a single grab sample is likely to be appropriate. However, where a wastewater is expected to vary over time, then a composite sample may be appropriate.

Note: Although composite samples may be advantageous in some scenarios, composite samples may not provide information on the maximum concentration of contaminants which may be of interest in a DTA.

Follow the laboratory instructions for requirements for sampling wastewater for DTA analysis. For the actual collection of the wastewater, follow the steps provided in the *Manual collection of surface water samples (including field filtration)* document.

Additional requirements specific to collecting wastewater for DTA analysis include:

- Collect wastewater from a specific release point. Wastewater needs to be collected directly from a water off-take or at the end-of-pipe prior to mixing with any receiving environment waters. Ensure GPS co-ordinates are taken and noted.
- Wastewater samples collected for the purpose of DTA are typically not filtered prior to storage for transport, unless specifically required by the analyst.
- Water samples must be delivered to the analyst within the prescribed holding time – this is generally within 24 hours to limit the degree of sample degradation.

Note: Delay in the delivery of water samples to the analyst can also result in significant losses or transformations of toxicants (e.g. chlorine, ammonia, cyanide, pesticides), which need to be considered when interpreting the results of DTA. This is particularly important where the time for transport exceeds maximum holding times for those analytes. Therefore, where feasible, all relevant indicators need to be measured at the site and time of collection. Alternatively, additional water samples need to be collected and preserved in an appropriate manner for later analysis. Seek advice from the DTA analyst who can assess this requirement on a case-by-case basis.

It is important that physicochemical parameters of both the wastewater and the receiving environment are measured *in situ* (wherever possible), and then again prior to conducting any DTA in the testing laboratory (by laboratory staff).

The physicochemical parameters include:

- temperature
- pH
- electrical conductivity
- dissolved oxygen
- turbidity.

5.2 Diluent water

Toxicity testing requires the use of a series of different wastewater concentrations. This is achieved by dilution of the wastewater sample with water. Generally speaking, a dilution series is prepared using water collected from a location in the receiving waters known to be uncontaminated by the wastewater being investigated (i.e. upstream, up-current, or local control site). Ensure GPS co-ordinates are taken and noted.

Follow the laboratory instructions for requirements for collecting diluent water. For the actual collection of diluent water, follow the steps provided in the *Manual collection of surface water samples (including field filtration)* document.

Additional requirements specific to collecting diluent water for DTA analysis include:

- A large volume of diluent water (uncontaminated water) is required for DTA
- It is important that samples are collected up-current of significant in-stream structures and known point sources of pollutants, except where the contribution of these pollutants are to be included as a component of the toxicity tests.
- Water sample containers must be filled to the top, leaving no airspace.
- Water samples are not filtered unless required by the analyst.
- Water samples must be delivered to the analyst within the prescribed holding time – this is generally within 24 hours to limit the degree of sample degradation.

5.3 Sediment

Sediment samples are typically more heterogeneous (poorly mixed) than water and wastewater samples. In order to collect a representative sample consideration needs to be given to the exact location for collection, and whether a composite sample from a wide area, or multiple sub-samples, is more appropriate than a single discrete sample. Where multiple sub-samples are taken to produce a composite sample, or taken as discrete samples in preference to a composite sample, at least three sub-samples should be used to provide adequate replication.

Follow the laboratory instructions for requirements for collecting sediment (i.e. volume, bottle type, holding times etc.). For the actual collection of sediment, follow the steps provided in the *Collection and preservation of sediment* document.

Additional requirements specific to collecting sediment for DTA analysis include:

- A record of the number of sub-samples used in the creation of a composite samples and the depth at which they are taken must be standardised between sites and defined prior to collection.
- The distances between sub-sampling sites must be standardised between sites and defined prior to collection.
- Sediment samples must be delivered to the analyst within the prescribed holding time. Holding times will vary depending on how the sediment is to be used.
 - Where pore-waters are to be extracted for DTA, holding times should be as short as possible (i.e. 24 hours).
 - Where whole sediments are to be used for DTA, holding times and sample storage conditions should meet the requirements described for the specific compounds present, but generally no

longer than two weeks (with appropriate refrigeration; <6°C). Seek advice from the analysing laboratory.

It is important that physicochemical parameters of sediment, and the receiving environment are measured *in situ* (wherever possible), and then again prior to conducting any DTA in the testing laboratory (by laboratory staff).

The physicochemical parameters include:

- pH
- electrical conductivity
- reduction/oxidation (REDOX) potential

Note: Disturbance of sediments invariably results in changes to various other chemical equilibriums (i.e. ammonia/ammonium, sulphide speciation, metal speciation), which need to be considered when interpreting the results of DTA. Additional sediment samples may need to be collected and preserved in an appropriate manner for later analysis.

6 References and additional reading

ANZECC & ARMCANZ 2000, *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*, National water quality management strategy, Paper 4, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, ACT.

AS/NZS 1999, *Guidance on Sampling Bottom Sediments* AS/NZS 5667.12

Simpson, SL, Batley, GE and Chariton, AA 2013, *Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines*, CSIRO Land and Water Science Report 08/07, Prepared for the Department of Sustainability, Environment, Water, Population and Communities.

Simpson SL and Batley GE, 2016, *Sediment Quality Assessment*, Commonwealth Scientific and Industrial Research Organisation, Bangor NSW.

U.S. EPA 2001, *Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses: Technical Manual*, Office of Water, U.S. Environmental Protection Agency, Washington, DC.