# Direct Toxicity Assessment Proficiency Scheme (DTAPS)

## DTAPS Operational Manual

## DTAPS Document 3 - Issue 13

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Changes since last Issue in Italics

## Authority

This Operational Manual is issued by the Environment Agency to ensure that the Direct Toxicity Assessment Proficiency Scheme (DTAPS) is operated in accordance with the 'Guidelines for the Requirements for the Competence of Providers of Proficiency Testing Schemes; ILAC-G13:2000' and the 'Proficiency Testing by Interlaboratory Comparisons; ISO/IEC Guide 43-1 & 2: 1997'.

The DTAPS has been designed and implemented, and is operated, by the Environment Agency. The highest level of management at which decisions are taken on the operation of the DTAPS is that represented by the DTAPS Technical Manager

This Operational Manual is issued under the authority of the *DTAPS* Technical Manager.

……..Andrew Chappell
DTAPS Technical Manager

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## Background & Purpose

The Direct Toxicity Assessment Proficiency Scheme (DTAPS) will be used by the Environment Agency as part of the assessment process to evaluate the ability of laboratories to competently perform ecotoxicity tests used in the Direct Toxicity Assessment (DTA) of industrial effluents.

The DTAPS will form a component of the overall assessment process within the Environment Agency's Monitoring Certification Scheme (MCERTS) for DTA.

The DTAPS will be developed and operated by the Environment Agency but the assessment process may also utilise appropriate data generated by other interlaboratory ecotoxicity test comparisons.

The primary aims of the DTAPS will be to establish accuracy and precision (intra and inter laboratory) criteria for laboratories undertaking, or intending to undertake, the ecotoxicological test methods specified in DTA guidance.

This document has been produced by the Environment Agency to provide;

i) a Quality Management and Technical Systems manual for the operation of the scheme,
ii) guidance and instruction to participating laboratories on the specific conduct of the scheme,
iii) a guide to external assessors of the provider and scheme regarding its structure and operation.

# Section 1: General

## 1.1 Scope

This document sets out the criteria that the provider of the DTAPS (and associated collaborators and contractors) shall meet in the development and operation of the scheme.

The document also provides guidance and instruction on laboratory participation in the scheme.

The provider will take full responsibility for ensuring that the operational requirements (both technical and management systems) of the scheme are met by the provider itself and any collaborators or sub-contractors.

## 1.2 References

Environment Agency (2006) Integrated Pollution Prevention and Control (IPPC), Guidance on the use of Direct Toxicity Assessment in PPC Impact Assessments.

Environment Agency (2001) Assessment of the Options for a Regulatory Ecotoxicology Testing Quality Scheme (RETQS), Research & Development Technical Report P426.

Environment Agency (1996) Performance Standards for Ecotoxicity Tests, Research & Development Technical Report SR 4166/1.

Environment Agency (1999) Report on a ring test of the 48 h Tisbe battagliai lethality test, Research & Development Technical Report E90.

Environment Agency (1999) A Proposed Scheme to Ensure the Quality of Data Generated by Laboratories Undertaking Regulatory Ecotoxicological Testing, Research & Development Technical Report P166.

British Standards Institute (2005) General requirements for the competence of testing and calibration laboratories BS EN ISO/IEC 17025:2005.

Environment Agency Monitoring Certification Scheme (MCERTS) (2006) Performance Standard for Laboratories Undertaking Direct Toxicity Assessment of Effluents.

Environment Agency Monitoring Certification Scheme (MCERTS) (2006) Laboratory Assessment.

Environment Agency Monitoring Certification Scheme (MCERTS) (2007) MCERTS for DTA Steering Committee Terms of Reference.

ILAC (2000) Guidelines for the Requirements for the Competence of Providers of Proficiency Testing Schemes, ILAC-G13:2000.

British Standards Institute (1997) Proficiency testing by interlaboratory comparisons – Part 1: Development and operation of proficiency testing schemes, ISO/IEC Guide 43-1:1997.

British Standards Institute (1997) Proficiency testing by interlaboratory comparisons – Part 2: Selection and use of proficiency testing schemes by laboratory accreditation bodies, ISO/IEC Guide 43-2:1997.

### 1.3 Definitions

**Provider** The body that undertakes the design, conduct and operation of the proficiency scheme.

The provider of the DTAPS is the Environment Agency. Full contact details are given below:

Andrew Chappell
Environment Agency
Rose Kiln Lane
Reading
Berks RG2 0SF

**Collaborator** An organisation operating a similar proficiency scheme that supplies or allows the supply of proficiency testing data on its participants to the provider for assessment within the DTAPS.

**Contractor** An organisation undertaking contracted activities for the provider of the DTAPS.

**Coordinator** The person with responsibility for coordinating all of the activities involved in the operation of the proficiency testing scheme. The coordinator of the DTAPS shall be the DTAPS Technical Manager.

**Direct Toxicity Assessment Proficiency Scheme (DTAPS)**
An ecotoxicological interlaboratory testing scheme designed and operated to ensure the performance of laboratories undertaking DTA testing for regulatory submission.

**DTAPS Round** A single operation of the DTAPS.

# Section 2: Technical Systems

## 2.1 Expertise & Experience

The Environment Agency's Science Department (and it's associated and predecessor sections and laboratories) has been a leader in the development of all five test methods used within the DTA regime, and has also developed a series of test method guidelines for use by laboratories performing such tests for use in the regulation of industrial effluents.

As well as developing the tests themselves, the Environment Agency has also been very closely involved with previous research into the use of a proficiency testing scheme to quality assure the results of DTA tests performed by contract testing laboratories.

This includes the development of performance standards for ecotoxicology testing, and research into the structure and operation of a 'Regulatory Ecotoxicology Testing Quality Scheme (RETQS)'. Much of the structure and operational aspects of the DTAPS are based directly on the conclusions of this previous research, with respect to the proficiency testing of ecotoxicolgical tests.

## 2.2 Staff Training

The provider's formal training policy and procedures for the DTAPS are given in the DTAPS Training Manual (DTAPS 8).

## 2.3 Collaborators and Contractors

The provider may use suitably evaluated and selected collaborators and contractors to undertake specific tasks for the DTAPS. Collaborators are those individuals and/ or organisations providing reciprocal services to the DTAPS, such as the sharing of proficiency testing data with the providers of other similar schemes. Contractors are those individuals or organisations providing services to the DTAPS provider for a fee, such as the chemical analysis of reference materials.

Both collaborators and subcontractors will be selected on the basis of their ability to meet the technical and quality assurance requirements of the DTAPS relevant to the tasks they are contracted to, or have agreed to, undertake. In particular, collaborators and contractors will be selected on the basis of their compliance with the relevant clauses of the ILAC-G13 and ISO/IEC guidelines.

All collaborators and contractors involved in the provision of the DTAPS will be listed in the DTAPS Register, DTAPS 2, including full contact details. The organisational and technical responsibilities of collaborators and contractors involved shall be identified and regularly reviewed. All documentation pertaining to such reviews and assessments will be held in the DTAPS Register (DTAPS 2).

## 2.4 Organisation and Design Logistics

The provider will supply prospective participating laboratories with the following documentation on expression of interest:

- DTAPS 3 (current version);
- instructions on how to apply for registration onto the scheme.
- *Other documents can be provided on request.*

The provider will give all registered participants a minimum of 4 weeks notice of the commencement of each round of the DTAPS. At (or before) this time, the provider will distribute documented instructions to all registered participants which will include as a minimum:

- the nature of test materials to be supplied by the provider;
- conditions of storage of reference materials;
- tests to be proficiency tested;
- timings of the specific operational round;
- the number of registered participants for each method;
- result reporting instructions.

All registered participants will also be automatically issued with new versions of DTAPS 1 and 3 following any changes, amendments or additions to the scheme or DTAPS documentation.

The identity of each participant in the DTAPS will be known only to the provider but may be communicated to contractors if deemed necessary (and subject to a confidentiality agreement between contractor and Environment Agency). Participation details will be confidential to all other participants and collaborators unless individual participants elect to waive such confidentiality. A written confidentiality waiver must have been received and registered by the provider prior to any communication with others regarding participant identity or proficiency testing data sharing. Participants may wish to waive confidentiality within the participant/ collaborator for a number of reasons including discussion and mutual assistance (e.g. through the MCERTS for DTA Steering Committee), or for regulatory or accreditation purposes.

### 2.4.1 Scheme Objectives

The objectives of the scheme are to generate proficiency data allowing the inter-laboratory comparison and assessment of laboratories performing Direct Toxicity Assessment (DTA) testing for regulatory purposes. Laboratories participating in the scheme will be assessed in terms of accuracy, defined as the proximity of results to a target (reference) value, and precision, defined as the variability over a number of repeat measurements, for each test method.

### 2.4.2 Participation in the Scheme

Any laboratory undertaking or wishing to undertake ecotoxicological test methods will be eligible for participation in the DTAPS.
The DTAPS also forms a primary component of the overall assessment process within the Environment Agency's Monitoring Certification Scheme (MCERTS) for Direct Toxicity Assessment (DTA).
Laboratories wishing to be MCERTS registered for DTA testing must participate in the DTAPS.

Participants are generally expected to be laboratories with experience of performing ecotoxicological test methodologies, but this does not preclude laboratories or organisations wishing to establish such a capability. The numbers of participants may vary between the different test methods to be proficiency tested, and the methods of statistical treatment of results may depend on the number of participants in each round of testing.

### 2.4.3 Scheme Design

The scheme will comprise two components as described below.

**i) Accuracy Assessment**

Accuracy assessments will be made for both intra and inter-laboratory performance. Accuracy assessments will be based on a combination of internal quality control data (supplied directly by participants) and data derived from an inter-laboratory proficiency testing programme.

**ii) Precision Assessment**

Precision assessments will also be made for both intra-laboratory performance. Precision assessments will be based on internal quality control data (supplied directly by participants).

Each round of the DTAPS will include full assessments for both accuracy and precision for each laboratory/ method.

### 2.4.4 Planning of DTAPS Rounds

A plan and schedule for the operation of each round of the DTAPS will be drafted by the coordinator, reviewed and agreed by the MCERTS for DTA Steering Committee, and provided to all registered participants prior to the commencement of each DTAPS round. The plan and schedule for each round will also be published as an Appendix to the DTAPS Operational Manual (DTAPS 3).

The DTAPS round plan shall be used in conjunction with the DTAPS Quality Manual (DTAPS 1) and Operational Manual (DTAPS 3) and will comprise round specific information detailing:

- a full description of the manner in which test items (reference toxicant solutions) are prepared, processed, checked for homogeneity/ stability and distributed;
- a time schedule for the various phases of the DTAPS round;
- information on test methodologies or guidelines to be followed;
- an outline of any data analysis required (by the participant) to determine assigned values, including statistical techniques;
- a full description of all data or information to be returned to the provider (or contractor) following testing;

- a full description of the methods and procedures used to analyse the proficiency scheme data and assess individual laboratory performance.

Technical aspects of the DTAPS will be reviewed periodically by the MCERTS for DTA Steering Committee. This will include a review of proficiency testing procedures to ensure their continuing suitability and effectiveness and to introduce any necessary changes or improvements. Reviews will also account for any reports from the provider's quality management, the outcome of recent internal audits, feedback or complaints from participants and any other factors relevant to the operation of the DTAPS. Copies of minutes of all MCERTS for DTA Steering Group meetings will be retained in the DTAPS Register (DTAPS 2).

### 2.4.5 Preparation of Test Items (Reference Toxicants)

For each round of the DTAPS batches of reference toxicant solutions will be prepared and distributed by a nominated contractor (under contract to the provider). Preparation will be by means of dissolving appropriate weights of a reference chemical in a suitable carrier solvent (e.g. distilled/ deionised water, methanol). Batches of reference toxicant may also be stabilised by the addition of other chemicals (e.g. conc. Hydrochloric acid for the acidification of solutions of metal salts).

Each batch of reference toxicant will be prepared prior to commencement of the DTAPS round. The batch will then be divided into sub-samples which will be stored in the dark at 2-8°C before being distributed to participants. Participants should store provided sub-samples of reference toxicant in the dark at 2-8°C until used for testing.

Sub-samples of each batch of reference toxicant will be provided to participants in acid washed glass or plastic bottles that are clearly labelled with batch code and date of preparation. Details of chemical composition and concentration may be provided (where appropriate). All sub-samples should be treated identically to effluent samples of unknown composition (including health and safety considerations), unless stated otherwise in the instructions accompanying the distributions. The sub samples will be securely packaged to minimise the potential for breakage and distributed to participants using an appropriate service provider.

### 2.4.6 Homogeneity, Stability and Verification testing of Test Items (Reference Toxicants)

Prior to distribution to participants, and during the proficiency testing phase, each batch of reference toxicant will be chemically analysed by a UKAS accredited chemical analysis laboratory to provide data supporting the;

- correct preparation/ concentration of each batch (e.g. concentration as zinc),
- homogeneity and stability of batches and sub-samples.

The laboratory used to provide this service will be fully detailed in the DTAPS Register (DTAPS 2).

The analysis will be performed in duplicate;

- on two randomly selected sub-samples prior to despatch to participants,
- at appropriate intervals on further sub-samples.

All information and data pertaining to the preparation, distribution, and homogeneity, stability and verification testing of reference toxicants will be recorded in accordance with the provider's recording and document control procedures, and will be retained in the DTAPS Operational Record (DTAPS 4).

## 2.5 Test Methods

The methods to be used within the DTAPS will be, as a minimum, those methods that are prescribed in the MCERTS performance standard for generation of DTA data to be submitted to the Environment Agency for regulatory purposes.

From time to time, the DTAPS may additionally be used as a vehicle to provide inter-laboratory quality support to programmes of work (involving the use of biological effects analysis methods) beyond the regulatory application of DTA. In such cases, non-DTA biological effects analysis methods (i.e. not prescribed in the MCERTS for DTA Performance Standard) may be included. The type and purpose of all such methods will be clearly defined in the appropriate Appendix relating to the specific round of the DTAPS within which the additional method is to be included.

The prescribed methods will be published by the Standing Committee of Analysts (SCA) or provided by the Environment Agency. Published methods may be downloaded from the Environment Agency website at www.environment-agency.gov.uk.

Participants will be expected to perform tests to their own standard operating procedures which conform to the prescribed SCA guidelines.

Participation of a laboratory in the scheme may extend from one to all of the prescribed methods. A laboratory must participate in the DTAPS for all the methods for which they wish to be MCERTS for DTA approved.

## 2.6 Conduct of a DTAPS Round

All registered participants for each round of the DTAPS will be given a minimum of four weeks notice of the distribution date of proficiency test samples.

Inter-laboratory testing samples will be distributed directly from the nominated contractor, using an appropriate transportation service.

The inter-laboratory testing sample containers will be appropriately packaged so as to prevent them (as far as is possible) from becoming damaged or otherwise compromised (e.g. contaminated). The bottles containing the samples will be unambiguously labelled to indicate their purpose and to uphold traceability. The samples should be treated by participants in an identical manner to effluent samples of unknown composition in all respects (including regard to health and safety considerations) unless stated otherwise in the instructions accompanying the samples (see below).

Detailed instructions on the testing of provided proficiency samples along with appropriate data recording sheets, instructions on data analysis, full data return requirements (i.e. inter-laboratory reference testing and internal quality control data) and deadlines for submission will be provided with the proficiency testing samples.

Participants must complete the tests they have nominated to undertake using the inter-laboratory testing samples, and then return the raw and processed test results and specified internal quality control data to the provider or specified contractor within the specified deadlines (detailed in the round-specific Appendix of this document).

The provider or nominated contractor will then collate the data returns, perform the required data analyses and provide feedback on individual laboratory performance (according to the performance criteria specified in the DTAPS) prior to drafting a full report on the results of the DTAPS round. This report will be reviewed and discussed by the MCERTS for DTA Steering Committee prior to being finalised and distributed to participants.

## 2.7 DTAPS Performance Criteria

The performance criteria for both accuracy and precision will be derived by the provider and reviewed and approved by the MCERTS for DTA Steering Committee. The agreed performance criteria will be specified in the appropriate appendix of this document (dependant on the annual round currently taking place).

The criteria (e.g. target values) used for performance assessment within the DTAPS will be derived independently of the DTAPS round currently being undertaken. Valid proficiency testing data derived in each round of the DTAPS will be used to update the criteria (if appropriate) for each subsequent round of the scheme.

Laboratories will not be automatically penalised (e.g. removed from the MCERTS for DTA approved laboratory register) by failure to meet the specified DTAPS performance criteria. Participation in the DTAPS for each method for which MCERTS for DTA approval is sought will be sufficient for a laboratory to satisfy the initial proficiency testing requirements of the MCERTS for DTA Standard.

Laboratories must, however, establish a procedure to investigate and address any proficiency test results submitted to the scheme provider which fail to meet the specified performance criteria. Laboratories which fail to meet the specified performance criteria will receive feedback from the scheme provider and will be required to provide possible reasons and potential solutions for the compromised test performance as part of their internal investigative process. The provider will support this process where possible and may be able to provide assistance to the participant in solving any problems in the testing process which may have been highlighted in the investigations.

The Environment Agency reserves the right to remove any laboratory from the MCERTS for DTA register of approved laboratories which repeatedly fails to meet the specified performance criteria and/ or does not appear to be adequately addressing such failures.

## 2.8 Data Treatment and Analysis

### 2.8.1 Test Data Processing

Participating laboratories will be required to calculate (and report) an EC50 value (and appropriate confidence limits) for each test undertaken with the proficiency testing samples. This should be achieved using the method or procedure that is routinely used for calculating such values in tests with environmental samples and internal quality control tests (reference testing).

Test results should be expressed as a nominal percentage volume/ volume of each proficiency testing sample as provided, unless otherwise specified in the detailed instructions provided to participants.

### 2.8.2 Laboratory Performance Assessment

The provider or nominated contractor will undertake analysis and assessment of the proficiency scheme data as generically outlined below. Further detail concerning such data analysis and laboratory performance assessment (including specific performance criteria) is provided in the appropriate appendix of this document depending on the annual round currently taking place.

#### i) Accuracy

Accuracy assessments (both intra and inter-laboratory) will be based on a Z scoring method utilising a suitable (independently derived) reference value and error target for each method.

The equation used to calculate a Z score for each laboratory/ method/ sample combination will be as follows:

*Z* = [Laboratory Result – Ref. Value] / Target Standard Deviation Value

Inter-laboratory accuracy assessments will be based on a Z score that compares a laboratory's individual proficiency testing results to the specified reference value.

Intra-laboratory accuracy assessments will be based on a Z score comparing the laboratory's mean internal quality control (reference testing) result to the specified reference value.

**ii) Precision**

Intra-laboratory precision assessments will be based on a comparison of variances using a $\chi^2$ test.

The variance ($S^2$) of a number of repeat measurements can be compared with an independently derived target variance value ($\sigma^2$) (accounting for the sample size) using the equation:

$$\chi^2 = [S^2 / \sigma^2] \times [n-1]$$

The estimated $\chi^2$ value is then compared to a critical $\chi^2$ value for the desired level of confidence and accounting for the sample size.

An acceptable level of variance is indicated by an estimated $\chi^2$ value which is less than the critical $\chi^2$ value. That is, the variability between repeat tests is less than expected based on the selected reference value.

## 2.9 Reporting

### 2.9.1 Reporting by DTAPS Participants

Laboratories participating in the DTAPS will be required to report both proficiency testing and internal quality control data. Both sets of data must be submitted to the provided within the timescales specified in the appropriate appendix of this document (depending on the annual round taking place).

Submissions must comprise:

i) raw data and calculated results from the testing of the provided proficiency test samples, and

ii) internal quality control data which must include, as a minimum;

- a current internal process control chart that includes:
  - control (warning & action) limits which have been derived from at least six reference tests with the toxicant zinc sulfate ($ZnSO_4$.7H2O),
  - a minimum of six plotted reference results derived from tests with the toxicant zinc sulfate ($ZnSO_4$.7H2O) - performed between the dates specified in the appropriate appendix of this document (depending on the DTAPS round currently being undertaken) and independent of the results used to derive the chart limits,
  - raw data for the reference tests plotted on the chart.

It is recognised that some laboratories may have a considerable reference testing data set and that precision measurements are likely to be more reliable if based on larger data sets (i.e. n > 6). For this reason, participants are encouraged to submit as much recent (last 12 months) reference data as possible. Such data should be submitted as raw test data and calculated results, and as plotted points on the appropriate process control chart.

### 2.9.2 Reporting by the DTAPS Provider

The provider will report the results of each DTAPS round in the following manner.

Individual laboratory performance summary reports will be provided to each participant on completion of inter-laboratory testing, data collation and analysis and laboratory assessments. These summary reports will only contain information that is directly relevant to individual laboratories, i.e. feedback on individual laboratory in comparison to the specified performance criteria.

A full report detailing the operation and results of each round of the DTAPS, and incorporating any other reports (e.g. from contractors performing tasks within the DTAPS), will be drafted by the provider. This report will be reviewed and discussed by the MCERTS for DTA Steering Committee prior to being finalised and distributed to participants.

## 2.10 Communication

Communication with regard to all aspects of each operational round of the DTAPS will be between provider and individual participants, collaborators or contractors. Contractors undertaking specific DTAPS tasks for the provider (e.g. distribution of inter-laboratory testing samples or collation of participant data returns) may be authorised by the provider to communicate directly with participants but the permitted communication will be fully defined prior to the operation of each round of the DTAPS and limited to essential communication only.

## 2.11 Collusion and Falsification of Results

All appropriate steps will be taken by the provider to ensure there is as little opportunity as possible for collusion and falsification of proficiency testing results.

It must be recognised that although the provider will do as much as possible to prevent collusion and falsification, the primary responsibility for such prevention lies with the participants in the scheme. In the event of participant collusion or result falsification being discovered and substantiated by the provider, the participant(s) in question will be expelled from the DTAPS, and thus are likely to also lose (or fail to gain) their MCERTS status for DTA testing.

## Appendix A

### 1. Test Methods

The tests to be proficiency tested in support of regulatory DTA activities will be the following:

*Daphnia magna* 48 hour immobilisation test,
*Tisbe battagliai* 48 hour mortality test,
Oyster (*Crassostrea gigas*) 24 hour embryo-larval development test,
Freshwater algae (*Pseudokirschneriella subcapitata*) 72 hour inhibition of growth test,
Marine algae (*Skeletonema costatum*) 72 hour inhibition of growth test.

The methods to be used in the performance of the tests will be those published by the Standing Committee of Analysts (SCA).

Published SCA methods are available from the Environment Agency website at www.environment-agency.gov.uk.

## 2. Test Items

The reference chemical solutions to be used by participants for proficiency testing will be prepared, sub-sampled and distributed to participants by the Environment Agency or their nominated contractors.

The reference chemical used for the DTA tests will be zinc sulfate ($ZnSO_4.7H_2O$) dissolved in reverse osmosis (RO) water and acidified/ stabilised with concentrated Hydrochloric Acid.

Samples at two different reference concentrations (Samples A & B) will be prepared and sub-samples of each provided to participants. The zinc solutions will be provided in 500 mL volumes in plastic bottles. Additional sub-samples will be held by the provider and chemically analysed for concentration, homogeneity and stability.

Sub-contractors nominated by the provider will be used to prepare, distribute and chemically analyse the test items.

The zinc reference solutions to be used by participants for internal laboratory reference testing must be prepared by the participating laboratory. The participating laboratory must also generate data that demonstrates the zinc concentration (and homogeneity and stability) of their internal reference solutions.

## 3. Instructions for Participation

### 3.1 Inter-laboratory Proficiency Testing

Each registered participant will receive two proficiency testing reference chemical samples (A & B) for the DTA tests plus any further instructions on their treatment and storage. Data sheets for the recording of proficiency testing data will be provided separately from the samples (by the provider).

Participants will be required to test both samples using each method they have elected to undertake for the scheme. The samples should, upon receipt at the participating laboratory, be treated identically to effluent samples undergoing DTA analysis, including all health and safety considerations.

Inter-laboratory tests using the supplied samples must be completed and results submitted to the Environment Agency by the date specified in Section 6 of this Appendix.

### 3.2 Intra-laboratory Testing

Internal reference testing data comprising (as a minimum),

- a current internal process control chart that includes:
    - control (warning & action) limits which have been derived from at least six reference tests,
    - a minimum of six plotted reference results derived from tests performed between *4th October 2010 and 3rd October 2011* and independent of the results used to derive the chart limits,
    - raw data for the reference tests plotted on the chart.
- chemical analysis data to demonstrate zinc concentration, homogeneity and stability of the internal reference standard(s) used,

must be submitted to the Environment Agency by the date specified in Section 6 of this Appendix.

## 4. Data Analysis

### 4.1 Participants

Participants must calculate and report an EC50 value (as % v/v sample) for both proficiency testing samples (A & B). The EC50 values should be calculated using an identical statistical method and/or software package as used by the participant to calculate results for effluent samples undergoing DTA analysis.

Internal reference test results (EC50s as nominal or measured zinc) used to construct process control charts and monitor internal quality control must also be calculated using an identical statistical method to that used to calculate results for effluent samples undergoing DTA analysis.

### 4.2 Provider/ Contractor

The provider will chemically measure the zinc concentrations of the proficiency testing samples (A & B) as well as chemically monitoring the homogeneity and stability of the samples over the duration of the sample testing phase.

The Environment Agency will collate the participant returns and convert each participant's results from % v/v sample (A or B) into a zinc concentration, as appropriate.

## 5. Performance Criteria

### 5.1 Accuracy

The Environment Agency will calculate two inter-laboratory Z scores for each laboratory for each method. There will be one score for each sample (A and B) and it will be based on the participant laboratory's internally calculated EC50. The Environment Agency will assess individual laboratory performance based on a comparison of the calculated Z scores with the accuracy performance criteria shown below.

The Environment Agency will additionally calculate an intra-laboratory Z score for each laboratory for each method based on their internal mean EC50 (nominal or measured zinc concentration). Laboratory performance will be assessed based on a comparison of the calculated Z score with the accuracy performance criteria shown below.

| **Accuracy Performance Criteria (All Methods)** | | |
| --- | --- | --- |
| 2 > **Z** > -2 | | |
| | **Z score Reference Value (mg/l Zn)** | **Standard Deviation Target** |
| Daphnia magna 48hr immobilisation | *1.58* | 0.51 |
| Tisbe battagliai 48hr mortality | *0.30* | *0.14* |
| Oyster embryo-larval development | *0.199* | 0.06 |
| Freshwater algae inhibition of growth | 0.076 | 0.03 |
| Marine algae inhibition of growth | 0.1 | 0.03 |

## 5.2 Precision

The provider will calculate a variance ($S^2$) for each laboratory for each method based on their internal quality control data returns.

Laboratory performance in terms of variability over repeat measurements will be assessed based on a comparison of these values to the internal precision performance criteria shown below.

| Method | Internal Target Variance ($\sigma^2$) |
| --- | --- |
| Daphnia magna 48hr immobilisation | 0.03 |
| Tisbe battagliai 48hr mortality | 0.03 |
| Oyster embryo-larval development | 0.03 |
| Freshwater algae inhibition of growth | 0.03 |
| Marine algae inhibition of growth | 0.04 |

## 6. Schedule

| Dates | Tasks |
| --- | --- |
| *18th February 2011* | Distribution of invitations to participate. |
| *1st March 2011* | Deadline for participant registration |
| *March 2011* | Finalise participants and total numbers for each method. Arrange sample distribution. |
| ***Week Commencing: 6th June 2011*** | Distribution of proficiency testing samples. |
| ***6th June 2011 – 3rd October 2011*** | Testing of samples. |
| ***10th October 2011*** | Deadline for submission of data returns (proficiency testing & internal) by participants to the Environment Agency. |
| *11th October – 15th November 2011* | Provider Data Analysis. |
| *16th – 30th November 2011* | Laboratory Summary Report Drafting. |
| ***Week Commencing: 5th December 2011*** | Individual Round 6 reports to participants. |
| *December 2011* | Participant internal investigations into non-compliant results. |
| ***February 2012*** | DTAPS Rounds 6 Final Report dissemination |

# Please Read these instructions carefully before undertaking any tests.

1. The proficiency testing samples you have received comprise 4 x 500mL solutions of zinc sulfate ($ZnSO_4$) in deionised water (Labelled A & B). The samples have been acidified and stabilised with an appropriate volume of conc. Hydrochloric acid.

   Please contact Andrew Chappell (01189535332) immediately if you have not received 2 x 500mL of Sample A and Sample B. Additional samples are available on request, please contact Andrew Chappell to request additional bottles of sample.

2. On receipt, the proficiency testing samples should be treated identically to industrial effluent samples received for DTA testing (this includes all appropriate Health & Safety considerations).

3. After receipt, samples should be stored in the dark at 2-8ºC.

4. Sample A must be tested as received. Sample B must be diluted 1:100 (1%) with test media (which will depend on the test method being undertaken) prior to use in proficiency tests.

5. Both samples should be tested with each DTA test method your laboratory has opted to perform. The test should be designed according to the specified guidelines, and in such a way that both an EC50 value, interpolated within the range of test concentrations (as % v/v sample), and a No Observed Effect Concentration (NOEC) can be calculated.

6. Raw data for proficiency tests should be recorded on the test specific datasheets provided with the samples.

   If you have not received copies of all the datasheets for the tests your laboratory has opted to perform, please contact Andrew Chappell immediately.

7. On completion of the proficiency tests, EC50 and NOEC values should be calculated for each test/ sample combination (as % v/v sample).

8. Please remember to account for the initial dilution of Sample B in your calculations.

9. For each test/sample combination the following information/ data must be reported/ returned:

i) Raw data for each test recorded on the appropriate DTAPS test datasheet.
ii) EC50 and NOEC calculations for each test as software print-out (or other method of derivation).

All DTAPS proficiency testing data must be submitted to Andrew Chappell by *10th October 2011*.

10. An additional element of the DTAPS is the further assessment of participants proficiency (precision) based on a laboratory's internal analytical quality control data (See MCERTS for DTA Performance Standard, DTAPS 1.0 and DTAPS 3.0).

This data (as outlined in the above documents) must also be submitted to Andrew Chappell by *10th October 2011.*

11. All tests should be undertaken according to the published SCA methods.

These are available at:

http://www.environment-agency.gov.uk/business/regulation/38783.aspx

12. Please note especially the requirements of the Freshwater and Marine Algal Growth method guidelines with respect to the composition of test media (particularly EDTA) and calculation of test endpoints (using biomass-based data only).

13. Daphnia tests must be undertaken using the artificial media described in Section 4.3.1 and Table 4 of the SCA guideline for the Daphnia immobilisation test. This has been highlighted as a significant (but easily controlled) contributor to inter-laboratory variability for this test.