Joint Test Plan for Verification of HACH-LANGE GmbH LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer

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FOREWORD

DHI DANETV Water Test Centre (DHI) which operates a Danish verification scheme, DANETV, supported by the Danish Ministry for Science, Technology and Innovation; the United States Environmental Protection Agency Environmental Technology Verification Program's Advanced Monitoring Systems (U.S. EPA ETV AMS) Center operated by Battelle (Battelle AMS Center) under a Cooperative Agreement with the U.S. EPA; and Ontario Centre for Environmental Technology Advancement (OCETA) through ETV Canada which operates the Canadian ETV Program on behalf of Environment Canada are conducting an international joint verification test of the HACH-LANGE GmbH water toxicity technologies named LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer.

This document, which is a compilation of three separate documents, will be used by DHI, the U.S. EPA ETV AMS Center, and ETV Canada to jointly verify the performance of these technologies. It is composed of a process document, a verification protocol, and a test plan for joint verification. Combined, these three documents satisfy the requirements of a U.S. EPA ETV approved verification test/QA plan and ensure that the requirements of all three programs are met. The verification protocol and test plan were developed by DHI with input from Battelle, U.S. EPA, ETV Canada, and other stakeholders. Together these documents satisfy DANETV's programmatic requirements. The process document was developed by the Battelle AMS Center with input from DHI, ETV Canada, and U.S. EPA. It was developed as a supplement to the DHI documents, to ensure that all of the U.S. EPA ETV programmatic requirements are met. All three documents were reviewed and approved via the ETV process prior to the start of testing.

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EXECUTIVE SUMMARY

DHI DANETV Water Test Centre (DHI) which operates a Danish verification scheme, DANETV, supported by the Danish Ministry for Science, Technology and Innovation; the United States Environmental Protection Agency Environmental Technology Verification Program's Advanced Monitoring Systems (U.S. EPA ETV AMS) Center operated by Battelle (Battelle AMS Center) under a Cooperative Agreement with the U.S. EPA; and Ontario Centre for Environmental Technology Advancement (OCETA) through ETV Canada which operates the Canadian ETV Program on behalf of Environment Canada are jointly verifying the HACH-LANGE GmbH water toxicity technologies named LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer.

Under this joint effort, DHI was responsible for developing the verification protocol, preparing the test plan including quality assurance (test/QA), testing, and generating the verification report/verification statement in accordance with their requirements. The Battelle AMS Center provided technical and quality assurance oversight throughout the DHI process to ensure U.S. EPA ETV requirements were also met, and facilitated reviews and audits by QA personnel, U.S. EPA Program management, and stakeholders of the verification protocol, test plan, testing, and verification report. The Battelle AMS Center also developed a process document to supplement the protocol and test plan developed by DHI to ensure that all of the U.S. EPA ETV programmatic requirements were met. The process document, combined with the protocol and test plan, satisfy the requirements of a U.S. EPA approved verification test/QA plan. This document is a compilation of these three documents. ETV Canada also provided technical and quality assurance oversight throughout the DHI process to ensure that the Canadian ETV program requirements were met.

The purpose of this verification is to evaluate the performance of a wastewater rapid toxicity technology that could be used to monitor industrial or domestic wastewater. The verification protocol covers two products from the same vendor, both are acute toxicity tests with luminescent bacteria. In the bioluminescence testing with LUMIStox and ECLOX, a strain of naturally occurring luminescent bacteria, *Vibrio fischeri*, is used. *Vibrio fischeri* is a non-pathogenic, marine, luminescent bacterium which is sensitive to a wide range of toxicants. When properly grown, luminescent bacteria produce light as a by-product of its cellular respiration. Any inhibition of cellular activity results in a decreased rate of respiration and a corresponding decrease in the rate of luminescence. The light emission/luminescence can be measured with a LUMIStox or ECLOX luminometer. Inhibition of the light emission in the presence of a sample is determined against a non-toxic control. Verification parameters will include determination of the criterion of detection, the range of application, precision as measured by repeatability and reproducibility, agreement with accepted values, and robustness. Other parameters such as an evaluation of the user manual, product costs, and environmental and occupational health and safety aspects will also be included.

Environmental Technology Verification Program

Advanced Monitoring Systems Center

Process Document for
U.S. EPA ETV AMS Center
DHI DANETV Water Centre
and
ETV Canada
Joint Verification of the
HACH-LANGE GmbH
LUMIStox 300 Bench Top Luminometer
and
ECLOX Handheld Luminometer

Process Document for

U.S. EPA ETV AMS Center

DHI DANETV Water Centre

and

ETV Canada

Joint Verification of the

HACH-LANGE GmbH
LUMIStox 300 Bench Top Luminometer
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January 12, 2010

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1 INTRODUCTION

DHI DANETV Water Test Centre (DHI) which operates a Danish verification scheme, DANETV, supported by the Danish Ministry for Science, Technology and Innovation; the United States Environmental Protection Agency Environmental Technology Verification Program's Advanced Monitoring Systems (U.S. EPA ETV AMS) Center operated by Battelle (Battelle AMS Center) under a Cooperative Agreement with the U.S. EPA; and Ontario Centre for Environmental Technology Advancement (OCETA) through ETV Canada which operates the Canadian ETV Program on behalf of Environment Canada will jointly verify the HACH-LANGE GmbH (Vendor) water toxicity technologies named LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer. This document establishes a process to ensure that the requirements of all three programs will be met for the joint verification to be successful.

The purpose of all three ETV programs is to provide objective and quality-assured performance data on environmental technologies, so that users, developers, regulators, and consultants can make informed decisions about purchasing and applying these technologies. Figure 1 describes the organizational relationships for this joint verification. DHI will perform the verification protocol preparation, the test plan preparation, including quality assurance (QA), testing, and verification report/test report/verification statement preparation in accordance with DANETV requirements. To ensure U.S. EPA ETV program acceptance of the verification, the Battelle AMS Center will provide technical and quality assurance oversight throughout DHI's process to confirm that each step meets the U.S. EPA ETV program requirements. The Battelle AMS Center will facilitate the necessary reviews and audits, coordinate stakeholders and QA personnel for the verification protocol, the test plan, the testing activities, and the verification reports and statements. This will provide the necessary oversight for the U.S. ETV program to ensure a quality process of evaluating, data collection, and reporting for this project, which was deemed an EPA QA Level III project by the EPA ETV AMS Center Project Officer. ETV Canada will facilitate the necessary reviews and audits by Environment Canada program management, stakeholders, and QA personnel for the verification protocol, the test plan, the testing activities, and the verification report. This will provide the necessary oversight for the Canadian ETV program to ensure a quality process of evaluating, data collection, and reporting. Because DHI is performing the technical work and preparing the drafts of the documents, it is assumed that DHI will follow

the necessary process to ensure a Danish ETV verification from their actions. Therefore, this document is focused on what Battelle, ETV Canada and DHI must do to ensure that the verification fulfills the requirements of the U.S. EPA ETV program and the Canadian ETV program. The efforts of DHI in testing and verification are described in a verification protocol and a test plan prepared in compliance with the DANETV Centre Quality Manual (CQM)[1]. Together the process document, verification protocol, and test plan are the equivalent of the U.S.EPA ETV AMS Center test/QA Plan and an ETV Canada technology specific test/QA plan. The roles of each participant shown in Figure 1 are described in the following section. The names of the key personnel and their roles during this verification are presented in Appendix A.

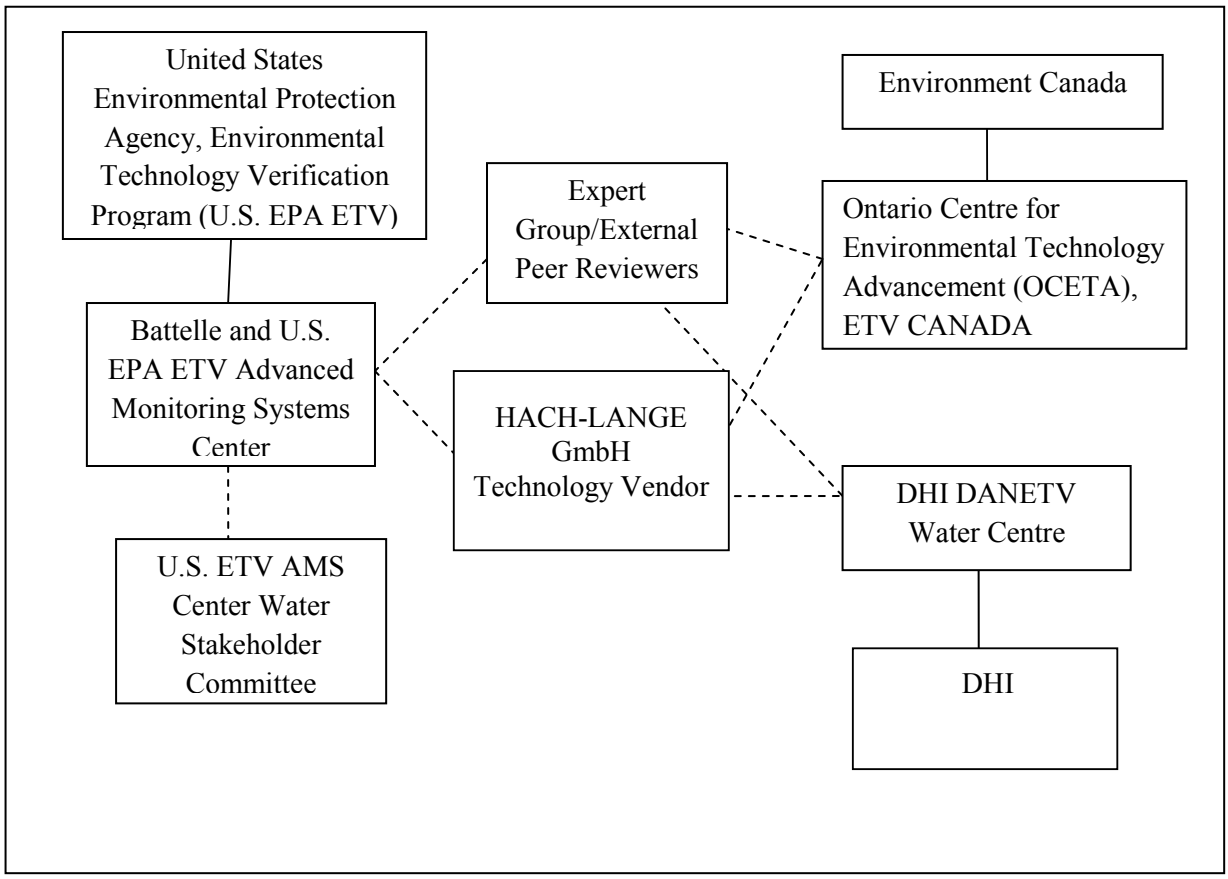


Figure 1. Organization Chart for the Joint Verification Test of HACH-LANGE GmbH LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer

1.1 HACH-LANGE GmbH Involvement

HACH-LANGE GmbH, the technology vendor, has entered into a Verification Contract with DHI and a joint verification agreement with all three parties, DHI, Battelle and ETV Canada. The vendor will provide the following support during joint verification of the vendor's technology:

- A person from vendor's organization to be Battelle's, ETV Canada's, and DHI's technical points of contact and to lead vendor's participation in verification of vendor's technology; and
- Review and comment on the process, plan, and report documents which will jointly satisfy documentation requirements of the U.S., Canadian, and Danish ETV programs; and other documents pertaining to verification of vendor's technology as requested by Battelle and/or ETV Canada and/or DHI; and
- Permission to post/cite information about vendor's technology, including the Verification Protocol, Test Plan, Verification Report, and Joint Verification Statement, on the U.S. ETV website (www.epa.gov/etv), ETV Canada website (www.etvcanada.ca), DANETV website (www.etv-denmark.com) and in other program publications; and
- At no cost to DHI, Battelle or ETV Canada, vendor's technology and associated equipment/materials for testing, appropriate training in its operation, and on-site support on an as-needed basis; and
- Written descriptions, diagrams, and/or photographs of vendor's technology, as input for the process, plan, and report documents.

1.2 DHI Involvement

During the verification of vendor's technology, DHI will:

- Provide a person from DHI's organization to be Battelle's, ETV Canada's and the vendor's point of contact and to lead DHI's participation in verification of the vendor's technology;
- Prepare and revise the Verification Protocol, Test Plan, Verification Report, Test Report, Verification Statement, and other documents pertaining to the verification of the vendor's technology and allow Battelle, U.S. EPA, ETV Canada, Environment Canada, and the Expert Group/Peer Reviewers the opportunity to review and comment on these documents;
- Provide input, review, and comment on the Process Document to be prepared by Battelle;
- Assemble a team of qualified technical staff to conduct the verification test in accordance with the Verification Protocol, Test Plan, and this document;

- Ensure that all quality procedures specified in the Verification Protocol, Test Plan, DANETV CQM1, and this document are followed;
- Participate in a (virtual) joint kick-off meeting, led by Battelle and including representatives from ETV Canada, (using checklist in Appendix B) prior to test initiation;
- Provide a summary of the QA activities performed by the DHI DANETV Water Centre Internal Auditor during the verification testing; and
- Provide permission to post/cite information about DHI's involvement in the joint verification, including the Joint Verification Protocol, Test Plan, Verification Report, Test Report and Verification Statement, on the U.S. ETV website and the Canadian ETV website and in other program publications.

1.3 Battelle Involvement

During the ETV verification of the vendor's technology, Battelle will:

- Provide a person from Battelle to be DHI's, ETV Canada's and the vendor's point of contact;
- Prepare a procedural document (Process Document) outlining the process of the vendor's technology verification for acceptance by the U.S. EPA (this document);
- Provide input, review, and comment on the Verification Protocol, Test Plan, Verification Report, Test Report, Verification Statement, and other documents pertaining to verification of the vendor's technology;
- Coordinate review of the Verification Protocol, Test Plan, Verification Report, Test Report, Verification Statement, and other documents pertaining to verification of the vendor's technology with U.S. EPA and U.S. expert reviewers and consolidate these comments for submission to DHI;
- Lead a (virtual) joint kick-off meeting with participants from DHI and ETV Canada (using checklist in Appendix B) prior to test initiation;
- Strive to obtain U.S. EPA approval for the final Verification Protocol, Test Plan, Test Report, Verification Report, including a Verification Statement; and
- Comply with all quality procedures and program requirements specified in the Verification Protocol, Test Plan, ETV AMS Center QMP2, and U.S. ETV Program QMP3, as follows:
    - Prepare and get U.S. EPA ETV approval of an audit checklist and provide the checklist to DHI prior to the audit;
    - Conduct a technical systems audit once during the verification test;
    - Audit at least 10% of the verification data;

- Prepare and distribute an assessment report for each audit;
- Verify implementation of any necessary corrective action; and
- Provide a summary of the quality assurance/quality control (QA/QC) activities and results for the verification reports.

1.4 ETV Canada Involvement

During the ETV verification of the vendor's technology, ETV Canada will:

- Provide a person from ETV Canada to be DHI's, Battelle's and the vendor's point of contact;
- Provide input, review, and comment on the Process Document, Verification Protocol, Test Plan, Verification Report, Verification Statement, and other documents pertaining to verification of the vendor's technology;
- Coordinate review of the Verification Protocol, Test Plan, Verification Report, Test Report, Verification Statement, and other documents pertaining to verification of the vendor's technology with Environment Canada and Canadian expert reviewers as needed, and consolidate these comments for submission to DHI;
- Participate in a (virtual) joint kick-off meeting, led by Battelle and including representatives from DHI, (using checklist in Appendix B) prior to test initiation;
- Inform Environment Canada of the final Verification Protocol, Test Plan, Verification Report, including the Verification Statement; and
- Comply with all quality procedures and program requirements specified in the Verification Protocol, Test Plan, and ETV Canada General Verification Protocol (GVP)[4], as follows:
  - Review Test Plan;
  - Review test data;
  - Provide summary of review; and
  - On site audit - might not be required by the Canadian ETV Program.

1.5 DANETV Involvement

DANETV responsibilities are based on the requirements stated in the DANETV CQM[1] and include the following:

Table 1.0 DANETV Responsibilities Concerning Reviews and Audits.

	DHI Internal		External Expert
Function	Technical expert	Trained auditor QA staff	Technical expert
Tasks			
Plan document with verification protocol and test plan	Review	-	Review
Test system	-	Audit	-
Report document with test report and verification report	Review	-	Review

1.6 U.S. EPA Involvement

A complete list of U.S. EPA's responsibilities for the AMS Center are based on the requirements stated in the AMS Center ETV QMP.[2] The U.S. EPA will provide technical and quality review for this joint verification activity to ensure the test planning and reporting documents are in compliance with the U.S. ETV program requirements.

1.7 Environment Canada Involvement

The Canadian ETV program is delivered by ETV Canada under a license agreement from Environment Canada. ETV Canada will consult with Environment Canada as required.

1.8 Stakeholder Committee and Expert Group/Peer Reviewer Involvement

The AMS Center's Water Stakeholder Committee is made up of buyers and users of water monitoring technologies. This committee assists in prioritizing the types of technologies to be verified and in specific cases, provides testing support. It also has representatives that assist in review of the Test/QA plans, Verification Reports, Test Report and Verification Statements. The AMS Center Water Stakeholder Committee provided concurrence for the Center to proceed with testing in this area. The stakeholders have been

kept apprised of progress throughout the planning process for this test and have provided input during progress meetings on the test design.

In addition, the U.S. ETV AMS Center obtains the peer review of at least one external peer reviewer and one EPA peer reviewer who are not directly involved with the verification test. ETV Canada uses an independent technical expert to perform the peer review of documents and test results. Reviews will be documented using Review Report Form (Appendix C). Battelle will consolidate comments from the U.S. reviews into one Review Report form and ETV Canada will consolidate comments from Canadian reviews into one Review Report form, to ease DHI implementation of comments.

The DANETV program uses an Expert Group to perform the external peer review of the documents and give input on the verification. For this test, the Expert Group is made up of three individuals to fulfill the requirements of all three programs. These individuals are named with their affiliations in the Verification Protocol[5] and Test Plan[6].

2 QUALITY SYSTEMS

The DHI, Battelle, and ETV Canada quality systems to be implemented for this joint verification will conform with the specifications listed in:

- ANSI/ASQ E4-2004, "Specifications and Guidelines for Quality Systems for Environmental Data Collection and Environmental Technology Programs"[7]
- International Standards Organization (ISO) 9001[8].
- Laboratory accreditation ISO 17025.

Per the U.S. EPA ETV Program QMP[3], verification organization quality systems, such as DHI's quality systems, are to be reviewed and approved by verification organization management, the U.S.EPA AMS Center Manager, and the U.S. EPA AMS Center QA Manager. This process document will serve to define the specific quality activities that will be performed by Battelle, DHI, and ETV Canada for this joint verification and clearly identify the processes required to comply with the quality manuals for the three organizations.

Per the Canadian ETV Program, DHI's quality systems are to be reviewed and approved by ETV Canada.

3 VERIFICATION PLANNING

In performing the verification test, DHI, Battelle and ETV Canada will follow the technical and QA procedures specified in the Verification Protocol[5], Test Plan[6], and this process document. Because DHI is preparing the Verification Protocol, Test Plan, Verification Report, Test Report and Verification Statement, as well as conducting the testing activities, the procedures and expectations of the U.S. EPA ETV program and Canadian ETV program need to be clarified in a document that explains the process and requirements (this document).

3.1 Planning the Test Design

The verification test design will produce a Verification Protocol[5] and Test Plan[6] based upon the DANETV, U.S. EPA ETV, and ETV Canada processes. These two documents, together with this process document, represent the equivalent of a U.S. EPA ETV AMS Center Test/QA plan and ETV Canada's technology-specific test/QA plan. The protocol includes an Application and Performance Parameter Definition Document (Appendix 3 in the Verification Protocol[5]) that developed relevant parameters and ranges for verification considering the vendor stated performance, government standards, and other technologies and methods in the market. The Application and Performance Parameter Definition Appendix was not jointly produced and is a specific process within the DANETV program; therefore, it was not reviewed by the U.S. EPA ETV program or ETV Canada. In designing this verification test, DHI staff used consensus-accepted test design, a U.S. EPA ETV protocol for similar products, a standard for verification of online equipment (ISO 15839[9]) and a protocol from TESTNET[10]. All relevant activities pertaining to environmental data operations have been identified, as well as performance specifications and the appropriate controls. Additionally Battelle conducted a statistical review of the test plan design for calculating EC50 and EC20 generated by analyzing various sets of solutions with ranges between 10 and 90% inhibition which is included in Appendix G.

Finally, a process document (this document) was produced by Battelle to address the process and differences between the programs to ensure a successful joint verification. Collectively, these three documents (the Verification Protocol[5], Test Plan[6], and Process Document for the U.S. EPA ETV AMS Center, DHI DANETV Water Centre and ETV Canada Joint Verification of the HACH-LANGE GmbH LUMIStox 300 Bench Top

Luminometer and ECLOX Handheld Luminometer) are referred to as the "testing documents".

The U.S. EPA ETV process utilized its Water Stakeholder Committee to guide the test design process. The Water Stakeholder Committee provided concurrence for the Center to proceed with testing in this area. The stakeholders have been kept apprised of progress throughout the planning process for this test and have provided input during progress meetings on the test design. A U.S. EPA peer reviewer, who is also an ETV AMS Center Water Stakeholder, was identified to review the Verification Protocol and Test Plan. ETV Canada will review the Test Plan and Verification Protocol (an external expert will be involved if required). The DANETV program uses an Expert Group to perform the external peer review of the Verification Protocol and Test Plan documents and give input on the verification. For this test, the Expert Group is made up of individuals to fulfill the requirements of all three programs. It includes up to three individuals that have extensive experience in the field of water toxicity monitoring, one U.S. EPA reviewer, one European reviewer, and one Canadian reviewer. These reviewers have no direct involvement in the verification test beyond providing their reviews. The comments from the reviews performed by the Expert Group of the Verification Protocol, Test Plan, Verification Report, Test Report and Verification Statement will be reconciled by DHI. The review process will utilize the Review Report Form produced by DHI which is included in Appendix C.

4 VERIFICATION TEST IMPLEMENTATION

This technology performance verification will be implemented according to the Verification Protocol[5] and the Test Plan[6] (including technical procedural documents) prepared during planning. Generation of verification test data will not be initiated until the Verification Protocol and Test Plan are approved by all three programs. System control data can be produced before approval. Any data generated before the required documents are approved will have to be repeated. In performing the verification test, DHI will perform an internal audit of the data collection and handling that follows the technical and QA procedures specified in these documents, as well as, the DANETV CQM[1]. Battelle and ETV Canada will perform technical systems audits (TSA) as appropriate to be sure that these requirements are being met.

A virtual joint kick-off meeting will be held prior to the start of the verification test to review procedures for the test with all verification testing staff. The joint kick-off meeting checklist is provided in Appendix B.

Test personnel will have access to the approved testing documents, approved changes to testing documents, and all referenced documents. When a prescribed sequence for the work is defined in the testing documents, work performed shall follow that sequence. Changes to that sequence need to be documented by preparing a deviation (Appendix D). All verification test activities will be documented. Suitable documents are bound notebooks (e.g. laboratory record books, or LRBs), laboratory data sheets, spreadsheets, computer records, and output from instruments (both electronic and hardcopy). All documentation is implemented as described in the testing documents. All implementation activities are traceable to the testing documents and to the test personnel. The responsibilities of specific test personnel listed in these testing documents that leave the project before it is completed will be reassigned.

When work cannot be implemented according to the approved testing documents, DHI shall be responsible for providing a written deviation report for the test records. Substantial changes to the way work is being implemented compared to the way work is described in the testing documents will be discussed by a teleconference of representatives of the three programs or through an email exchange between the three program representatives, before substantial changes are implemented. As soon as Battelle becomes aware of a deviation, Battelle will also notify the U.S. EPA AMS Center Project Officer and QA Manager. A deviation report is produced for any changes to the testing document that occurred during the test. Deviation reports must be retained in the verification test records and summarized in the Verification Report. Frequent deviations from established procedures, which indicate that testing cannot be performed as written, should result in a retrospective review of the written document(s) and possible revision. Deviations will include all the information displayed on the forms shown in Appendix D.

All persons responsible for performing verification testing and HACH-LANGE GmbH will receive copies of the final versions of the Verification Protocol[5] and the Test Plan[6] and associated documentation provided by DHI. Current versions of the Verification Protocol[5] and the Test Plan[6] and any applicable methods and SOPs are required to be physically in place at the technology verification testing sites. Battelle oversight and inspection of the verification test will be provided by the Battelle AMS Center Quality

Manager or designee, ETV Canada oversight and inspection of the verification test will be provided by the Quality Manager. On site oversight and inspection activities by Battelle will occur during one week. An audit checklist for Battelle to use will be prepared by Battelle and approved by the U.S.EPA AMS Center Project Officer and U.S. EPA AMS Center QA Manager. If required, an audit checklist for ETV Canada will be prepared and approved by the ETV Canada Program Manager. The audit checklists will be provided to DHI prior to the audit. The audit will begin with an "In Briefing" conducted by the Battelle AMS Center Quality Manager and, if applicable, the ETV Canada Quality Manager to specify and clarify the necessary points of the audit. Testing during laboratory activities will be observed along with viewing the external laboratory performing the reference analyses. To verify full implementation of the testing documents, the inspection will include the testing process and any documentation associated with the process, such as sample chain of custody transfers, instrument maintenance and calibration, sample preparation and analysis, and data records. At the conclusion of the audit there will be an "Exit Briefing" held to discuss the findings and corrective actions necessary. The Battelle AMS Center Quality Manager and, if applicable, ETV Canada Quality Manager will also provide a written audit report, verify the completion of any corrective actions needed, and retain a copy of the report with permanent Battelle AMS Center Quality Manager records and a copy in the ETV project records. The audit report will be sent to DHI within 10 working days after the end of the audit. The audit report will be commented on by DHI and comments addressed before it is distributed. The Assessment Reporting Form is presented in Appendix E. The U.S. EPA AMS Center Project Officer and ETV Canada Program Manager will be included in the routing of the inspection results and a written copy provided to the U.S. EPA AMS Center Project Officer, the U.S. EPA AMS Center QA Manager, the ETV Canada Program Manager and Quality Manager, and DHI.

5 ASSESSMENT AND RESPONSE

Assessments will be planned, scheduled, conducted, and reported in order to measure the efficacy of the DHI, Battelle and ETV Canada quality procedures and verification execution. The testing will be audited internally by the DHI Internal Auditor in accordance with the Verification Protocol[5] and Test Plan[6]. The DHI Document reviewer will perform the technical review of the Test Plan and Verification Report documents. The DHI Internal

Auditor will perform an audit based upon identified critical points. The DHI Document Reviewer and Internal Auditor equate to the Battelle AMS Center Quality Manager and ETV Canada Quality Manager. The procedure includes two main steps:

- Check that the protocol/plan is prepared and followed in accordance with the DANETV CQM (horizontal audit).
- Check verification/test parameters and data at the identified critical points, i.e., a vertical audit in laboratory, office.

Data from the testing will be controlled by the DHI Verification Responsible and Test Responsible when received (see Appendix A for definition of roles of key personnel and an explanation of the parallel roles between DANETV, U.S. EPA ETV, and ETV Canada programs). Data integrity will be controlled by the Test Responsible (transfer of raw data to spreadsheets) and Verification Responsible (calculations as part of evaluations) as spot checks (5% of the data).

Monitoring of the work process to be conducted by the Battelle AMS Center Quality Manager and ETV Canada Quality Manager will be done to:

- Ensure satisfactory performance based on requirements,
- Ensure required actions (as specified in implementation documents) are performed so that routine measurements meet specifications,
- Ensure preventive maintenance is performed and documented as specified in facility and study records,
- Ensure calibrations are performed as planned and prescribed,
- Ensure corrective actions are implemented and documented as planned in response to items of nonconformance.

Assessment types, responsibility, and schedule for this joint verification will be as shown in Table 2.0, and are defined below. Battelle will conduct one technical systems audit (TSA) and one audit of data quality (ADQ) for this program. Battelle completed a quality systems audit (QSA) of DHI in February of 2009.

Quality Systems Audit (QSA), an on-site review of the implementation of the DHI quality procedures. This review is used to verify the existence of, and evaluate the adequacy of, the internal quality system. This review will be done in conjunction with the Technical Systems Audit.

Technical Systems Audit (TSA), a qualitative on-site evaluation of sampling and/or measurement systems associated with a particular verification test. The objective of the TSA is to assess and document the acceptability of all facilities, maintenance, calibration procedures, reporting requirements, sampling, and analytical activities, and quality control

procedures in the test. Conformance with the testing documents and associated methods and/or Standard Operating Procedures is the basis for this assessment. The Battelle AMS Center Quality Manager will prepare and use an audit checklist that is approved by the EPA AMS Center Quality Manager. If necessary, the ETV Canada Quality Manager will prepare an audit checklist and ETV Canada Program Manager will review. The checklists will be available to DHI before the audit takes place. This review will be done in conjunction with the QSA.

Performance Evaluation Audits (PE), a quantitative evaluation of the measurement systems used. The type and frequency of performance evaluation self-audits are specified in the Test plan for the joint verification test. The value or composition of reference materials must be certified or verified prior to use, and the certification or verification must be adequately documented. The Battelle AMS Center Quality Manager and ETV Canada Quality Manager will review results of PE audits during the TSA; however, it is most preferable for the PE results to be shared with Battelle and ETV Canada as soon as they are available, so that any issues can be resolved.

Audits of Data Quality (ADQ), an examination of the verification data after they have been collected and verified by project personnel. The Battelle Verification Test Coordinator will review 100% of the data and the Battelle AMS Center Quality Manager will audit at least 10% of all verification data, including equations and calculations. The ETV Canada Quality Manager will audit all test data including calculations. DHI will provide technology test data and associated records (e.g., data sheets; notebook records) from the first day of testing within one day and thereafter on a weekly basis to ETV Canada and Battelle. The first set of reference laboratory data will also be sent within one day of receipt by DHI, then on a weekly basis. Battelle will immediately forward these data to US.EPA upon receipt (i.e., for simultaneous review). The goal of this data delivery schedule is prompt identification and resolution of any issues.

Note that if it is determined by DHI, Battelle, U.S. EPA, or ETV Canada during any of the assessments that test objectives of acceptable quality cannot be achieved, a stop work order should be considered and discussed among all parties. See Section 5.2 for more information on stop work orders.

Table 2.0 Assessments for the Joint Verification

Assessment Tool	Assessors	Responders	Subject of Assessment	Minimum Frequency	Reason for Assessment	Report Reviewed by
Quality Systems Audit	Battelle AMS Center Quality Manager and ETV Canada Quality Manager	DHI	DANETV CQM	Once*, thereafter as requested	Assess quality management practices of verification collaborators	U.S. EPA directors of quality assurance, U.S. EPA AMS Center Project Officer, Battelle AMS Center Manager and Test Coordinator, ETV Canada Program Manager and Quality Manager, DHI Verification Responsible
Technical Systems Audits	Battelle AMS Center Quality Manager, ETV Canada Quality Manager and DHI Internal Auditor	DHI	Verification Protocol, Test Plan, and Process Document	Once	Assess technical quality of verification tests	U.S. EPA AMS Center Project Officer, U.S. EPA AMS Center QA Manager, Battelle AMS Center Manager and Test Coordinator, ETV Canada Program Manager and Quality Manager, DHI Verification Responsible
Performance Evaluation Audits	Battelle AMS Center Quality Manager, ETV Canada Quality Manager and DHI Internal Auditor	DHI	Verification Protocol, Test Plan, and Process Document	Once	Assess measurements performance	U.S. EPA AMS Center Project Officer, U.S. EPA AMS Center QA Manager, Battelle AMS Center Manager and Test Coordinator, ETV Canada Program Manager and Quality Manager, DHI Verification Responsible
Audits of Data Quality	Battelle AMS Center Quality Manager, ETV Canada Quality Manager and DHI Internal Auditor	DHI	raw data and summary data	At least 10% of the verification data	Assess data calculations and reporting	U.S. EPA AMS Center Project Officer, U.S. EPA AMS Center QA Manager, Battelle AMS Center Manager and Test Coordinator, ETV Canada Program Manager and Quality Manager, DHI Verification Responsible

*QSA of DHI completed by Battelle in February 2009.

5.1 Assessment Reports

Each assessment must be fully documented. Battelle completed a QSA of DHI in February 2009 and this report is available. Within 10 working days of the TSA and ADQ, the Battelle AMS Center Quality Manager will provide a draft audit report to the U.S. EPA AMS Center Project Officer and QA Manager. The draft audit report will summarize any potential issues or findings noted during the audits. The draft audit report may require additional

editorial review prior to submitting to testing staff for corrective actions and corrective actions will not be documented in the draft; however, the goal is to provide immediate information to the U.S. EPA AMS Center Project Officer and QA Manager on the types of corrective actions being requested as part of the assessments. The PE audits will be reviewed as part of the TSA. Also, DHI has been asked to share PE results with Battelle and ETV Canada as soon as they are available so that any issues can be resolved. If information on the PE audits is provided to Battelle outside of the TSA, Battelle will forward that information to the U.S. EPA AMS Center Project Officer and QA Manager within 10 days of receipt. The Battelle AMS Center Quality Manager, the ETV Canada Quality Manager and the DHI Verification Responsible will archive all assessment reports generated for this verification test.

Each assessment must be responded to by the appropriate level of management. The Battelle quality assessment reports shall require a written response by the person performing the inspected activity, and acknowledgment of the assessment by the Battelle AMS Center Verification Test Coordinator. The Assessment Reporting Form is provided in Appendix E. The audit report will be based on the forms in Appendix E, but a separate report will be prepared. An assessment report will also be prepared by the DHI Internal Auditor and provided to the Verification Center Test Coordinator for archive.

Corrective action must be documented and approved on the original assessment report, with detailed narrative in response to the assessor's finding. Initials and date are required for each corrective action response. Acknowledgment of the response will be provided by the Battelle AMS Center Verification Test Coordinator.

Implementation of corrective actions must be verified by the Battelle AMS Center Quality Manager or the DHI Internal Auditor to ensure that corrective actions are adequate and have been completed. This will be done in real-time if corrective actions can be immediately performed and signed off on the assessment report. Alternatively, should the corrective action require additional approvals not immediately available on-site, the DHI Internal Auditor may need to repeat the inspection, as the designee of the Battelle AMS Center Quality Manager, in order to corroborate the implementation and effectiveness of the corrective action.

Implementation of corrective actions will be verified by the ETV Canada Quality Manager to ensure that corrective actions are adequate and have been completed. This will be done during data review.

5.2 Stop Work

Assessor responsibility and authority to stop work during a verification test for quality considerations is delegated to DHI, Battelle, and ETV Canada. DHI must ensure compliance with all applicable Danish federal, state, and local safety policies during the performance of verification testing.

Should it be determined by Battelle, U.S. EPA, or ETV Canada during an assessment that test objectives of acceptable quality cannot be achieved during performance of verification testing, Battelle and ETV Canada are responsible for immediately notifying the DHI Verification Responsible of the need to consider a stop work order. The DHI Verification Responsible will then direct the staff accordingly. The U.S EPA AMS Center QA Manager will notify the U.S. EPA AMS Center Project Officer if work of inadequate quality is discovered.

During verification, the ETV Canada Quality Manager will notify the ETV Canada Program Manager if work of inadequate quality is discovered.

Documentation is required of any stop work order and the corrective action implemented and shall be maintained as part of the Battelle quality records, with a copy provided to the U.S. EPA AMS Center Project Officer and U.S. EPA AMS Center QA Manager and as a part of ETV Canada quality records, with a copy provided to the ETV Canada Quality Manager, and DHI.

5.3 Response

Responses to TSA adverse findings should be addressed within 10 working days after the TSA report is completed. However, it is expected that findings that have a direct impact on the conduct of a verification test will be corrected immediately following notification of the finding.

Responses to each adverse finding will be documented in the assessment report. Ideally, assessment reports will provide space after each adverse finding for a response to be recorded. The response will indicate the corrective action taken or planned to address the adverse finding. The response should be signed and dated by the staff responsible for implementing the corrective action.

Any corrective action that cannot be immediately implemented will be verified following completion by the Battelle AMS Center Quality Manager or designee and by the

ETV Canada Quality Manager. Once all corrective action associated with an assessment report has been taken, the Battelle AMS Center Quality Manager or designee and the ETV Canada Quality Manager will initial the corrective action in the assessment report thus documenting verification of the corrective action. Any impact that an adverse finding had on the quality of verification test data should be addressed in the verification report.

The TSA report, with responses to adverse findings recorded within, will be sent to U.S. EPA within 10 working days after the Battelle AMS Center Quality Manager has verified all corrective actions.

6 DOCUMENTATION AND REPORTING

6.1 Documentation and Reporting Responsibilities

Responsibilities for activities concerning documentation and reporting are summarized in Table 2.0 and are detailed below.

6.1.1 Preparation

Individual case requirements and this document shall guide document and record content and/or format. Guidance for content and/or format are derived by the U.S. EPA ETV, ETV Canada and DANETV directives and the following documents:

- ANSI/ASQ E4-2004[7].
- U.S. ETV AMS Center QMP[2].
- U.S. EPA ETV Program QMP[3].
- U.S. EPA document "EPA QA/R-2, *EPA Requirements for Quality Management Plans*," March 2001.
- ETV Canada GVP[4], February 2007.
- DHI CQM[1].
- Nordic Water Technology Verification Centers. NOWATECH. Final technical report. Nordic Innovation Centre Project 06223. August 2009.

6.1.2 Review/Approval

Record review/approval for joint verification testing documents shall be performed by qualified technical and/or management personnel as described in Table 3.0. The individual reviewer shall have access to all needed references.

6.2 Reporting

The end result of the joint verification process will be a Verification Report, Test Report and Verification Statement for the HACH-LANGE GmbH LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer. The review and approval procedures for the verification report and statement for U.S. EPA ETV program and ETV Canada purposes are given in Table 2.0. The Verification and Test Report will be reviewed by external reviewers in the Expert/ Peer Reviewer Group and the Verification Statement will be signed by an U.S. EPA laboratory director, Battelle management, ETV Canada management (OCETA), the DHI Director of Research and Quality Management and the DANETV Steering Committee Head. Appendix F presents a preliminary template for a Verification Statement. This document will be expanded and organized to meet U.S. EPA ETV, ETV Canada and DANETV program requirements.

All logos will appear on the Verification Statement. These will include: U.S. ETV, Battelle, Environment Canada and ETV Canada, DANETV, DHI, and U.S. EPA logos. All logos except the U.S. EPA logo will appear on the cover page of all other joint testing documents (Test Plan, Verification Protocol, Test Report, Verification Report). All of these testing documents will be made publicly available on the U.S. EPA ETV Web site (www.epa.gov/etv), the DANETV Web site (www.etv-denmark.com), and ETV Canada Web site (www.etvcanada.ca) regardless of the technology's performance.

The vendor will comply with the DANETV, U.S. EPA ETV and ETV Canada policies on referencing the verification documents of their technology.

Table 3.0 Document and Reporting Responsibilities for the Joint Verification*

Record Type	Preparation/ Updating	Review	Approval	Finals Distributed to:
Verification Protocol and Test Plan (including SOPs, amendments and deviations)	DHI	Battelle AMS Center Manager, Battelle AMS Center Quality Manager, U.S. EPA AMS Center QA Manager, U.S. EPA AMS Center Project Officer, ETV Canada Program Manager, ETV Canada Quality Manager, ETV Canada External Technical Expert if required, DHI Verification Responsible, DHI Document Reviewer, Stakeholders/Expert Group, Vendor	U.S.EPA AMS Center Project Officer, U.S. EPA AMS Center QA Manager, U.S. ETV Program Director ETV Canada Program Manager, ETV Canada Quality Manager, DHI Verification Responsible	Testing Staff, Vendor, U.S. EPA AMS Center Project Officer, U.S.EPA AMS Center QA Manager, ETV Canada Program Manager, ETV Canada Quality Manager DHI Verification Responsible
Raw data	DHI	DHI Internal Auditor	N/A	U.S.EPA can request copies
Verification Report	DHI	Battelle AMS Center Manager, Battelle AMS Center Quality Manager, U.S. EPA AMS Center QA Manager, U.S. EPA AMS Center Project Officer, ETV Canada Program Manager, ETV Canada Quality Manager, ETV Canada External Technical Expert if required, DHI Verification Responsible, DHI Document Reviewer, Stakeholders/Expert Group, Vendor	U.S.EPA AMS Center Project Officer, U.S. EPA AMS Center QA Manager, U.S. ETV Program Director ETV Canada Program Manager, ETV Canada Quality Manager DHI Verification Responsible	U.S. ETV Program Director, U.S. EPA AMS Center Project Officer, U.S. ETV Webmaster, ETV Canada Program Manager, ETV Canada Quality Manager Vendor, DHI Verification Responsible
ETV Verification Statement	DHI	Battelle AMS Center Manager, Battelle AMS Center Quality Manager, U.S. EPA AMS Center QA Manager, U.S. EPA AMS Center Project Officer, ETV Canada Program Manager ETV Canada Quality Manager ETV Canada External Technical Expert if required DHI Verification Responsible, DHI Document Reviewer, Vendor, U.S. ETV Program Director Stakeholders/Expert Group	U.S. EPA Laboratory Director, U.S. ETV Program Director, Battelle Management, U.S. EPA AMS Center Project Officer, U.S. EPA AMS Center QA Manager, ETV Canada Program Manager, ETV Canada Quality Manager DHI Director R&D and Quality Management DANETV Steering Committee Head	U.S. ETV Program Director, U.S. EPA AMS Center Project Officer, Battelle AMS Center Manager, ETV Canada Program Manager, ETV Canada Quality Manager DHI Verification Responsible, U.S. ETV Webmaster, Vendor
Audit Reports	DHI Internal Auditor	DHI Test Responsible DHI Verification Responsible	N/A	DHI Verification Responsible, Battelle AMS Center Quality Manager, Battelle AMS Center Manager, Battelle Verification Test Coordinator, U.S. EPA AMS Center Project Officer, U.S. EPA AMS Center QA Manager, ETV Canada Program Manager, ETV Canada Quality Manager
Audit Reports	Battelle AMS Center Quality Manager	Battelle AMS Center Manager, Battelle Verification Test Coordinator, DHI Verification Responsible DHI Test Responsible U.S. EPA AMS Center QA Manager	N/A	U.S.EPA AMS Center Project Officer, U.S.EPA AMS Center QA Manager, Battelle AMS Center Manager, Battelle Verification Test Coordinator, DHI Verification Responsible ETV Canada Program Manager, ETV Canada Quality Manager
Audit Reports	ETV Canada Quality Manager	ETV Canada Program Manager ETV Canada Quality Manager		

*See Appendix A for the roles and names of the individuals filling these roles.

7 REFERENCES

1. ETV Test Centre and Test Organization. Centre Quality Manual – Water technology. Version 2. October 2009.

2. Battelle. Quality Management Plan for the ETV Advanced Monitoring Systems Center. Version 7.0. November 2008.

3. United States Environmental Protection Agency. Environmental Technology Verification Program Quality Management Plan. Version 3.0. January 2008.

4. ETV CANADA General Verification Protocol (GVP). February 2007

5. DHI. HACH-LANGE GmbH LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer, Joint Verification Protocol. Review Version. November 2009.

6. DHI. HACH-LANGE GmbH LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer, Joint Test Plan. Review Version. November 2009.

7. American Society for Quality. ANSI/ASQ E4-2004. Quality systems for environmental data and technology programs – Requirements with guidance for use. 1-4-2004.

8. International Standardization Organization. ISO 9001. Quality management systems – Requirements. 11-15-2008.

9. International Standard Organization. ISO 15839. Water quality–On-line sensors/analyzing equipment for water–Specifications and performance tests. First Edition 2003-10-15.

10. TESTNET Evaluation Report Test case 1a:TOXcontrol BioMonitor for Surface water Workpackage 3. 01-03-2008.

APPENDIX A

ROLES OF KEY PERSONNEL

DANETV Role:

DANETV person who signs the Verification Statement: DANETV Steering Committee Head – Christian Grøn

DHI DANETV Water Centre (DHI) Roles:

DHI person who signs the Verification Statement: Director of Research & Development and Quality – Jørn Rasmussen
DHI Verification Responsible: Mette Tjener Andersson
DHI Test Responsible: Claus Jørgensen
DHI Document Reviewer: Margrethe Winter-Nielsen
DHI Internal Auditor: Bodil Mose Pedersen
DHI Verification and Test staff: several – see protocol and plan

U.S. EPA ETV Roles:

U.S. EPA person who signs the Verification Statement: National Risk Management Research Laboratory (NRMRL) Director: Sally Gutierrez
U.S. EPA ETV Program Director, Teresa Harten
U.S. EPA AMS Center Project Officer: John McKernan
U.S. EPA AMS Center QA Manager: Michelle Henderson

Battelle Advanced Monitoring Systems (AMS) Center Roles:

Battelle person who signs the Verification Statement: Chemical, Environmental and Materials Operations Manager: Lisa McCauley
AMS Center Manager: Amy Dindal
AMS Center Quality Manager: Zack Willenberg
AMS Center Verification Test Coordinator: Mary Schrock

Environment Canada Roles:

Environment Canada person who oversees the Canadian ETV Canada Program: Head of Technology Program: Raymond Klicius.

ETV Canada Roles:

Ontario Center of Environmental Technology Advancement person who signs the Verification Statement: President and CEO: Kevin Jones
ETV Canada Program Manager: John Neate
ETV Canada Quality Manager: Mona El Hallak

Parallel Roles between the DANETV, U.S. EPA ETV and ETV Canada programs:

Verification Responsible = Center Manager = Program Manager
Test Responsible = Center Test Coordinator = Quality Manager
Internal Auditor + Document Reviewer = Center Quality Manager = Quality Manager

APPENDIX B

KICK OFF MEETING CHECKLIST

ETV JOINT VERIFICATION TEST KICK-OFF MEETING

PURPOSE

To prepare verification testing staff for the DANETV, U.S. EPA ETV, ETV Canada joint verification test and review critical logistical, technical, and administrative aspects of the test. The kick-off meeting will be scheduled prior to the start of testing. It should be near the start of the test but allow time for DHI to address any lingering issues.

FORM

The kick off meeting will be virtual, i.e. based upon phone and WebEx sharing of documents.

STAFF TO ATTEND

- Verification test coordinator/responsible (DHI, Battelle)
- Verification Test quality staff (DHI, Battelle)
- Representative of ETV Canada approved by ETV Canada
- U.S. EPA ETV, Environment Canada, and DANETV program staff (invited but optional)

All testing staff involved in all phases of test will subsequently have a kick-off meeting on-site with the DHI Verification Responsible. The external laboratory is informed through requisitions of analyses only.

PROJECT MANAGEMENT

- Review roles/responsibilities of all staff attending meeting
- Review test schedule, review notifications of testing schedule (e.g., U.S. ETV stakeholders, others?)
- Documentation of all pertinent forms.
    - Vendor-Collaborator agreement
    - Peer review forms on Verification Protocol and Test Plan. Must include one U.S. EPA reviewer/two non-U.S. EPA peer reviewers (preferably one from Canada and one from Europe).
    - Final Test Plan approved by vendor.
    - Documentation that the vendor is satisfied that the staff operating the technology are proficient in its use.

QUALITY ASSURANCE

- Copies of all standard methods cited or included in the Test Plan, the final Test Plan, and the final Joint Verification Protocol are available to verification testing staff and in the laboratory where test will be performed?
- U.S. EPA QA staff pre-notified of test start date and ETV Canada Quality Manager.
- Remind testing staff to sign and date everything.

- If samples are to be transported between labs, DHI should bring an example chain-of-custody form to the meeting, review how to complete, and where to obtain form.
- Review deviation/amendment procedures at meeting – what to do in the middle of a test if testing document cannot be followed – who to notify/what forms to file.
- Review testing document at meeting – identify key testing procedures and critical steps to ensure no ambiguity or questions.
- Are or will there be copies of the certificates of analysis in the verification test records (documentation from vendor attesting to the quality and concentration of stock standard solutions used in the test)?
- If applicable, discuss when performance evaluation (PE) audit(s) will be performed? Who will perform? Have materials/equipment been purchased or obtained for the PE audit? What are quality control (QC) limits will be considered acceptable? What should be done if QC limits are not met? Who should be contacted?
- Will regular communication between DHI, Battelle and ETV Canada be maintained? If so, how? Daily/weekly email updates?

TECHNICAL

- Emphasize to testing staff to document anything and everything that is observed about the technologies, particularly if there are unusual sample results (e.g., sample color).
- Are provisions made to handle daily preparation of solutions/standards, if necessary?
- Take digital photos of all test activities.

DATA/REPORTING

- Review data recording forms or sheets at meeting or discuss how/where will data be recorded for each testing activity
- How are data going to be converted electronically? Are data saved in technology undergoing verification and then exported to Excel? Or will data be recorded manually by the operators? If so, how will transcription errors be avoided?
- Data review – can data be sent out every two weeks as collected for review? Who will be doing review for each data set sent out? If Battelle or ETV Canada staff are not on-site, how will data be transmitted to them?
- Distribute and review report schedule. Reporting should begin at the same time as testing.

APPENDIX C

REVIEW REPORT FORM

Review report

Document title:		Document date:	
Reviewer name:		Review date:	
Name:			
Organization:			
Address:			
Telephone:			
E-mail			

Review results					
Rate items	*Satisfactory*	*Unsatisfactory*		*Overall recommendation*	
Contents					
Scope				Acceptable as is	
Organization				Minor revisions	
Data quality				Major revisions	
Method validity				Not acceptable	
Conclusions					
Other (specify)			Reason		

Revision details				
Topic	*Report chapter, section, page*	*Revision required*	*Reason*	*Revision action (to be filled in by document owner during revision after review)*

Add additional rows, if pertinent.

APPENDIX D

JOINT VERIFICATION TESTING DOCUMENT

DEVIATION FORM

DEVIATION REPORT

TESTING DOCUMENT TITLE AND DATE:

DEVIATION NUMBER: _____

DATE OF DEVIATION: _____

DESCRIPTION OF DEVIATION:

CAUSE OF DEVIATION:

IMPACT OF DEVIATION ON THE TEST:

CORRECTIVE ACTION:

ORIGINATED BY:

_____
DHI Test Responsible, Battelle AMS Center Verification Test Coordinator or ETV Canada

_____
DATE

ACKNOWLEDGED BY:

_____
DHI Quality Manager

_____
DATE

_____
Battelle AMS Center Quality Manager

_____
DATE

_____
ETV Canada Quality Manager

_____
DATE

Required Distribution with documentation - All individuals/organizations listed below:

Battelle AMS Center Manager
DHI Verification Responsible
Battelle AMS Center Quality Manager
DHI Internal Auditor
ETV Canada

APPENDIX E

ASSESSMENT REPORTING FORM

Quality Assurance Routing Sheet

Verification Test:

Audit Type:

Test Coordinator:

Vendor:

Auditor: **Date**:

Test Coordinator, please complete the attached form indicating CORRECTIVE ACTION TAKEN (IF NEEDED), sign and date this Routing Sheet in the space provided beside your name, and return the entire set when completed to the Battelle AMS Center Quality Manager no later than _____.

Route To	**Signature**	**Date**
DHI Test Responsible		
Battelle AMS Center Test Coordinator		
ETV Canada		
Approval		
Battelle AMS Center Manager		
Battelle AMS Center Quality Manager		
DHI Verification Responsible		
ETV Canada Quality Manager		

Audit Comment Sheet

Instructions: The Battelle AMS Center Quality Manager or ETV Canada Quality Manager will fill out the first column for the audit indicated above. The Verification Test Coordinator (or assigned responder) will respond to the comments and initial and date the response in column three. The Battelle AMS Center Quality Manager or ETV Canada Quality Manager will verify and document that the response/corrective action has been completed by initialing and dating the final column.

QA Comment	Testing Coordinator Response/Corrective Actions	Responder Initials/ Date	QA Initials/ Date

APPENDIX F

EXAMPLE JOINT VERIFICATION STATEMENT

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

This is a preliminary template of a Verification Statement that may be expanded and organized to meet DANETV, U.S. EPA ETV, and ETV Canada program requirements.

ETV Joint Verification Statement

TECHNOLOGY TYPE:

APPLICATION:

TECHNOLOGY NAME:

COMPANY:

ADDRESS:

PHONE:
FAX:

WEB SITE:
E-MAIL:

- Description of EV and the organizations involved in this joint verification.
- Name technology category and technology (product) that was jointly verified.

VERIFICATION AND TEST DESCRIPTION

- Describe the verification test- when, how
- Describe the performance parameters
- Describe the QA performed

TECHNOLOGY AND PRODUCT DESCRIPTION

- Describe the technology (product)

VERIFICATION RESULTS

- Summary of results by performance parameters

Signature blocks for:

DANETV Steering Committee Head – Christian Grøn

DHI Director of Research and Quality – Jørn Rasmussen

Battelle Chemical, Environmental and Materials Operations Manager - Lisa McCauley

U.S. EPA National Risk Management Research Office of Research and Development – Sally Gutierrez

OCETA, President and CEO- Kevin Jones

NOTICE: ETV verifications are based on an evaluation of technology performance under specific, predetermined criteria and the appropriate quality assurance procedures. U.S. EPA, Battelle, DANETV, DHI, Environment Canada and ETV Canada make no expressed or implied warranties as to the performance of the technology and do not certify that a technology will always operate as verified. The end user is solely responsible for complying with any and all applicable federal, state, and local requirements. Mention of commercial product names does not imply endorsement.

APPENDIX G
STATISTICAL EVALUATION OF TEST PLAN
TESTS A AND C

Testing is designed to follow Hach Lange operating manual procedures for testing under ISO 11348-3. The 10-90% inhibition range used in the Hach Lange protocol is specified in the ISO 11348-3 method in Section 9.2 Determination of EC-values. This section of the ISO method also specifies calculating and reporting EC20 and EC50, which is why these two EC values are used as the reported result. The EC values are generated in the Hach Lange protocol using a nine dilution scheme from 1:2 to 1:32 of the highest inhibition solution. This dilution scheme (discussed in Test Plan section 3.2.1) is also specifically recommended in ISO11348-3 Annex B , Section B.2, on sample preparation (shown in the ISO Method Annex B Table B.1).

Additionally, a Battelle statistician reviewed the test design where EC20 and EC50 are calculated with various solutions in the 10-90% inhibition range to provide additional information on reasonableness of this approach. The results of this evaluation are discussed below:

Objective

Throughout the test design, scenarios are provided for EC50 and EC20 values to be calculated based on between three and nine samples. The calculation algorithm for EC50/EC20 is applied only to samples which result in fluorescence inhibition between 10 and 90 percent. The upper limit of nine samples is achieved when the planned nine point dilution series (32, 24, 16, 12, 8, 6, 4, 3, and 2 fold dilutions) all result in inhibition results between 10 and 90 percent. Fewer than nine samples may be used when a specific test calls for it or when the standard nine point dilution series produces inhibition results outside the 10 to 90 percent range. In these cases, the concentrations with results outside the 10 to 90 percent range are excluded from the EC50/EC20 calculation. A question of interest is whether a nine point dilution curve is adequate to accurately estimate the EC50/EC20 and to what extent fewer than nine dilutions may negatively impact the accuracy.

To evaluate the concerns about EC50/EC20 accuracy, a statistical simulation study was performed.. Data were randomly generated from an assumed statistical model under different scenarios for numbers of test dilutions and corresponding expected percent inhibition results. The EC50/EC20 values were calculated at each iteration of the simulation analysis and the entire set of values was summarized to provide 95 percent confidence limits on the expected accuracy of the responses.

Results

For Test A which will involve a nine point curve with the lowest concentration expected to yield 10% inhibition and the highest concentration expected to yield 90% inhibition using the nine dilution scheme provided in the test plan the EC50 would be estimated, with 95% confidence to (0.92, 1.09) times the true value if the true variability in measured results at 50 percent inhibition for a particular sample is a standard deviation of 10 percent. For EC20, the estimated value should be within (0.89, 1.11) times the true value.

When less than nine points are used, because the planned dilution series did not perfectly span the 10 to 90 percent inhibition range, a penalty in accuracy is seen. As an example, instead of (0.92, 1.09) times the true EC50, a test where only five of the values are within the 50 to 90 percent inhibition range would be (0.79, 1.18). For EC20, it can be seen that missing values in the upper range (i.e., only five points ranging from 0.1 to 0.5) does not negatively impact accuracy (0.88, 1.12).

Test C proposes to estimate EC50 with values of 60 percent and below. In this case, it is likely only about five points will be usable, but the EC 50 at (0.88, 1.20) times the true value and the EC20 at (0.84, 1.18) times the true value have about 50% more uncertainty. A test to estimate EC20 with values of 30 percent and below may yield only three usable test points in the nine dilution series, and the EC20 would be estimated at 95 percent confidence to (0.86, 1.19) times the true value, not too dissimilar to the (0.89, 1.11) times for the full nine point curve.

All the results were also calculated under two other assumed variability models: one with the 50 percent inhibition having a lower variance estimate of 5 percent (i.e., two standard deviation would be 45 to 55 percent inhibition, and one with the 50 percent inhibition having a higher variance estimate of 20 percent (i.e., two standard deviation would be 30 to 70 percent inhibition). The lower variance resulted in less uncertainty in EC50 and EC20 estimation, while the higher variance resulted in greater uncertainty.
However, the comparative results for the different testing scenarios were similar.

All results assume 16-fold dilution series covers from 10 to 90 percent inhibition						
Dilution series is 32, 24, 16, 12, 8, 6, 4, 3, and 2 fold						
Scenarios for Evaluation		full 9	upper 5	lower 5	max 60	max 30
dilution	1/dilution					
32	0.03	0.10	0.50	0.01	0.02	0.01
24	0.04	0.15	0.61	0.02	0.03	0.01
16	0.06	0.25	0.75	0.04	0.05	0.02
12	0.08	0.34	0.83	0.06	0.08	0.02
8	0.13	0.50	0.90	0.10	0.14	0.05
6	0.17	0.61	0.93	0.15	0.21	0.07
4	0.25	0.75	0.96	0.25	0.33	0.13
3	0.33	0.83	0.98	0.34	0.44	0.18
2	0.50	0.90	0.99	0.50	0.60	0.30
		95% Confidence Interval of Ratio Curve Fit EC to True EC				
	% Std@50% Inhibition	EC 20				
	5%	(0.94,1.06)	(0.81,1.18)	(0.94,1.06)	(0.93,1.07)	(0.93,1.08)
	10%	(0.89,1.11)	(0.62,1.36)	(0.88,1.12)	(0.84,1.14)	(0.86,1.19)
	20%	(0.76,1.23)	(0.26,1.68)	(0.73,1.25)	(0.64,1.27)	(0.53,2.07)
	% Std@50% Inhibition	EC 50				
	5%	(0.96,1.04)	(0.9,1.09)	(0.92,1.11)	(0.93,1.09)	N/A
	10%	(0.92,1.09)	(0.79,1.18)	(0.84,1.28)	(0.88,1.2)	N/A
	20%	(0.84,1.19)	(0.53,1.33)	(0.74,1.95)	(0.78,1.65)	N/A

Statistical Methodology

The statistical model for the simulation study is:

*Log(p_i/(1-p_i)) = b0 + b1*log(c_i) + e_i*

Where

p_i is the fluorescence inhibition for a single dilution

c_i is the concentration of the analyte for the dilution

i=1 to 9 (or less)

b0 is the intercept

b1 is the slope

*e_i is the error between the observed log(p/(1-p)) response and the regression predicted b0+b1*log(c) value.*
The errors are assumed to be independent and normally distributed with mean 0 and variance σ^2

Note 1: This logistic regression model appears to be appropriate based on the documentation found in the LUMIStox 300 Operating Manual of January 2008. This is based on Section 4.4, "Calculating the EC Value", which references calculating % inhibition divided by (1 - % inhibition) values and using the associated concentration values and plotting them on a two dimensional logarithmic coordinate system. It is presumed that data have been collected from this instrument and shown to exhibit the desired linear relationship in the logarithmic coordinates. However, no such data are presented for reference.

Note 2: The assumed constant variance σ^2 in the model is an important one. The implication of the assumption is that in the natural scale (%inhibition vs concentration), the variability of results is greatest for the concentration corresponding to 50% inhibition and diminishes toward zero as concentrations lead to %inhibition results of either 0% or 100%. If this assumption is not appropriate, a different model may be preferable.

From the model, the desired quantities of EC50 and EC20 are calculated as follows:

EC50: Since log(0.5/(1-0.5)) = log(1) = 0, the EC50 is the concentration given by exp(-bo/b1)
EC20: Since log(0.2/(1-0.2)) = log(1/4), the EC20 is the concentration given by exp((log(1/4)-bo)/b1)

In order to perform the statistical simulation analysis, several assumptions needed to be made. For the statistical model provided, the b0, b1, and σ^2 are unknown values that jointly define the "true" relationship between % inhibition and concentration. To determine the b0 and b1 values, "Table 6.1 Results from HACH-LANGE test of LUMIStox and ECLOX" in the LUMIStox 300 Bench Top Luminometer ECLOX Handheld Luminometer Joint verification protocol was consulted. This table showed 10 and 90 percent inhibition concentrations as well as the EC50 for several different compounds identical or related to those to be evaluated in the upcoming tests. From Table 6.1, the p-Cresol result was chosen as typical. With an approximate 90% inhibition of 6.0 mg/L and an EC50 (50% inhibition) of 1.5 mg/L, it was calculated that the b0 would be -0.64 and that the b1 would be 1.58. Since the difference between 0.38 and 6.0 is approximately 16-fold, it can be assumed that a perfectly selected nine point dilution series as specified in the test plan will produce percent inhibition results ranging from 10 percent for the highest dilution and up to 90 percent for the lowest dilution. The results for many of the

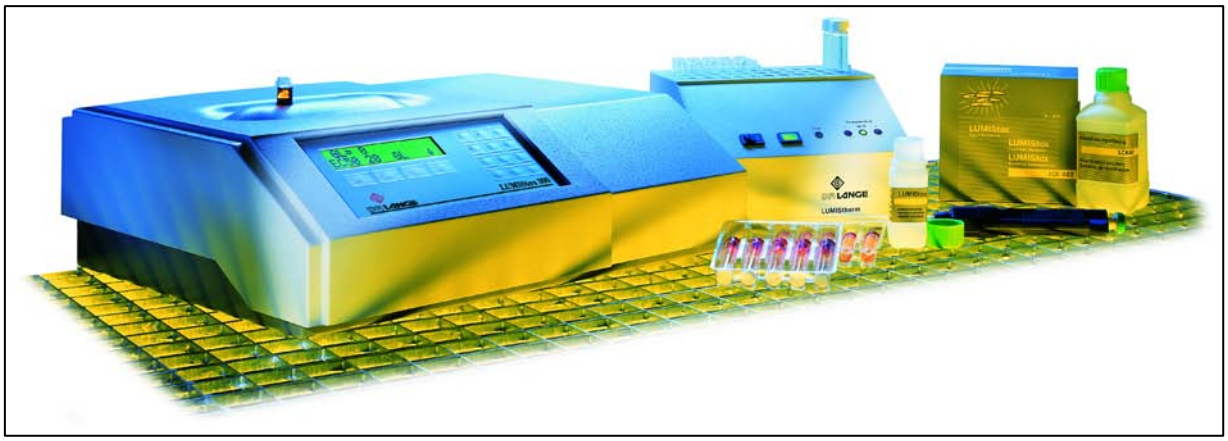
compounds in Table 6.1 were similar. The 10 to 90 percent inhibition range was as low as 6-fold in a couple cases (corresponding to larger b1 values) and as wide as 18 fold in one case (corresponding to smaller b1 values), and the entire analysis completed here could easily be repeated with values derived from other assumed true relationships, but the p-Cresol result seemed satisfactory as a start. To determine the variance estimate, it would be best to have a single run in which the same dilution was evaluated in all nine points, and a corresponding mean and standard deviation of the percent inhibition values calculated. This should be done across a range of average percent inhibition values, with multiple compounds, and perhaps under different test conditions to assure consistency. Lacking any of these data, a simple assumption had to be made and it was decided that in the original units of percent inhibition, repeated measurement of the same sample that averages 50 percent inhibition might reasonably have a standard deviation of 10 percent of the 50 percent (i.e., two standard deviations would be anywhere from 40 percent inhibition to 60 percent inhibition). For sensitivity, the analysis was repeated for a standard deviation of five percent and one of 20 percent.

With the b0, b1, and σ^2 estimates, the simulation required different dilution series to be defined. A standard nine point series with dilutions as defined above with the 32-fold dilution producing on average 10 percent inhibition and the 2-fold dilution producing 90 percent inhibition was the starting point. From the assumed statistical relationship and with the normally distributed variance, one iteration of the simulation consisted of producing the nine percent inhibition values centered at the corresponding values of the relationship and randomly perturbated using a normal distribution with the assumed variance. The nine randomly determined (consistent with the model) points were then fit to a logistic regression relationship using least squares estimation to get an estimated b0 and b1 (different from the true b0 and b1 assumed, but generally similar). Once the estimated b0 and b1 were obtained, they were used to calculate an estimated EC50 or EC20 using the equations above. This entire process was repeated 10,000 times for one scenario. The 10,000 EC50 or EC20 values were then divided by the true EC50 or EC20 as determined by the initially assumed b0 and b1, and the ratios ranked from smallest to largest. The 250th smallest and 250th largest were selected to provide a 95 percent confidence interval for the ratio of calculated EC50 or EC20 that might be expected to occur for a given true relationship between percent inhibition and concentration within a particular sample. This is variation that does not reflect circumstances where the underlying true relationship is not the same (i.e., any other sample). All calculations were performed in Excel.

LUMIStox 300 Bench Top Luminometer
ECLOX Handheld Luminometer

Joint verification protocol

Luminescent bacteria test for use in wastewater



LUMIStox 300

January 2010 **Approved**

LUMIStox 300 Bench Top Luminometer
ECLOX Handheld Luminometer

Joint verification protocol

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2 INTRODUCTION

Environmental technology verification (ETV) is an independent (third party) assessment of the performance of a technology or a product for a specified application, under defined conditions and quality assurance.

This verification is a joint verification between DANETV, the U.S. EPA ETV Advanced Monitoring Systems (AMS) Center and the Canadian ETV Program. The objective of the verification is to evaluate the performance of a wastewater rapid toxicity technology that could be used to monitor industrial or domestic wastewater.

This verification includes two products from one vendor.

2.1 Name of product

The verification protocol covers two products from the same vendor, both are acute toxicity tests with luminescent bacteria. The target products are respectively LUMIStox 300 bench top luminometer and ECLOX handheld luminometer. Both can operate in connection with a LUMIStherm thermostat and the PC software LUMISsoft4.

2.2 Name and contact of vendor

HACH-LANGE GmbH, Willstätterstrasse 11, 40549 Düsseldorf, Germany, phone +49 211 5288 0.

Contact: Dr. Elmar Grabert, email: elmar.grabert@hach-lange.de, phone +49 211 5288 241.

Web site: www.hach-lange.de

2.3 Name of center/verification responsible

Danish Centre for Verification of Climate and Environmental Technologies, (DANETV), DHI DANETV Water Centre, DHI, Agern Allé 5, DK-2970 Hørsholm, Denmark.

Verification responsible: Mette Tjener Andersson, email mta@dhigroup.com, phone +45 16 91 48.

U.S. EPA ETV Advanced Monitoring Systems Center (Battelle), Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693, U.S.A.

Verification responsible: Mary E. Schrock, email schrock@battelle.org, phone +1 614 424 4976.

ETV Canada, 2070 Hadwen Road Suite 201 A, Mississauga, Ontario L5K 2C9, Canada.

Verification responsible: Mona El- Hallak, email melhallak@etvcanada.ca, phone +1 905 822 4133 extension 239.

2.4 Verification test organization

The verification will be conducted as a joint verification between the Danish Centre for Verification of Climate and Environmental Technologies (DANETV), the Canadian Environmental Technology Verification Program (ETV Canada) and the U.S. Environmental Technology Verification (U.S. EPA ETV) program. The verification is planned and conducted to satisfy the requirements of the ETV scheme currently being established by the European Union (EU ETV) as well as the Canadian and U.S. ETV programs. Verification and tests will be performed by DHI as DANETV Water Technology ETV Center (DHI DANETV Water Centre) under contract with the Danish Agency for Science, Technology and Innovation. Battelle will participate as the manager of the ETV Advanced Monitoring Systems (AMS) Center through a cooperative agreement with the U.S. Environmental Protection Agency (EPA). ETV Canada will participate as manager of the Canadian ETV Program.

The day-to-day operations of the verification and tests will be coordinated and supervised by DHI personnel, with the participation of the vendor, HACH-LANGE. The testing will be conducted in the DHI laboratories, Hørsholm, Denmark. DHI will operate the luminometers during the verification. HACH-LANGE will provide the luminometers, the thermostats, bacteria, software, user manuals and operation instructions. They will participate in development of protocol and plans in cooperation with DHI. Battelle and ETV Canada will ensure that the verification and tests are planned and conducted to satisfy the requirements of the U.S. and Canadian ETV programs, including input and concurrence from their stakeholder groups, as described in the process document /20/ produced to ensure compliance of the verification with the requirements of the U.S. and the Canadian ETV programs. Battelle and ETV Canada will also participate in the development of the verification protocol and test plan for the verification and tests and perform quality assurance of the verification and tests. Verification protocol and test plan will be reviewed and approved by U.S. EPA ETV AMS Center and ETV Canada.

Three technical experts have been identified to provide independent expert review of the planning, conducting and reporting of the verification and tests.

The organization chart in Fig 2.1 identifies the relationships of the organization associated with this verification and tests.

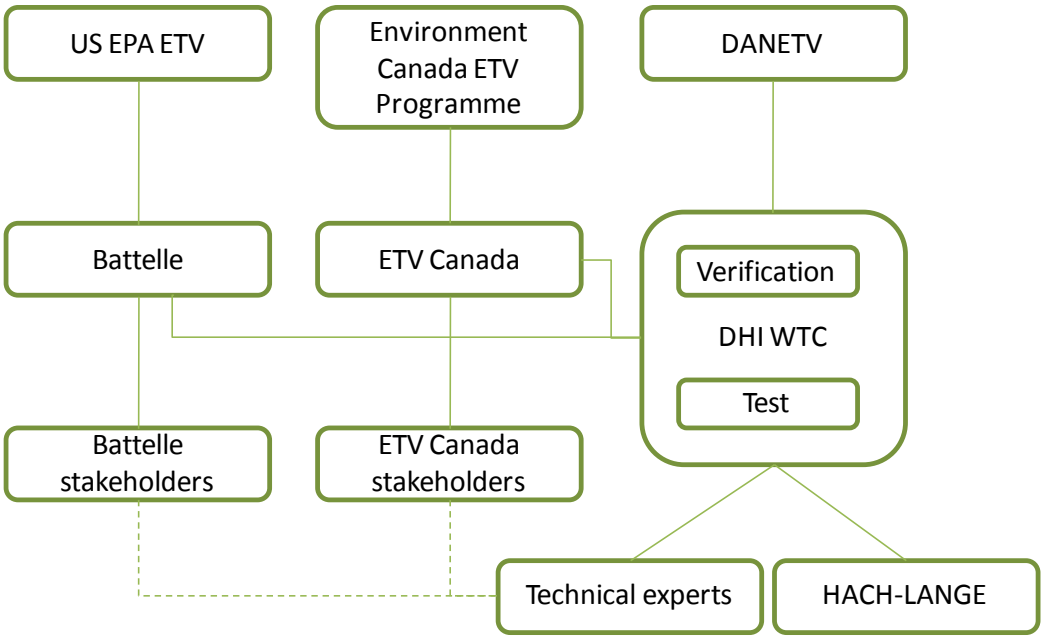

Fig 2.1 Organization of the verification and tests.

2.5 Technical experts

Three technical experts have been appointed. They are:

Dr. Joel Allen, email: allen.joel@epa.gov, phone +1 513 487 2806. U.S.EPA, Office of Research and Development/National Risk Management Research Laboratory/Water Supply and Water Resources Division/Water Quality Management Branch.

Associate Professor Kresten Ole Kusk, email: kok@env.dtu.dk, phone +45 4525 1569. Technical University of Denmark, Department of Environmental Engineering.

Dr. Ali Amiri, email: aamiri@oceta.on.ca, phone +1 905 822 41 33 ext 222. Ontario Center for Environmental Technology Advancement (OCETA).

2.6 Verification process

The principles of operation with the role of the verification and test documents and the different sub-bodies responsible are given in Fig 2.2.

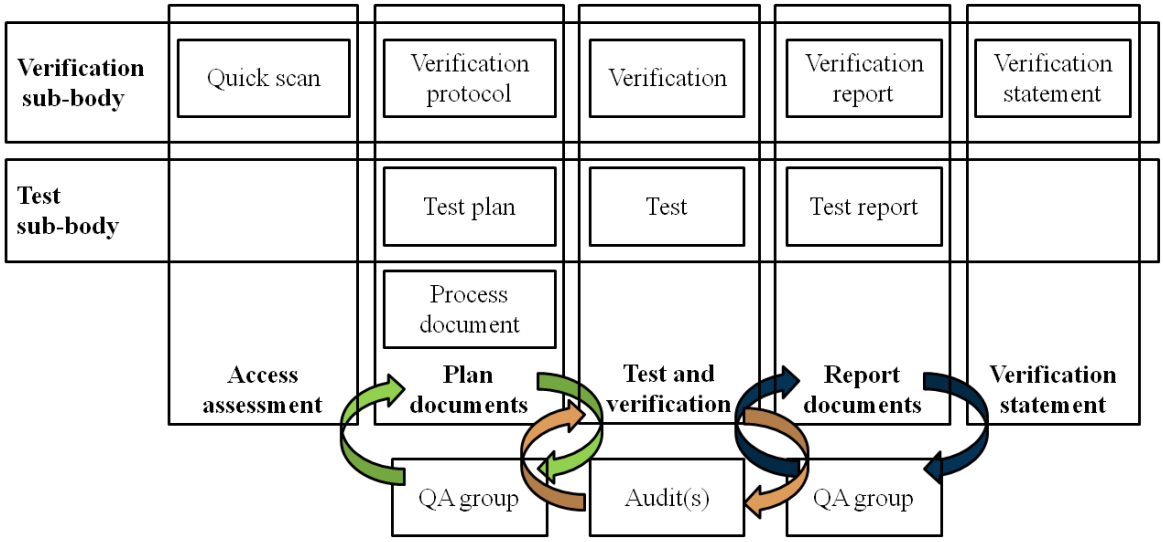

Fig 2.2 Principles of operation of the DANETV verification scheme for joint verification.

The QA group covers the expert group, Battelle, U.S. EPA ETV and ETV Canada. Audits will be performed internally by DHI, and optionally by Battelle, U.S. EPA or ETV Canada. Battelle is expected to perform an audit.

References for the verification process are the Quality Management Plan from Battelle /2/, the General Verification Protocol from ETV Canada /3/ and the Quality Manual for the ETV operations at DHI following the DANETV Quality Manual Template /1/.

The final verification protocol, the test plan and the above mentioned process document shall be seen as one consolidated verification description.

A joint U.S. EPA ETV, ETV Canada and DANETV verification statement will be issued after completion of the verification. One verification report and verification statement will cover both the LUMIStox 300 Bench Top Luminometer and the ECLOX Handheld Luminometer.

3 DESCRIPTION OF THE TECHNOLOGY

Luminometers such as LUMIStox and ECLOX are in vitro testing systems that use bioluminescent bacteria to detect toxic compounds in water, e.g. wastewater; river and lake water; leachate from soil, waste, rubble, etc.; or directly in fluent chemicals. Bioluminescence tests are metabolic inhibition tests that provide acute toxicity analyses. In the bioluminescence testing with LUMIStox and ECLOX a strain of naturally occurring luminescent bacteria, *Vibrio fischeri*, is used. *Vibrio fischeri* is a non-pathogenic, marine, luminescent bacterium which is sensitive to a wide range of toxicants. When properly grown, luminescent bacteria produce light as a by-product of its cellular respiration. Any inhibition of cellular activity results in a decreased rate of respiration and a corresponding decrease in the rate of luminescence. The light emission/luminescence can be measured with a LUMIStox or ECLOX luminometer.

Inhibition of the light emission in the presence of a sample is determined against a non-toxic control. The luminescence is measured after a contact time of 5 (optional), 15 and 30 minutes at 15°C, taking into account a correction factor, which is a measure of intensity change of control samples during the exposure time.

4 DESCRIPTION OF THE PRODUCTS

4.1 LUMIStox 300

The LUMIStox 300 is a bench top luminometer that has been developed as a measuring unit for the luminescent bacteria test. In combination with the LUMIStherm incubation block it conforms to the technical requirements of ISO 11348.

ISO 11348 describes determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri.* The ISO standard contains three parts, using freshly prepared bacteria, liquid-dried bacteria and freeze-dried bacteria respectively. For the LUMIStox (and ECLOX) freeze-dried bacterium is used (ISO 11348-3, /23/). ISO 11348-3, Section 9.2 specifies determining EC values using solution concentrations with inhibition values between 10% and 90% following a dilution scheme described in Annex B, Table B1 involving nine dilutions ranging from 1:2 to 1:32 and reporting the resulting EC_{20}- and EC_{50}-values.

The LUMIStox 300 has a built-in photometer function and an automatic measuring and evaluation routine, which enables it to recognize color effects in the luminescent bacteria test and to take these into account in the test result.

The photometer function also allows the color effect to be estimated in advance, and can be used to determine the extinction (as OD - optical density) of bacteria suspensions for the purpose of assessing light extinction.

The LUMIStox 300 can be connected to a PC running LUMISsoft4 that enables the operator performing and recording luminescent bacteria tests to conduct all of the ISO 11348 requirements. The results from LUMIStox are either LID or EC_{50}-values, representing Lowest Ineffective Dilution causing less than 20%[1] inhibition and Effective Concentration causing 50% inhibition, respectively. The EC_{50}-values are the commonly used result from toxicity tests, while the LID is used as a standard practice in Germany.

4.2 ECLOX

The ECLOX is a portable instrument designed to provide data appropriate for risk assessments in the event of environmental releases, emergency situations, preventive security measures, and regulatory monitoring.

The ECLOX is designed in particular to be used for the Luminescent Bacteria Toxicity Test and to be used with a Chemiluminescence Toxicity Test. Both tests will give results

[1] LID of 20% inhibition is stated in ISO 11348-3, Annex B, Section B.5.

in a short-term and simple way in the field or in the laboratory. The ECLOX used in the field gives values of % inhibition.

Additionally the ECLOX can be used to deliver luminescence values. If the ECLOX is connected to LUMISsoft4, a PC program for the luminescent bacteria test according to ISO 11348, LID and EC_{50}-values can be calculated and recorded.

5 APPLICATION AND PERFORMANCE PARAMETER DEFINITIONS

The application is defined as detailed in the application definition appendix, Appendix 3, in terms of matrix/matrices for use, targets of monitoring and effects.

5.1 Application definition

An overview of matrix, effect, target and technology for the LUMIStox and ECLOX is given in Table 5.1.

Table 5.1 Description of matrix, effect, targets and technologies for LUMIStox and ECLOX.

Matrix	**Effect**	**Targets**	**Technologies**
LUMIStox and ECLOX are applied for wastewater; river and lake water; leachate from soil, waste, rubble, etc.; or directly in fluent chemicals. Verification testing will be conducted on domestic and industrial wastewater effluents	Measurement of toxicity as indicated by inhibition of luminescent bacteria by a variety of compounds including metal ions, organic pesticides, inorganic and organic pollutants and surfactants **Additional parameters:** User manual quality, product cost, environmental health and safety	The target for the application is measurement of toxicity, specifying criterion of detection (CD), range of application, precision (repeatability and reproducibility), agreement with accepted values and robustness	ECLOX and LUMIStox analyses for inhibition of light emitting luminescent bacterium *Vibrio fischeri*

5.2 Performance parameters for verification

The performance parameters relevant for the application, as derived in Appendix 3, are presented in Table 5.2. The ranges presented for these parameters are used for planning the verification and testing only.

Table 5.2 Relevant ranges of performance parameters in effluent industrial and domestic wastewater.

	Criterion of detection	**Range of application**	**Precision (RSD)** %		**Agreement with accepted values**	**Robustness**
			Repeatability	**Reproduce-ability**		
	% inhibition	L/L			%	%
LUMIStox	< 10	>1/2 - < 1/32	< 20	< 30	100 ±50	100±50
ECLOX	< 10	>1/2 - < 1/32	< 20	< 30	100 ±50	100±50

For toxicity testing it is not possible to determine the limit of detection (LoD). Instead it is chosen to determine the criterion of detection (CD) based on the standard deviation of blanks (2% NaCl solution and bacteria suspension, no toxic compound added).

The range of application for a chemical analysis is usually the range of analyte concentration from the limit of detection to the highest concentration with linear response. This concept is not meaningful for a toxicity test of a water sample, because the test does not measure a concentration but an inhibitory effect as a function of the dilution of the sample. The range of application for determining EC_{50} therefore has to be considered in terms of dilution. According to the HACH-LANGE manual estimation of an EC_{50} of a water sample requires a minimum of three measurements where the inhibition is between 10% and 90%. In addition one of the three measurements must be above 50%. If the standard dilution row is considered as described in the LUMIStox 300 Operation manual and in Annex B of the ISO 11248-3:2007 with 9 dilutions (2, 3, 4, 6, 8, 12, 16, 24, 32 times dilution in the test suspension) then EC_{50} should be in the range of dilutions > 2 and < 32 times dilution assuming three measurements with inhibition between 10 and 90%. Based on test results will be given ranges of concentrations of the compounds tested in this study, which will give an inhibition within the range of application.

Precision shall be evaluated under repeatability and reproducibility conditions. Repeatability is defined as the relative standard deviation of measurements done with the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time. Reproducibility is defined as the relative standard deviation of measurements under different conditions such as different locations, operators, measuring systems, and replicate measurements on the same or similar objects. In laboratory terminology, repeatability is the within-series precision and the reproducibility the between-series precision. For reproducibility of luminescent toxicity testing, the difference in bacteria batches is considered to be the greatest source of deviation. Precision will be determined as the relative standard deviation of the EC_{20} and EC_{50} results generated during testing.

Trueness is generally the closeness of agreement between the (mean) concentrations found in measurements and the true or accepted concentration. According to ISO 11348-3 the true or accepted EC_{50}-value of a substance is obtained, as long as the criteria in the ISO are met. Reference testing with purpose of gaining true toxicities will therefore not be performed. For this verification it is chosen to determine ”trueness”, as “agreement with accepted values”. This agreement will be the inhibition results (EC_{50}-values) obtained in

the tests compared to robust literature values for EC_{50}-values, with clear reference to tests being performed according to the ISO 11348-3 method, for the same compound. The agreement with accepted values will only be determined for test substances where robust literature values are available.

The parameters of robustness to be verified are pH change, temperature change, presence of color or turbid material in sample, difference in start concentration, matrix variation, and type of cuvette. Robustness is basically the trueness as found for different values of the robustness parameters.

Samples will be tested with different concentration of color and turbid material, since the ISO standard specifies that they will cause interference. Correction methods are available for both LUMIStox and ECLOX, these methods will be verified.

The ISO 11348-3 recommends test to be performed at a pH range of 7±0.2, but states that pH values of 6.0-8.5 are acceptable. Tests will be performed comparing the three pH-values.

The ISO 11348-3 specifies that a thermostat or similar shall cool the test vials to 15±1°C. The thermostat will be monitored. Tests will be performed comparing temperatures of 14°C, 15°C and 16°C. In case the test of the thermostat shows greater variation than 14-16°C, the minimum and maximum temperatures will be used instead of 14°C and 16°C.

When testing wastewater samples it may not be possible to cover the range from 10-90% inhibition, which is ideal. Therefore tests are performed with maximum concentrations of approximately 30% and 60% inhibition (EC_{30} and EC_{60}), to see how that affects the determination of EC_{20} and EC_{50}. The start concentration of causing approximately 30% and 60% inhibition, are chosen to ensure determination of EC_{20} and EC_{50}, where the last will only be possible to determine with the start concentration of approximately 60% inhibition.

Testing of industrial and domestic effluent wastewater samples is included. These tests will include testing of the wastewater as received as well as spiked non-inhibitory wastewater. The tests will be performed to see the effect of the wastewater matrix on the luminescent test.

Generally glass cuvettes are used in the LUMIStox and plastic cuvettes are used in the ECLOX. HACH-LANGE has though stated that plastic cuvettes also can be used in LUMIStox, to ease the testing all test will therefore be performed with use of plastic cuvettes except for test L where LUMIStox robustness towards cuvettes (glass or plastic) will be tested.

5.3 Additional parameters

Besides the performance parameters to be obtained by testing, compilation of parameters describing user manual, product costs and occupational health & safety issues of the product are required as part of the verification.

6 EXISTING DATA

6.1 Summary of existing data

The vendor has recently performed tests with the LUMIStox and ECLOX instruments for determination of precision expressed by the relative standard deviation (RSD). The results are given at a contact time of 15 minutes and are listed in Table 6.1.

Table 6.1 Results from HACH-LANGE test of LUMIStox and ECLOX.

Compound	**Range 10-90% inhibition** mg/L	**LUMIStox**			**ECLOX**		
		No. of bacteria batches/ no. of replicates	**EC_{50}** mg/L	**RSD** %	**No. of bacteria batches/ no. of replicates**	**EC_{50}** mg/l	**RSD** %
Cr^{6+}	1.7-27	3/5	6.6	38	1/3	8.6	26
Zn^{2+}	1.5-9.0	2/4	4.3	25	1/3	4.2	15
Pb^{2+}	0.21-2.5	2/4	0.49	8.0	1/3	0.48	8.7
SDS	0.14-2.3	3/6	0.66	16	1/3	0.55	2.8
CTAB	0.33-6.0	2/4	0.84	5.8	1/3	1.1	16
Formaldehyde	4.4-35	2/4	15	9.5	1/3	14	5.1
Hydroquinone	0.03-0.20	2/7	0.093	46	Not tested		
p-Cresol	0.38-6.0	2/4	1.5	33	1/3	1.6	6.6
CN^-	0.51-8.1	2/6	2.7	74	Not tested		

The range 10-90% inhibition is the measurement interval used for calculating the EC_{50}-values. 10% inhibition equals to EC_{10}, while 90% equals to EC_{90}. This range will for compound included in this verification be used as guidance for test range.

Notice that the test of LUMIStox was performed on 2-3 different bacteria batches, while the test of ECLOX was performed on one bacteria batch only. This results in higher relative standard deviations for LUMIStox than for ECLOX.

It should be mentioned that the relative standard deviation is calculated by the vendor with no reference to number of samples tested in each bacteria batch.

The vendor has made a note on results for cyanide regarding cyanide to be difficult to work with in the laboratory at a pH of 7.

At pH 7 almost all cyanide is in the volatile and toxic HCN form and evaporation of HCN can occur.

6.2 Quality of existing data

The tests are performed by the vendor and not by an independent body. Furthermore, the analyses are not conducted by a laboratory with ISO 17025 accreditation. The data can therefore only be used for planning of the verification, and not as verification data.

6.3 Accepted existing data

No existing data are accepted for use as part of this verification test. However, these data provide useful background for planning the test.

7 TEST PLAN REQUIREMENTS

Based upon the application and performance parameter identification, Section 5, the requirements for test design have been set up, see below. The detailed test plan is prepared separately, based upon the test requirements specified below.

7.1 Test design

The outline of the required tests is shown in Table 7.1, more details of the test design are found in the Test Plan /25/. The principle behind the design is that three test set-ups are used:

- LUMIStox 300 bench top luminometer with LUMIStherm thermostat and LUMISsoft4 PC software. According to ISO 11348-3.
- ECLOX handheld luminometer with LUMIStherm thermostat and LUMISsoft4 PC software. Conditions similar to ISO 11348-3.
- ECLOX handheld luminometer with use of firmware.

Three matrices will be used in the testing: spiked 2% NaCl MilliQ water, domestic effluent wastewater, and industrial effluent wastewater.

Table 7.1 Test design and associated performance parameters.

Test no.	Performance parameters	Equipment			Matrix	
		LUMIStox	ECLOX incl. thermostat and software	ECLOX incl. firmware	2% NaCl MilliQ	Wastewater
A	Range, Repeatability, Agreement with accepted values	x	x		x	
B	Criterion of detection	x	x		x	
C	Robustness, effect of start conc. on repeatability	x	x		x	
D	Reproducibility	x	x		x	
E	Robustness, sample temperature at field use			x	x	
F	Robustness, sample temperature at laboratory use	x	x		x	
G	Robustness, pH	x	x		x	
H	Robustness, color	x	x		x	
I	Robustness, turbidity	x	x		x	
J+K	Robustness, matrix	x	x			x
L	Robustness, cuvettes	x			x	

Tests will be performed with specific compounds in 2% NaCl MilliQ water to determine their EC_{20}- and EC_{50}-values. The tests will show the range of responses towards these specific toxic compounds. Secondly, tests will be performed on effluent wastewater with and without spiking with a toxic compound. This will show the robustness of the luminescent tests towards the wastewater matrix. The last test will evaluate the effect on results between use of glass cuvettes and plastic cuvettes in the LUMIStox Benchtop.

According to ISO 11348-3 three reference substances shall be tested for each batch of bacteria. These tests will be performed solely on the LUMIStox.

7.2 Reference tests and analysis

The true value of a toxicity test cannot be determined since no bacteria vials are fully identical, and therefore the test results will react slightly different in every test. The reference tests will therefore not be used as true values as seen in other verifications, but will give an identification of the level of toxicity and e.g. false negative tests performed with the LUMIStox or ECLOX equipment (false negative: no response observed where there should be a response). A false negative result should be investigated. False negative results could be caused by improper handling of samples and test equipment, or could be because test equipment is not responding as expected. As a result the test of reference samples will be limited.

Luminescent bacteria reference tests must be done under ISO 17025 accreditation /16/ using the ISO 11348-3 Luminescent bacteria test method, e.g. with Microtox® and must be documented to satisfy performance parameters as derived for LUMIStox and ECLOX in Table 5.2 and Appendix 3.

Reference analysis of stock solutions must also be done under ISO 17025 accreditation /16/ with appropriate methods *by an independent laboratory*.

7.3 Data management

Data storage, transfer and control must be done in accordance with the requirements of ISO 9001 /17/ enabling full control and retrieval of documents and records. The filing and archiving requirements of the DHI Quality Manual must be followed (10 years archiving).

7.4 Quality assurance

The quality assurance of the tests must include control of the test system at DHI DANETV Water Centre, control of the reference test and reference analysis performed at external laboratories and control of the data quality and integrity.

The test plan and the test report will be subject to review by the expert group as part of the review of this verification protocol and the verification report, see Fig 2.2.

As this verification is a joint verification with the U.S. EPA ETV and ETV Canada, an on-site audit by Battelle AMS Center is to be included in the quality assurance.

7.5 Test report

The test report must follow the principles of the template of the DHI DANETV verification center quality manual template /1/ with data and records from the tests presented. For this joint verification, the principles of the U.S. EPA ETV and ETV Canada formats must be complied with as well.

One joint test report will be prepared for LUMIStox and ECLOX.

8 EVALUATION

The evaluation includes calculation of the performance parameters, see Section 5.2 for definition, evaluation of the data quality based upon the test quality assurance, see Section 7.4, and compilation of the additional parameters as specified in Section 5.3.

The calculations behind the EC_{20} and EC_{50} determination in the software LUMISsoft4 are not verified as part of this test, but will be spot-checked by a graphical check of the EC_{20}- and EC_{50}-values for one dilutions series.

8.1 Calculation of performance parameters

By testing a dilutions series with inhibitions in the range from 10-90%, EC_{20}- and EC_{50}-values can be calculated according to principles in ISO 11348-3. This is performed by the PC software LUMISsoft4 connected to the HACH-LANGE instruments. To estimate EC_{50}-values a minimum of 3 measurements have to be in the range from 10-90%

inhibition. Furthermore, one concentration has to give responses above 50% inhibition and one concentration has to give response below 50% for determination of valid EC_{20}-values.

For use of the ECLOX without connection to PC, the results will be recorded as % inhibition and EC-values cannot be determined directly.

Calculations of EC_{20}- and EC_{50}-values (and in the case of ECLOX using firmware, % inhibition) are performed according to generally accepted statistical principles such as those described in /9/ and as described in Table 8.1, referring also to the test design shown in Table 7.1.

Table 8.1 Calculations used for the test results

Parameter	**Calculation**	**Explanations**
Criterion of detection	$CD = t_{0.95}(f) s_k (1 + \frac{1}{n})^{½}$	CD is criterion of detection; $t_{0.95}(f)$ is the Student's t factor for f where f= n-1 degrees of freedom. n is number of measurements; s_k is a pooled estimate for standard deviation of luminescent in control glasses
Range of application	Minimum: just above 2*EC_{50} Maximum: just less than 32*EC_{50}	EC_{50}: Concentration causing 50% inhibition
Precision (repeatability or reproducibility), as relative standard deviation, RSD	$D_i = \lvert x_{i\max} - x_{i\min} \rvert$ $\bar{x}_i = \frac{\sum x_i}{n}$ $d_i = \frac{D_i}{\bar{x}_i}$ $\bar{d} = \frac{\Sigma d_i}{m}$ $RSD = \frac{\bar{d} * 100}{1.693}\%$	D_i is the range at level i; x_{imin} and x_{imax} are the lowest and highest measurements at level i; $\bar{x}_i$ is the average of n measurements; m is the number of levels; d_i is the relative range at level i; $\bar{d}$ is the mean relative range for all m levels used with three replicates, i=3 in x_i
Agreement with accepted values, A Based on robust literature values (obtained by use of ISO 11348-3)	$\bar{x}_i = \frac{\sum x_i}{n}$ $\bar{y}_i = \frac{\sum y_i}{n}$ $A_i = \frac{\bar{x}_i}{\bar{y}_i} \times 100\%$ $A = \frac{\sum A_i}{m}$	$\bar{x}_i$ is the mean of measurements at level i, $x_{i;}$ $\bar{y}_i$ is the literature value at level i, $y_{i;}$ Ai is the agreement at level i; A is the mean agreement for all levels
Robustness, R	$R = \frac{\bar{x}_{ro}}{\bar{x}_{re}} \times 100\%$	$\bar{x}_{ro}$ is the average of measurement under conditions of robustness test; $\bar{x}_{re}$ is the average of measurements under reference conditions

Calculations on performance parameters will be performed in Excel 2007 set up for the purpose with the equations required.

8.2 Evaluation of test data quality

The information of the test report on the reference test and analysis, the test system and data quality and integrity control will be evaluated against the requirements set in this protocol and the objectives set in the test plan.

The spreadsheet used for the calculations will be subject to control on a sample basis (spot validation).

The internal audit report and the external audit report prepared by Battelle AMS Center, see Section 7.4, will be evaluated and major findings compiled and reported.

8.3 Compilation of additional parameters

8.3.1 User manual

The verification criterion for the user manual is that it describes the use of the equipment adequately and understandable for the typical laboratory technician and test coordinator. This criterion is evaluated through evaluation of a number of specific points of importance; see Table 8.2 for the parameters to include.

A description is complete, if all essential steps are described, if they are illustrated with a figure or a photo, where relevant, and if the descriptions are understandable without reference to other guidance.

Table 8.2 Criteria for user manual evaluation.

Parameter	Complete description	Summary description	No description	Not relevant
Product				
Principle of operation				
Intended use				
Performance expected				
Limitations				
Preparations				
Unpacking				
Transport				
Assembly				
Installation				
Function test				
Operation				
Steps of operation				
Points of caution				
Accessories				
Maintenance				
Trouble shooting				
Safety				
Chemicals				
Power				

Cost prices for LUMIStox, ECLOX, thermostat, the software LUMISoft4 and additional equipment as cuvettes, bacteria and chemicals will be given in verification report.

8.3.2 Product costs

The capital investment costs and the operation and maintenance cost, could be seen as the sustainability of the product, will be itemized based upon a determined design basis /28/, see Table 8.3 for the items that will be included.

Table 8.3 List of capital cost items and operation and maintenance cost items per product unit.

Item type	Item	Number	None
Capital			
Site preparation			
Buildings and land			
Equipment			
Utility connections			
Installation			
Start up/training			
Permits			
Operation and maintenance			
Materials, including chemicals			
Utilities, including water and energy			
Labor			
Waste management			
Permit compliance			

The design basis will be described and the cost items relevant for the LUMIStox and ECLOX listed. Note that the actual cost for each item is not compiled and reported.

8.3.3 Occupational health and environment

The risks for occupational health and safety and for the environment associated with the use of the products will be compiled. The compilation will list chemicals used during product operation and classified as toxic, T, or very toxic, Tx, for human health and/or very environmentally hazardous (N) according to /29/. The information will be given as amount used per product unit (sample), see Table 8.4 for format.

Table 8.4 Compilation of classified chemicals used during product operation.

Compound	CAS number	Classification	Amount used per product unit

Additional risks from installing, operating and maintaining the product will be evaluated, compiled and reported, if relevant. In particular, risks for human health associated with power supply and danger of infections will be considered.

9 VERIFICATION SCHEDULE

The verification is planned for 2009-2010. The overall schedule is given in Table 9.1.

Table 9.1 Verification schedule.

Task	Timing
Quick scan	October 2009
Verification protocol and test plan	October to December 2009
Test	January and February 2010
Test reporting	January and February 2010
Verification	February 2010
Verification report	February 2010
Report document review	March 2010
Verification statement	March and April 2010

10 QUALITY ASSURANCE

The quality assurance of the verification is described in Table 10.1 and Fig 2.2. The quality assurance of the tests are described in the test plan but are summarized here, as well as in the process document prepared by Battelle /20/.

Table 10.1 QA plan for the verification.

	DHI		Battelle AMS Center	U.S. EPA ETV	ETV Canada	Expert Group
Initials	MWN	BOP	ZW	JMK, MH	MEH	KOK, JA, AA
Tasks						
Plan document with verification protocol and test plan	Review	-	Review	Review	Review	Review
Test system	-	Audit	Audit	-	-	-
Report document with test report and verification report	Review	-	Review	Review	Review	Review

An internal review of plan and report documents is conducted by the Head of Innovation, Margrethe Winther-Nielsen (MWN). A test system audit (see test plan) is conducted following GLP audit procedures by a trained auditor: Senior Chemical Engineer Bodil Mose Pedersen (BOP).

The Battelle Quality Manager, Zachary Willenberg (ZW) will perform a technical systems audit (TSA) during this verification and test.

U.S. EPA staff, John McKernan (JMK) and Michelle Henderson (MH), and Mona El-Hallak (MEH) from ETV Canada will review the plan and report documents.

The expert group, Kresten Ole Kusk (KOK), Joel Allen (JA) and Dr. Ali Amiri (AA) will review the plan and report documents.

Reviews will be done using the DANETV review report template.

A P P E N D I X 1

Terms and definitions used in the verification protocol

The abbreviations and definitions used in the verification protocol are summarized below.

Where discrepancies exist between DANETV and U.S. EPA ETV terminology, definitions from both schemes are given.

Word	DANETV	U.S. EPA ETV
Agreement with accepted values	Here defined as the % agreement between literature values and test results	
AMS Center	Advanced Monitoring Systems Center at Battelle	
Analytical laboratory	Independent analytical laboratory used to analyze reference samples	
Application	The use of a product specified with respect to matrix, target, effect and limitations	
CD	Criterion of detection	
CTAB	Cetyl trimethyl ammonium bromide	
DANETV ETV	The Danish Centre for Verification of Climate and Environmental Technologies	
EC	Effect concentration, e.g. causing 50% inhibition (EC_{50})	
ECLOX	ECLOX handheld luminometer from HACH-LANGE	
Effect	The way the target is affected	
EN	European standard	
ETV	Environmental technology verification (ETV) is an independent (third party) assessment of the performance of a technology or a product for a specified application, under defined conditions and adequate quality assurance	EPA program that develops generic verification protocols and verifies the performance of innovative environmental technologies that have the potential to improve protection of human health and the environment
EU	European Union	
Evaluation	Evaluation of test data for a technology product for performance and data quality	An examination of the efficiency of a technology
Experts	Independent persons qualified on a technology in verification or on verification as a process	Peer reviewers appointed for a verification
GLP	Good Laboratory Practice	
ISO	International Standardization Organization	
LID	Lowest ineffective dilution. Often seen as the dilution in a dilution series causing less than 20% inhibition	
Limit of detection LoD	Calculated from the standard deviation of replicate measurements at less than 5 times the detection limit evaluated. Corresponding to less than 5% risk of false blanks	
LUMISsoft4	PC software from HACH-LANGE , produced for LUMIStox	
LUMIStherm	Thermostat from HACH-LANGE , produced for LUMIStox	
LUMIStox	LUMIStox 300 bench top luminometer from	

Word	DANETV	U.S. EPA ETV
	HACH-LANGE	
Matrix	The type of material that the product is intended for	
Method	Generic document that provides rules, guidelines or characteristics for tests or analysis	
OD	Optical density	
PC	Personal computer	
Performance claim	The effects foreseen by the vendor on the target (s) in the matrix of intended use	
Performance parameters	Parameters that can be documented quantitatively in tests and that provide the relevant information on the performance of an environmental technology product	
Precision	The relative standard deviation obtained from replicate measurements, here measured under repeatability or reproducibility conditions	
(Environmen-tal) product	Ready to market or prototype stage product, process, system or service based upon an environmental technology	(Environmental) technology
QA	Quality assurance	
Range of application	Generally: the range from the LoD to the highest concentration with linear response. For this verification the range is based on range of dilution of a test sample	
Reference analyses	Analysis by a specified reference method in an accredited (ISO 17025) laboratory	
Repeatability	The precision obtained under repeatability conditions, that is with the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period of time	
Reproducibility	The precision obtained under reproducibility conditions. Measurement performed at different locations, operators, measuring systems, and replicate measurements on the same or similar objects	
Robustness	% variation in measurements resulting from defined changes in matrix properties	
RSD	Relative standard deviation in %	
SDS	Sodium lauryl sulphate	
Stakeholder		Buyers and users of technology, technology developers/vendors, the consulting engineers, the finance and export communities, government permitters, regulators, first responders,

Word	DANETV	U.S. EPA ETV
		emergency response, disaster planners, public interest groups, and other groups interested in the performance of innovative environmental technologies
Standard	Generic document established by consensus and approved by a recognized standardization body that provides rules, guidelines or characteristics for tests or analysis	
Target	The measurable property that is affected by the product	
(Environmen-tal) technology	The practical application of knowledge in the environmental area	An all-inclusive term used to describe pollution control devices and systems, waste treatment processes and storage facilities, and site remediation technologies and their components that may be utilized to remove pollutants or contaminants from, or to prevent them from entering the environment
Test/testing	Determination of the performance of a product by parameters defined for the application	
Trueness	The % recovery of true value obtained either from knowledge on the preparation of test solutions or from measurements with reference methods	
TSA	Technical system audit	
U.S. EPA	United States Environmental Protection Agency	
Vendor	The party delivering the product or service to the customer	The technology developer, owner, or licensee seeking verification
Verification	Evaluation of product performance parameters for a specified application under defined conditions and adequate quality assurance	Establishing or proving the truth of the performance of a technology under specific, predetermined criteria, test plans and adequate data QA procedures
Vibrio fischeri	Light producing bacteria used in luminescent bacteria test	

A P P E N D I X 2

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A P P E N D I X 3

Application and performance parameter definitions

This appendix defines the applications and the relevant performance parameters used to verify the performance of an environmental technology following the DANETV Program.

1 Applications

The intended application of the product for verification is defined in terms of the matrix, the targets and the effects of the product.

The LUMIStox and ECLOX are luminometers which measure light from the light producing bacteria *Vibrio fischeri*, as indicator of acute toxicity.

1.1 Matrix/matrices

The luminometers are sold for testing of wastewater; river and lake water; leachates from soil, waste, rubble, etc.; or directly in fluent chemicals. The matrix in which the application is being verified is wastewater effluent from both domestic and industrial sources.

1.2 Effect

The luminometers can measure any acute toxicity that causes an effect on the light emission from *Vibrio fischeri*. In the ISO 11348-3 /23/ standard, which the LUMIStox is being tested according to, three compounds are listed as reference substances to be included in validity testing. These are 3,5-dichlorophenol, zinc(II) as zinc sulphate heptahydrate and chromium (VI) as potassium dichromate.

The verification will include these reference substances as well as selected metal ions, organic pesticides, organic toxic compounds, industrial chemicals and surfactants.

1.2.1 Compounds to be tested

The vendor has suggested a list of compound to be included in the verification, these are listed in Appendix Table 1.

Appendix Table 1 List of compounds suggested by vendor.

Group	Compound
Heavy metals	Hg-complexes as $HgCl_2$ Pb^{2+} as $Pb(NO_3)_2$ Zn^{2+} as $ZnSO_4+7H_2O$ $Cr_2O_7^{2-}$ as $K_2Cr_2O_7$
Organic pesticide	2,4,5 Trichloroanilin
Organic pollutants	Formaldehyde p-Crecol Hydroquinone (benzene-1,4-diol)
Industrial pollutant	Cyanide (CN-) as KCN
Surfactants	SDS (sodium lauryl sulphate) CTAB (cetyl trimethyl ammonium bromide)

The vendor has performed tests on all suggested compounds except $HgCl_2$ and 2,4,5 Trichloroanilin.

Each of the target groups and vendor suggested compounds was evaluated as follows:

Hg is banned in the EU, it is therefore not likely to be found in European domestic wastewater today. Hg is difficult to work with in the laboratory. For these reasons Hg is excluded.

Copper is included since it is a good representative for heavy metals in both domestic and industrial wastewater, and since it is found in wastewater as many different ions.

The ISO 11348-3 uses 3,5-dichlorophenol, Zn^{2+} (as $ZnSO_4+7H_2O$) and Cr^{6+} (as $K_2Cr_2O_7$, in water resulting in $Cr_2O_7^{2-}$) as reference substances for testing the quality of delivered bacteria batches. $Cr_2O_7^{2-}$ will be included giving the possibility to do some reference to the standard and the precision test which is described in Appendix Table 7. Zn^{2+} will be included since good literature values exist.

Having two positive metals ions (Cu^{2+} and Zn^{2+}), seems sufficient and Pb^{2+} has therefore been excluded from the test program.

2,4,5 Trichloranilin is not a regularly used pesticide. Instead a pesticide produced by the Danish company Cheminova and included in their standard effluent wastewater analyses is included. The specific pesticide, flutriafol, has been chosen in cooperation with Cheminova.

Hydroquinone is not seen as a compound with special relevance for effluent wastewater and is therefore excluded.

Formaldehyde and p-cresol are easily degradable and relatively volatile. It is therefore unlikely that they will remain in the wastewater effluent after treatment in the plant. Instead, triclosan, which is widely used in household products and found in domestic wastewater, is included. Triclosan is toxic to bacteria.

U.S. EPA ETV has performed verification of similar equipment, but to be used on a chlorinated drinking water matrix. The selection of compounds for those tests was made with a different focus than in this verification. However, the U.S. EPA ETV verification included cyanide, which also is included in the list of compounds suggested by vendor. The vendor has found cyanide to be difficult to work with at pH 7. Cyanide will be included as target compound, but special actions will be taken to ensure and monitor loss of cyanide from test solutions.

In addition to the listed surfactants, nonylphenol ethoxylate will be included in the test since it is a well know surfactant that is very toxic to aquatic organisms and is unwanted in the water environment. By including nonylphenol the three surfactants will represent anionic, cationic and nonionic detergents.

The final list of compounds to be included in the verification is listed in Appendix Table 2.

Appendix Table 2 List of compounds to be included in test with notification on whether compound is typical for domestic or industrial wastewater.

Group	Compounds suggested by vendor	Chosen compounds	Domestic	Industrial
Heavy metals	Hg-complexes as $HgCl_2$ Pb^{2+} as $Pb(NO_3)_2$ Zn^{2+} as $ZnSO_4+7H_2O$ $Cr_2O_7^{2-}$ as $K_2Cr_2O_7$	Cu^{2+} as $Cu(NO_3)_2$ CrO_7^{2-} as K_2CrO_7 Zn^{2+} as $ZnSO_4+7H_2O$	X X X	X X X
Organic pesticides	2,4,5 Trichloroanilin	Flutriafol		X
Organic pollutants	Formaldehyde p-Crecol Hydroquinone (benzene-1,4-diol)	Triclosan	X	X
Industrial pollutant	Cyanide (CN-) as KCN	Cyanide (CN^-) as KCN		X
Surfactants	SDS (sodium lauryl sulphate) CTAB (cetyl trimethyl ammonium bromide)	SDS (sodium lauryl sulphate) CTAB (cetyl trimethyl ammonium bromide) Nonylphenol ethoxylate	X X X X	X X X X

Appendix Table 3 is a list of EC_{50}-values for the selected compound found in the literature.

Appendix Table 3 EC_{50}-values from literature for the selected compounds.

Group	CAS no.	Compound	EC_{50} (*Vibrio fischeri*) mg/L	According to ISO 11348-3	Reference
Heavy metals	7758-98-7	Cu^{2+} (Cupper sulfate)	7.1 (0.35 – 19.5, n=3)	to be determined	/26/
	7778-50-9	$Cr_2O_7^{2-}$	18.7 mg/L ±11% (potassium dichromate)	Yes	/23/
	7733-02-0	Zn^{2+}	2.2 mg/l ± 23% (zinc sulphate heptahydrate)	Yes	/23/
Organic pesticides	7667-21-0	Flutriafol	no data found		
Organic pollutants	3380-34-5	Triclosan	0.28	Yes	/21/
Inorganic pollutant	57-12-5	Cyanide (CN^-)	4	to be determined	/6/
Surfactants	151-21-3	SDS	2.09	unknown	/22/
	57-09-0	CTAB	0.97[2]	Yes	/27/
	104-35-8	Nonylphenol ethoxytale	no data found		

[2] 30 minutes incubation time.

1.3 Target(s)

The targets for the application are generally reported in terms of limit of detection (LoD), precision (repeatability and reproducibility), trueness, range of application and robustness. For toxicity testing the limit of detection is not possible to determine. Instead it is chosen to determine the criterion of detection (CD) based on the standard deviation of blanks. The trueness of the inhibition is difficult to measure, and therefore the verification of trueness will be replaced a verification of agreement with accepted values, which will be evaluated by comparing the measured value to available robust literature values obtained by use of the ISO 11348-3 method, for same compound. The range of the application cannot be determined directly by identification of linear range as for regular measurements. For this verification range is based on the inhibitions needed to determine EC_{50}-values, see description in Section 4 Performance parameter definitions.

The values of the targets claimed by the vendor are given in Appendix Table 4 for the products.

The vendor has incorporated equipment in the LUMIStox for color correction of inhibition. With the use of the color correction on colored samples a robustness of 95-113% was shown. Without color correction, the robustness was 109-148% /24/.

The robustness is the change in results due to defined variations in e.g. concentration level, temperature, pH, color, turbidity, cuvette types, matrix (pure water versus wastewater). The ISO 11348-3 standard includes the possibility of testing (marine) saltwater samples; however, saltwater samples are not included in robustness testing of the products.

Appendix Table 4 Vendor claim of performance /4/.

	Criterion on detection[3] % inhibition	**Precision (RSD)** %		**Range of application (linear screening range)** % inhibition	**Agreement with accepted values** %	**Robustness** %
		Precision of instrument	**Precision of test**[4]			
LUMIStox	(10)	0.7	< 20	10-90	Not specified	Not specified
ECLOX	(10)	2	< 20	10-90	Not specified	Not specified

The vendor has recently tested selected compounds. The results can be found in Table 6.1, in Section 6.1 Summary of existing data.

In the ECLOX manual the vendor states the following:

> *Due to nature of the simplified procedure and that the test is carried out at ambient temperatures the results may differ if compared directly with results [derived] for the same sample using the ISO 11348 method.*

[3] Given as part of linear range.
[4] Is not clearly stated from vendor as repeatability or reproducibility.

1.4 Exclusions

The verification is to be performed on one effluent domestic wastewater and one industrial wastewater, other media are excluded. However, individual test substances are tested in 2% NaCl MilliQ-water.

According to the vendor, samples containing chlorine as a result of drinking water chlorination will interfere with the test results by affecting the viability of the bacterial agents. Chlorine containing samples are excluded from the test.

2 General performance requirements

No formal performance requirements for the application have been identified in the European Union or the U.S. and Canada.

The conventional performance parameters of analytical and monitoring methods and equipment are limit of detection (LoD), precision (repeatability and reproducibility), trueness, specificity, linearity and matrix sensitivity. The uncertainty of measurements may be used to summarize the performance. Parameters may be added to characterize variations of equipment, e.g. on-line or on-site monitoring instruments.

2.1 Regulatory requirements

No regulatory requirements exist for measurement of luminescent toxicity. The new Water Framework Directive 2009/90/EC of 31 July 2009 contains a minimum performance criteria of 25% RSD, applicable for all methods of analysis.

In Germany, wastewater regulations include results from luminescent bacteria tests (LID, lowest ineffective dilution) as quality criteria for several industries including the chemical industry, the rubber industry, cooling towers and waste treatment plants /24/. For the chemical industry a LID = 32 is accepted, meaning that the wastewater has to be diluted a maximum of 32 times to obtain a toxicity below 20% inhibition towards the luminescent bacteria.

For a few of the compounds, environmental quality standards for surface waters are given by the EU /13/. These are listed in Appendix Table 5.

Appendix Table 5 Environmental quality standards stated by EU /13/ and Denmark. For Denmark values in normal writing are effective /14/, while values in italic are planned to come in force within 2010 /15/.

Group	**Compound**	**EU**		**Denmark**	
		Inland surface water µg/L	**Other surface water** µg/L	**Fresh water** µg/L	**Marine water** µg/L
Heavy metals	Cr(VI)			*4.9 (dissolved)*	*3.4 (dissolved)*
	Cu			1 (dissolved) max 12	1 (dissolved) max 2.9
	Zn			*7.8 (dissolved) Soft water: (H<24 mg $CaCO_3$/L) 3.1 (dissolved)*	*7.8 (dissolved)*
Organic pesticides	Flutriafol			*31*	*3.1*

2.2 Application based needs

A validity check is required according to ISO 11348-3. The validity check involves analysis of three reference standards which should cause 20 to 80% inhibition after 30 minutes of contact time. The results from the validity check are shown in Appendix Table 6, as reported for the LUMIStox by vendor.

Appendix Table 6 Vendor quality data for LUMIStox according to ISO 11348-3 /5/.

	3,5 dichlorophenol	**Zn^{2+}**	**$Cr_2O_7^{2-}$**
Standard concentration	3.4 mg/L	2.2 mg/l (zinc sulphate heptahydrate)	18.7 mg/L (potassium dichromate)
No. of data set	70	60	70
Range of inhibition	22.46-63.53%	20.82-49.19%	47.90-78.77%
Mean inhibition	44.1%	30.6%	63.4%
RSD	27%	23%	11%

In ISO 11348-3, results from an interlaboratory trial with the three reference standards are listed for information. The results are shown in Appendix Table 7.

Appendix Table 7 Interlaboratory trial, Annex C, ISO 11348-3.

	3,5 dichlorophenol		**Zn^{2+}**		**$Cr_2O_7^{2-}$**	
	EC_{20}	EC_{50}	EC_{20}	EC_{50}	EC_{20}	EC_{50}
No. of laboratories	14	13	15	14	15	14
Average conc.	2.32 mg/L	3.36 mg/L	1.08 mg/L	2.17 mg/L	3.60 mg/L	18.71 mg/L
RSD	18.6%	9.6%	43.6%	33.6%	52.4%	32.9%

3 State of the art performance

Other similar luminometers exist on the market. Some selected luminometers are listed in Appendix Table 8. Information as to whether they have been verified is included.

Appendix Table 8 Luminometers and verification of these.

Name	Verification	Reference
Portable		
BioFix Lumi-10	None known	/10/
Triathler	None known	/11/
ToxScreen-II	U.S. EPA ETV	/8/
Deltatox	U.S. EPA ETV	/7/
Laboratory		
Microtox	U.S. EPA ETV	/6/
Field installation		
TOXcontrol BioMonitor	TESTNET	/9/

The three U.S. EPA ETV verifications have all been performed using drinking water with a focus on chemical compounds toxic to humans. One compound, cyanide, is also relevant with regards to wastewater. Performance on cyanide measurements for the three products is listed in Appendix Table 9. The toxicity threshold is the lowest concentration of the tested dilutions where toxic effects were significant. For ToxScreen-II a special set-up was used and EC_{50} could therefore not be retrieved.

Appendix Table 9 Results from U.S.EPA ETV verification on cyanide.

Luminometer	Microtox	Deltatox	ToxScreen-II
Cyanide EC_{50} at 5 minutes	8 mg/L	7.6 mg/L	Not measured
Cyanide EC_{50} at 15 minutes	4 mg/L	Not measured	Not measured
Repeatability. Range of relative standard deviation	0-4%	1-4%	0-29%
Toxicity threshold	0.25 mg/L	0.25 mg/L	0.25 mg/L

For the TOXcontrol BioMonitor the LoD, RSD, repeatability etc. were tested and reported for several test set-ups. The compounds used were Zn^{2+} and 3,5 dichlorophenol. Some of the results are summarized in Appendix Table 10.

Appendix Table 10 Results from TESTNET verification of TOXcontrol BioMonitor.

	Range	Comment
Lowest detectable change	7.2-17.4% inhibition	Calculated based on solution of approximately 20%, 50% and 80% inhibition
RSD	5.7-39.3%	
Repeatability	2.4-5.8% inhibition	
Day-to-day repeatability	2.5-31.2% inhibition	Calculated based on solution of approximately 20% and 80% inhibition
Memory effect	Not relevant	No significant effect
Interference (Tropaeolin-color)	Not relevant	Increased inhibition was significant at concentrations from 0.25 mg/L

Vendors of *Vibrio fischeri* test the bacteria lots and state an interval for EC_{50} for selected standard parameters. They also test each lot before shipment. An example of such a test from an anonymous vendor including user laboratory reference testing is shown in Appendix Table 11.

Appendix Table 11 EC_{50} performance of Vibrio fischeri *on standard parameters stated by vendor and tested by vendor and user laboratory.*

Standard parameter	**Phenol**	**Zinc sulfate**	**Zinc^{2+} (ion)**
Specification from vendor			
EC_{50} interval at specification	13.0-26.0 mg/L	3.0-10.0 mg/L	0.6-2.2 mg/L
Test time	5 minutes	15 minutes	15 minutes
Vendor test result			
No. of LOTs	9	9	9
Mean	18.0 mg/L	4.88 mg/L	1.04 mg/L
RSD	19%	27%	25%
User laboratory test result			
No. of LOTS	9	9	Not tested
No. of tests	14	15	-
Mean	18.3 mg/L	5.52 mg/l	-
RSD	10%	20%	-

4 Performance parameter definitions

Based on the above-mentioned performance requirements, a set of relevant ranges of performance parameters for activated sludge tanks (and treated wastewater) have been set up and are listed in Appendix Table 12.

Appendix Table 12 Relevant ranges of performance parameters in effluent wastewater.

	Criterion of detection	**Range of application**	**Precision (RSD)** %		**Agreement with accepted values**	**Robustness**
			Repeatability	**Reproduce-ability**		
	% inhibition	L/L			%	%
LUMIStox	< 10	>1/2 - < 1/32	< 20	< 30	100 ±50	100±50
ECLOX	< 10	>1/2 - < 1/32	< 20	< 30	100 ±50	100±50

The limit of quantification is set to 10% because this is equal to the vendor claim for linear range and because EC_{10}-values often are used for reporting ecotoxicological results.

The range of application for a chemical analysis is usually the range of analyte concentration from the limit of detection to the highest concentration with linear response. This concept is not meaningful for a toxicity test of a water sample, because the test does not measure a concentration but an inhibitory effect as a function of the dilution of the sample. The range of application for determining EC_{50} therefore has to be considered in terms of dilution. According to the HACH-LANGE manual estimation of an EC_{50} of a water sample requires a minimum of three measurements where the inhibition is between

10% and 90%. In addition one of the three measurements must be above 50%. If the standard dilution row is considered as described in the LUMIStox 300 Operation manual and in Annex B of the ISO 11248-3:2007 with 9 dilutions (2, 3, 4, 6, 8, 12, 16, 24, 32 times dilution in the test suspension) then EC_{50} should be in the range of dilutions > 2 and < 32 times dilution assuming three measurements with inhibition between 10 and 90%. Based on test results will be given ranges of concentrations of the compounds tested in this study, which will give a inhibition within the range of application.

Repeatability in Appendix Table 9 and Appendix Table 10 is less than 6% in all cases, except for the ToxScreen-II, where a repeatability of 0-29% is seen. The vendor claims a precision for the products of < 20%, see Appendix Table 4. A repeatability of less than 20% is chosen, since the vendor claims to fulfill this.

The day-to-day repeatability for TOXcontrol BioMonitor, as shown in Appendix Table 10, lists RSD values up to 31.2%. The vendor states, as mentioned, a test precision of < 20%, while the quality check of LUMIStox in Appendix Table 6 shows a reproducibility of up to 27%. Here a reproducibility of 30% is chosen.

The agreement with accepted values is evaluated by looking at the EC_{50}-values specified by a vendor of *Vibrio fischeri* LOTs in Appendix Table 11. The largest relative interval is given for $zinc^{2+}$, the “mean” here is 1.4 mg/L with an acceptable range of ± 57%. The ISO standard 11348-3 requires inhibition of 20-80% of specified concentrations. These numbers cover both reproducibility and repeatability. The agreement with accepted values is set to ± 50%.

Robustness has been tested directly for the TOXcontrol BioMonitor, where the dye chemical tropaeolin was added. The results showed a significant interference at 0.25 mg tropaeolin/L, where an increased inhibition was seen. Color correction is part of the LUMIStox product, see section 1.3 Target(s). The robustness can be interfered by other parameters. The general robustness is set to the level seen without color correction, here values of 148% of true value were seen.

LUMIStox 300 Bench Top Luminometer
ECLOX Handheld Luminometer

Joint test plan

Luminescent bacteria test for use in wastewater

Handheld ECLOX

January 2010 **Approved**

LUMIStox 300 Bench Top Luminometer
ECLOX Handheld Luminometer

Joint test plan

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2 INTRODUCTION

Environmental technology verification (ETV) is an independent (third party) assessment of the performance of a technology or a product for a specified application, under defined conditions and quality assurance.

This verification is a joint verification between DANETV, the U.S. EPA ETV Advanced Monitoring Systems (AMS) Center and the ETV Canada. The objective of the verification is to evaluate the performance of a wastewater rapid toxicity technology that could be used to monitor industrial or domestic wastewater.

This verification and test plan includes two products from one vendor.

2.1 Verification protocol reference

This test plan is prepared in response to the test design established in the LUMIStox and ECLOX, verification protocol, for luminescent bacteria test for use in wastewater, 2009 /1/.

2.2 Name and contact of vendor

HACH-LANGE GmbH, Willstätterstrasse 11, 40549 Düsseldorf, Germany, phone +49 211 5288 0.

Contact: Dr. Elmar Grabert, email: elmar.grabert@hach-lange.de, phone +49 211 5288 241.

Web site: www.hach-lange.de

2.3 Name of center/test responsible

The Danish Centre for Verification of Climate and Environmental Technologies, DANETV), DHI DANETV Water Centre, DHI, Agern Allé 5, DK-2970 Hørsholm, Denmark.

Test responsible: Claus Jørgensen, email clj@dhigroup.com, phone +45 16 95 62.

U.S. EPA ETV Advanced Monitoring Systems Center (Battelle), Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693, U.S.A.

Test responsible: Mary E. Schrock, email schrock@battelle.org, phone +1 614 424 4976.

ETV Canada, 2070 Hadwen Road Suite 201 A, Mississauga, Ontario L5K 2C9, Canada.

Test responsible: Mona El-Hallak, email melhallak@etvcanada.ca, phone +1 905 822 4133 extension 239.

2.4 Expert group

The expert group assigned to this test and responsible for review of the test plan and test report includes:

Dr. Joel Allen, email: allen.joel@epa.gov, phone +1 513 487 2806. U.S.EPA, Office of Research and Development/National Risk Management Research Laboratory/Water Supply and Water Resources Division/Water Quality Management Branch.

Associate Professor Kresten Ole Kusk, email: kok@env.dtu.dk, phone +45 4525 1569. Technical University of Denmark, Department of Environmental Engineering.

Dr. Ali Amiri, email: aamiri@oceta.on.ca, phone +1 905 822 41 33 ext 222. Ontario Center for Environmental Technology Advancement (OCETA).

3 TEST DESIGN

Test compounds are selected as described in the joint verification protocol Appendix 3 /1/, and are summarized in Table 3.1. The pesticide flutriafol has been chosen together with the Danish company Cheminova. Flutriafol is a pesticide included in their effluent wastewater analyses.

Table 3.1 Selected test compounds.

Group	Compound
Heavy metals	Cu^{2+} as $CuSO_4$ Zn^{2+} as $ZnSO_4+7H_2O$ $Cr_2O_7^{2-}$ as $K_2Cr_2O_7$
Organic pesticides	Flutriafol
Organic pollutants	Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether)
Industrial pollutants	Cyanide (CN^-) as KCN
Surfactants	SDS (sodium lauryl sulphate) CTAB (cetyl trimethyl ammonium bromide) Nonylphenol ethoxylate

Compounds which are easy to handle in the laboratory and are toxic to *Vibrio fischeri* will be used. For the specific test set-up, use of a non-colored compound in the test of color robustness or use of a chemical not having a pKa value in the range of 6.0-8.5 for the pH robust test will be considered.

The test design outlined in the test protocol /1/ is summarized in Table 3.2. Acronyms are explained in Appendix 1.

Test results will be EC_{20} and EC_{50} values, representing the concentration causing respectively 20% and 50% inhibition of luminescence of the *Vibrio fischeri* population. For tests where the luminometer is not connected to a PC with LUMISsoft4 software, results will be as % inhibition. This will be the case for a few tests performed with the ECLOX.

All test will be performed with use of plastic cuvettes except for test L were LUMIStox robustness towards cuvettes (glass or plastic) will be tested.

Table 3.2 Test design.

Test No.		**Equipment to be tested**			**Matrix**	**Criterion of detection (CD)**	**Range**	**Precision**		**Agreement with accepted values**	**Robustness**
		LUMIStox	**ECLOX incl. thermostat and software**	**ECLOX incl. firmware**				**Repeatability**	**Reproducibility**		
A	Test of dilution series (9 dilutions) for all compounds. 3 test replicates (includes 2 measurement replicates each). Optimal concentrations will result in inhibitions of 10-90%.	x	x		2% NaCl MilliQ		x	x		x	
B	Test of series of 9 blanks (incl. bacteria suspension, but no sample).	x	x		2% NaCl MilliQ	x					
C	Test of 2 dilution series (9 dilutions) for 1 compound. Max concentrations in dilution EC_{30}, and EC_{60}, respectively. 3 tests replicates (includes 2 measurement replicates each).	x	x		2% NaCl MilliQ						Effect of start concentration on repeatability
D	Test of dilution series for 1 compound. Dilution as used in test A. 3 test replicates (includes 2 measurement replicates each). Repeated on 4 different days with 4 different bacteria batches (test A is equal to first test day).	x	x		2% NaCl MilliQ				x		
E	3 concentrations ~ EC_{20}, EC_{50}, EC_{80} for 2 compounds (metal and organic). 3 test replicates (no further replicates). Performed at 3 possible outdoor temperatures: room temperature, 15°C climate room, and outdoor temperature in Denmark at wintertime (~0-5°C). Measurement only after 15 minutes of inhibition.			x	2% NaCl MilliQ			x			Sample temperature at field use

Test No.		Equipment to be tested			Matrix	Criterion of detection (CD)	Range	Precision		Agreement with accepted values	Robustness
		LUMIStox	**ECLOX incl. thermostat and software**	**ECLOX incl. firmware**				**Repeatability**	**Reproducibility**		
F	Just for concentration ~ EC_{20} for 1 compound. For temperatures of 14°C, 15°C and 16°C. 3 test replicates (includes 2 measurement replicates each). If test of thermostat shows greater variation than from 14-16°C, these temperatures will be used instead.	x	x		2% NaCl MilliQ						Sample temperature at laboratory use
G	Just for concentration ~ EC_{20} for 1 compound. For pH 6.0, 7.0 and 8.5, 3 test replicates (includes 2 measurement replicates each).	x	x		2% NaCl MilliQ						pH
H	Just for concentration ~ EC_{20} for 1 compound. Addition of color in three concentrations and 1 with no color. 3 test replicates (includes 2 measurement replicates each). Include blind with color and no sample. LUMIStox with color correction, ECLOX with correction cuvettes according to ISO 11348-3.	x	x		2% NaCl MilliQ						Color
I	Just for concentration ~ EC_{20} for 1 compound. Addition of turbid reagent/material in three concentrations and 1 with no material. The third being visibly turbid. 3 test replicates (includes 2 measurement replicates each). Include blind with turbid reagent/material and no sample.	x	x		2% NaCl MilliQ						Turbidity
J	Spiked non- inhibiting domestic and industrial wastewater. Just for concentration ~ EC_{20} for 5 compounds. 3 test replicates (includes 2 measurement replicates each). If needed wastewater is diluted to be non-inhibitory.	x	x		Wastewater						Matrix

Test No.		Equipment to be tested			Matrix	Criterion of detection (CD)	Range	Precision		Agreement with accepted values	Robustness
		LUMIStox	**ECLOX incl. thermostat and software**	**ECLOX incl. firmware**				**Repeatability**	**Reproducibility**		
K	Test of dilution series for undiluted and unspiked industrial and domestic wastewater. 3 test replicates (includes 2 measurement replicates each).	x	x		Waste-water						Matrix
L	Just for concentration ~ EC_{20} for 2 compounds. 3 replicates. Test of use of glass and plastic cuvettes.	x			2% NaCl MilliQ						Cuvettes

3.1 Test sites

Both the laboratory tests and outdoor dependent test will be conducted at DHI, Hørsholm, Denmark.

3.1.1 Types

Domestic wastewater samples for laboratory testing are planned to be obtained from Lundtofte wastewater treatment plants. Industrial wastewater will be retrieved from an industrial wastewater treatment plant at Cheminova, a producer of pesticides in north-western Jutland, Denmark.

The wastewaters will be collected by the personnel at the treatment plant. The sample from Cheminova is sent cold to DHI. The sample from Lundtofte wastewater treatment plant is stored cold at the plant and will picked up by DHI personnel. Both wastewater samples will be stored at DHI at 5 °C until use.

MilliQ water from the DHI laboratory, added NaCl to a concentration of 2%, will be used for standard dilution series.

3.1.2 Addresses

Addresses of all sites are listed:

Laboratory and outdoor test: DHI, Agern Alle 5, DK-2970 Hørsholm.

Domestic wastewater: Renseanlæg Lundtofte, Hjortekærsbakken 12, DK-2800 Lyngby.

Industrial wastewater: Cheminova, Thyborønvej 78, DK-7373 Harboøre.

3.2 Tests

The test program is designed to comply with ISO 11348-3. Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test). /2/ and to retrieve information needed to determine performance parameters, as described in ISO/TR 13530 guide to analytical quality control for water analysis /12/, ISO 15839, Water Quality - On-line sensors/analysing equipment for water - Specifications and performance tests. /3/, ICH Harmonised Tripartite Guideline for validation of analytical procedure /11/ as well as previous verifications of similar equipment for drinking water performed by U.S. EPA ETV and described in a public testplan /4/.

The test design, as described in Table 3.2, includes three test set-ups:

- LUMIStox 300 bench top lumiometer with LUMIStherm thermostat and LUMISsoft4 PC software.
- ECLOX with LUMIStherm thermostat and LUMISsoft4 PC software.
- ECLOX with use of firmware.

The main focus is on the laboratory set-up of LUMIStox 300 bench top and ECLOX in connection with LUMIStherm thermostat and LUMISsoft4 PC software, while ECLOX using firmware is tested to a less extent.

The test will be performed mainly in the laboratory, while one test on the ECLOX will be performed outdoors at DHI.

3.2.1 Test methods

Luminescence tests with *Vibrio fischeri* are described in a three-part standard ISO method /2/. Part 1 requires use of freshly prepared bacteria, part 2 uses liquid-dried bacteria, while part 3 uses freeze-dried bacteria. The LUMIStox and ECLOX use freeze-dried bacteria. Therefore the following applies:

> ISO 11348-3 Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) – Part 3: Method using freeze dried bacteria.

This standard is incorporated in the manuals for LUMIStox and ECLOX.

Dilution series of 9 dilutions will be performed for all selected compounds in test A. The dilution series will be prepared according to Annex B in ISO 11348-3. Each dilution will be prepared and analyzed twice and the average will be used as result. This will be repeated 3 times. All other tests will be performed on selected compounds of the 9 target compounds at various dilutions described in Table 3.2 except criterion of detection (Test B), which will be performed on NaCl dilution. Robustness for temperature at lab use (Test F), pH (Test G), color (Test H), turbidity (Test I) and type of cuvettes (Test L) will be performed at one concentration (EC_{20}), while robustness towards start concentration will be performed at two concentrations (EC_{30} and EC_{60}). Robustness towards wastewater matrix with undiluted and unspiked industrial and domestic wastewater (Test K) will be performed on dilutions series with 9 dilutions.

Stock solutions will be prepared in 2% NaCl MilliQ water. Solid NaCl will be added to wastewater to obtain the salt concentration required for testing with the saltwater bacteria, *Vibrio fischeri*. Dilution series will be prepared with dilution saltwater (2% NaCl) provided from HACH-LANGE.

The LUMISsoft4 PC software calculates EC_{50}-values as an overall result of the testing of a dilution series. When a certain test concentration gives 0% or 100% inhibition, the result cannot be used in the determination of EC_{50}. Usually only results between 10% and 90% inhibition are used in the calculation of EC_{50}. For compounds not previously tested it will therefore be necessary to perform a range finding test to determine concentrations within 10-90% inhibition. The software requires a minimum of three values between 10 and 90% to perform the calculation of EC_{50}.

The principle of the dilution series in the thermostat is shown in Figure 3-1. In the rows C and B are the two measurement replicates included in all tests where the thermostat is used. When performing the test, bacteria suspension is added doubling the volume. The final dilution series is therefore 32, 24, 16, 12, 8, 6, 4, 3, 2.

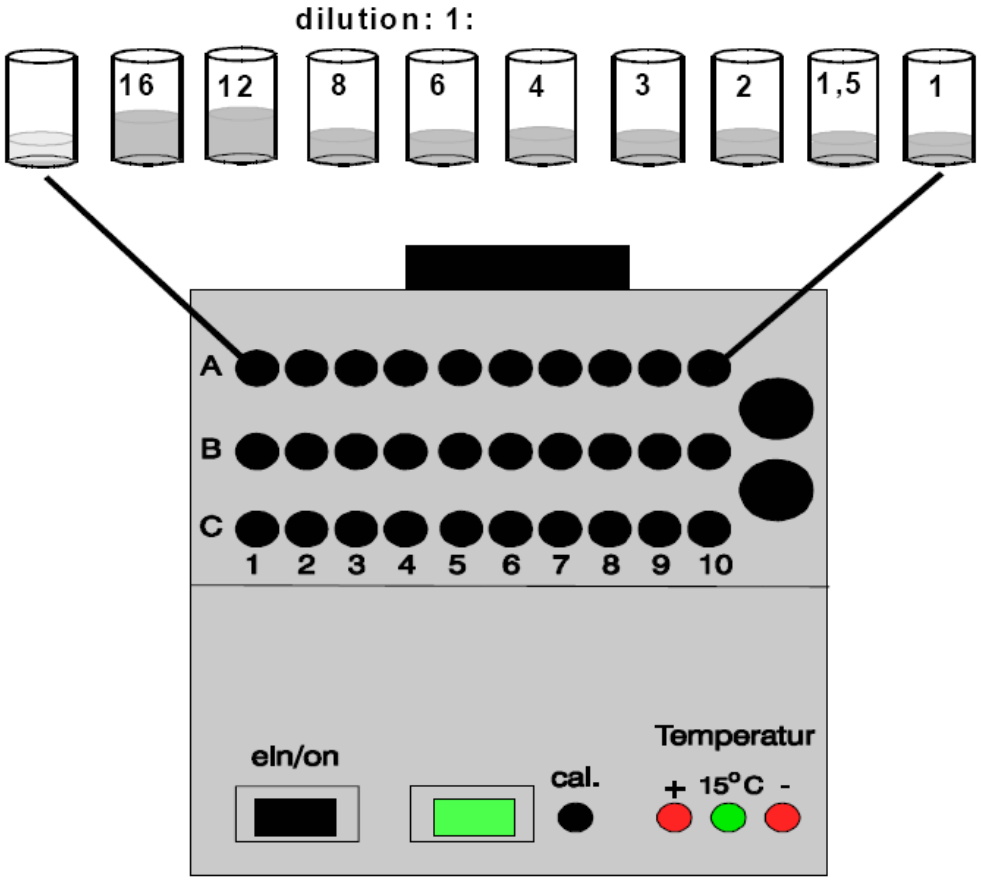

Figure 3-1 LUMIStherm thermostat and dilution series.

Tests will be performed on effluent industrial and domestic wastewater. The toxicity (as indicated by inhibition) of these samples will be tested prior to being used in the spiking test (Test series J and K). If the wastewater is toxic, it will be diluted with 2% NaCl MilliQ water to a non-toxic concentration, and then spiked with the selected compounds.

For field portability (Test E), the ECLOX without the LUMISsoft4 software is used (i.e., no PC is taken along). The firmware only shows readings of % inhibition. The goal of this test is to assess how stable individual inhibition measurements are under three different temperatures, a controlled 15 °C, ambient room temperature, and outdoor temperature (ambient and outdoor temperatures to be recorded at time of testing). Solutions generating three inhibitions (20%, 50% and 80%) will be measured at each temperature to give a sense of variability over a range of inhibitions in temperatures that might be encountered in real-world field testing.

3.2.2 Test staff

The test responsible is Claus Jørgensen, and test technicians are Jane Bergstrøm and Connie Seierø.

3.2.3 Test schedule

The test schedule is given in Table 3.3, see Table 3.2 for identification of experiment labels.

Table 3.3 Test schedule.

Task	Week no. 2009			Week no. 2010														
	51	52	53	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Test plan review	X	Vacation																
Set-up of equipment	X																	
Test preparation	X																	
Testing				X	X	X	X	X										
Test report draft											X							
Test report QA												X	X	X				
Test report																X		
Test report review																		X

3.2.4 Test equipment

The test equipment and manuals include:

- LUMIStox 300 bench top luminometer and LUMIStherm thermostat. Described in:
  - LUMIStox 300. Manual. HACH-LANGE. Version 3.02 and above. BDA 356. January 2008.
  - Luminescent bacteria test with freeze-dried bacteria according to EN/ISO 11348-3. Luminescent bacteria test LCK 491. DR LANGE.
- ECLOX handheld luminometer with LUMIStherm thermostat and LUMISsoft4 PC software or with firmware. Described in:
  - Luminescent bacteria test using the ECLOX^{TM} instrument. User Manual. Hach Company. Edition Beta 2. September 2009.
- LUMISsoft4 PC software. Described in:
  - Dr. Lange LUMISsoft 4. Manual. Version 1.001. LZV 093. 2000.

The DR. LANGE manual LCK 491 for LUMIStox 300 specifies use of glass cuvettes (LZP 187) for the testing. HACH-LANGE informs that both glass cuvettes and plastic test tubes (LZP 1480) can be used with LUMIStox 300. For ECLOX, HACH-LANGE specifies that plastic test tubes (LZP 1480) must be used. /13/

General laboratory equipment procedures including cleaning and calibration are those described and ISO 17025 accredited /5/ for the DHI laboratories under the laboratory services manual of the DHI Quality Management System /6/.

3.2.5 Type and number of samples

The types and number of samples are summarized in Table 3.4.

Table 3.4 Summary of analyses of samples and blanks.

Test No.	Performance parameters	Analyses of samples		Analyses of blanks	
		LUMIStox	ECLOX	LUMIStox	ECLOX
A	Range Repeatability Agreement with accepted values	486	486	54	54
B	Criterion of detection			9	9
C	Robustness, start concentration	108	108	12	12
D	Reproducibility	162	162	18	18
E	Repeatability, field		54		18
F	Robustness, sample temperature	18	18	18	18
G	Robustness, pH	18	18	18	18
H	Robustness, color	30	30	30	30
I	Robustness, turbidity	30	30	30	30
J	Robustness, matrix	60	60	60	60
K	Robustness, matrix	108	108	12	12
L	Robustness, cuvettes	24		24	
Total		**1044**	**1074**	**286**	**280**

Except for test B, the number of blanks may be changed slightly during the tests if some blanks can be used for several tests.

3.2.6 Operation conditions

The operation conditions applied during the verification of the product are generally as required in ISO 11348-3. EC-values will be determined for 15 and 30 minutes.

When using the ECLOX in the field, it is possible to fulfill the requirements in the ISO 11348-3 on adjustment of pH and salinity, and settling of turbid samples; however, it is not possible to adjust the temperature of the testing samples. Therefore a test on variation in sampling temperatures (indoor and outdoor in Danish winter time) is included.

3.2.7 Operation measurements

During operation, the following conditions are recorded, when relevant, see Appendix 6 for data recording and reporting forms:

- Conductivity/salinity of stock solutions.
- pH of stock solutions.
- Temperature in the thermostat, will be done on a daily basis.

Salinity and pH will be adjusted if required according to ISO 11348-3.

The vendor has experienced that cyanide is difficult to work with in the laboratory. Therefore, a regular determination of the concentration of cyanide in the dilutions will be performed with a test kit. This is expected to be done by adding a dilution with cyanide to a beaker before starting luminescence testing. The cyanide concentration of this solution will be determined with the test kit and after the end of luminescence testing, the cyanide concentration in the beaker will be determined again. The test kit,

LCK 315 from HACH-LANGE, uses a babituric acid-pyridine method and has a range from 0.01-0.6 mg/L.

If the loss of cyanide exceeds 20% the results of the cyanide test will not be included in the verification.

3.2.8 Product maintenance

The following storage information for the bacteria is provided by the vendor:

- The test reagents must be stored at –18°C until the date of expiry. Reactivated bacteria should be used within 4 hours if possible. Undiluted, reactivated bacteria should be placed only temporarily in a refrigerator. The sensitivity spectrum of reactivated bacteria may shift as time elapses. Tubes containing thawed but not reactivated freeze-dried luminescent bacteria can be refrozen.

For the LUMIStox the vendor provides the following information on product maintenance and optimal performance:

- The system diskette must be inserted into the drive before the instrument is switched on! Whenever the instrument is moved, the diskette must be removed from the drive first.

- The LUMIStox 300 measuring instrument should not be operated in an ambient temperature below 16°C or above 29°C, otherwise problems may occur with the cooling of the measuring shaft. Do not operate the instrument in direct sunlight!

- Soiling impairs the functioning of the cuvette lowering system. For this reason, do not pipette reagents into measuring cuvettes in the measuring shaft. The measuring shaft should also be closed in the <exit > mode when the measurements have been completed. When the LUMIStox 300 is in use, the measuring shaft is automatically closed after a 10-minute idle period. It can be opened again by pressing any key.

- Before any measurements the LUMIStox 300 must have been switched on for at least 30 minutes so that the photomultiplier and the cooled components are ready for operation.

For the ECLOX the vendor provides the following information on product maintenance and optimal performance:

- All cleaning and maintenance of the ECLOX™ Water Test Kit is to be performed in a suitable clean, dry area. Make sure the kit is clean before removing any access or battery covers. Do not permit foreign material to enter the kits as this can cause equipment damage.

- The ECLOX Water Test Kit is designed for field use. No routine maintenance is required, provided all cleaning, test, and calibration procedures are followed.

- The luminometer must be kept clean at all times. If the surface is dirty, wipe it down with a clean damp cloth. Do not let water get into the luminometer cell. If water gets into the cell, remove the cell insert and wipe out the moisture with a clean, dry cloth. Replace the cell insert.

- When replacing the battery a special procedure described in the manual must be followed.

3.2.9 Health, safety and wastes

The use of the product does not imply special health, safety and waste issues.

Laboratory work during testing will be done according to the DHI Safety Rules that are compliant with the Danish rules for safe occupational health and the European regulations of work with chemicals. The test substances will be handled carefully in accordance with material datasheets of the test substances. Wastewater will be handled according to DHI's safety rules.

Outdoor work will be done according to DHI's rules for safe field work included in DHI's safety rules.

Chemicals and test solutions are discarded according to Danish regulations for chemical waste by collection and destruction, *in casu* by collection and shipment to controlled destruction when required.

4 REFERENCE TESTS AND ANALYSIS

The true value of a toxicity test cannot be determined, since no bacteria vials are fully identical, and are prepared slightly differently from vendor to vendor. Therefore, the test results will react slightly differently in every test. According to ISO 11348-3, the true or accepted EC_{50}-value of a substance is obtained, as long as the criteria in the ISO are met. The reference tests will not be used as true values as seen in other verifications, but will give an indication of the sensitivity of the test organisms and will help to identify false negative tests performed with the LUMIStox or ECLOX equipment. As a result the test of reference samples will be limited. Reference tests are performed on selected samples:

- Test A: samples with start concentration for all 9 target compounds (expect cyanide). 3 replicates will be performed for one compound.

- Test K: samples of one spiked, non-inhibiting domestic wastewater and one spiked, non-inhibiting industrial wastewater. 3 replicates will be performed for one of the wastewater samples.

Reference samples will be sent to an independent laboratory for analysis under ISO 17025 accreditation /5/ using the ISO 11348-3 Luminescent bacteria test method. The equipment from Microtox® will be used. Microtox test will not be performed for cyanide. The volatility of cyanide is supposed to impact the quality of the results. Since cyanide is toxic there will also be difficulties by sending the sample and the reference laboratory will have to take special precautions working with a toxic compound. Due to the difficulties of working with a toxic sample and lack of confidence in the results, cyanide will be excluded from the reference testing.

Samples of stock solutions for confirmation of test concentration will be sent to an independent laboratory to be analysed under ISO 17025 accreditation /5/ with appropriate methods. When performing luminescent bacteria tests, solid NaCl salt is added to

the samples. Therefore this salt will be added to the stock solutions before shipment to ensure that measured concentrations are similar to the concentrations in the luminescent bacteria test. Stock solutions are not stored over longer periods (weeks). Analyses of stock solutions are done at start of toxicity testing, except for cyanide where a test kit will be used to monitor concentration changes in open vials.

4.1 Analytical laboratory

Reference test of toxicity will be performed by ALcontrol AB, Olaus Magnus väg 27, S-583 30 Linköping, Sweden. SWEDAC accreditation registry number 1006.

Contact Britt Aurell, email: britt.aurell@alcontrol.se, phone: +46 13-254987

Chemical analyses on stock solution will be done by Eurofins Danmark A/S, Ladelundvej 85, 6600 Vejen, Denmark. DANAK accreditation registry number 168.

Contact Vivi Handberg, email: vivihandberg@eurofins.dk, +45 70 22 42 66.

4.2 Analytical parameters

Samples are tested with Microtox®, results are EC_{20}- and EC_{50}-values.

All stock solutions will be analysed for the concentration of the added compound.

The wastewater samples will be analysed for general wastewater parameters as listed in Table 4.1.

Table 4.1 Analytical parameters for wastewater.

Analytical parameters	
Turbidity	COD
TOC	Suspended solids (SS)
Conductivity	Nitrogen (total)
Alkalinity	Phosphorus (total)
pH	BOD_5

4.3 Methods of test and analysis

The reference test method will be ISO 11348-3 Luminescent bacteria test method. The equipment from Microtox® will be used. The ISO 11348-3 method includes tests of reference substances, to ensure validity of the test. ALcontrol performs regular tests for zinc sulphate heptahydrate and 3,5-dichlorophenol. These test results will be available for review. ALcontrol has also participated in one interlaboratory trial, results from this will be available for review.

Stock solutions are analyzed according to the methods listed in Table 4.2, while wastewater parameters are analyzed according to methods listed in Table 4.3.

Table 4.2 Analytical methods and performance expectations from the contracted laboratory.

Group	Compound	Method	Limit of detection µg/l	Uncertainty %
Heavy metals	Cr	ISO 17294m - ICP-MS	1.0	15[1]
	Cu	ISO 17294m - ICP-MS	3.0	15[1]
	Zn	ISO 17294m - ICP-MS	0.5	10[2]
Organic pesticides	Flutriafol	GC/MS[3]	0.1	10
Organic pollutants	Triclosan[2]	-	-	-
Industrial pollutants	Cyanide (CN^-)	DS/EN ISO14403	1	10
Surfactants	Nonylphenol ethoxylate	MK0250-GC/MS	0.1	15
	SDS (sodium lauryl sulphate)	MK8230-LC-MS[3]	5.0	15
	CTAB (cetyl trimethyl ammonium bromide)[4]	VKI	100	20

Table 4.3 Method for analytical parameters analysed in wastewater.

Parameters	Method	Parameter	Method
Turbidity	DS 290	COD	ISO 15705
TOC	DS/EN 1484	Suspended solids (SS)	DS/EN 872
Conductivity	DS/EN 27888	Nitrogen (total)	DSENI 11905 Auto
Alkalinity	DS/EN I 9963	Phosphorus (total)	DS/EN ISO 6878
pH	DS 287	BOD_5	DS/EN 01899-1

For analyses performed under accreditation, internal and external quality control data will be available from Eurofins.

4.4 Analytical performance requirements

The analytical performance requirement for the reference test performed with Microtox® should be equal to performance parameters as derived for LUMIStox and ECLOX, summarised in Table 4.4. The available quality control data from ALcontrol, as described in Section 4.3, is not expected to give information on all these parameters.

[1] Eurofins states that salt content in samples can give higher RSD.
[2] The inclusion of triclosan in wastewater analyses is relatively new. Triclosan will be set up by Eurofins in December 2009. This method is therefore not included under Eurofins accreditation.
[3] Method is not included under Eurofins accreditation.
[4] CTAB will be analysed with a general method for cationic detergents. The method is calibrated with benzyl dimethyl tetradecyl ammonium chloride-dihydrate. The concentration of CTAB will be calculated based on the mole weight of the two compounds.

Table 4.4 Required analytical performance.

	Criterion of detection % inhibition	**Precision (RSD)**%	**Agreement with accepted values** %	**Robustness** %
LUMIStox	< 10	< 30	100 ±50	100±50
ECLOX	< 10	< 30	100 ±50	100±50

4.5 Preservation and storage of reference samples

Samples for Microtox® testing will be frozen before shipment according to instructions from the reference laboratory.

Stock solutions for chemical analyses will be preserved according to instructions of the reference laboratory. Wastewater samples will be stored cold at 5°C. The samples will be shipped on ice or according to the instructions of the lab.

5 DATA MANAGEMENT

Data filing and archiving will follow the procedures of the DHI Quality Management System. In here is stated that, the entire material is filed after the project has been completed. The project material comprises all documents, calculations, analyses, results etc. that will enable another DHI employee to scrutinise the work carried out. After 10 years, the project files should still be sufficiently complete to make possible a reconstruction of the work.

5.1 Data storage, transfer and control

The data to be compiled and stored are summarized in Table 5.1.

Analytical raw data will be filed and archived according to the specifications of the laboratory, Eurofins, quality management systems under their ISO 17025 accreditation.

Table 5.1 Data compilation and storage summary.

Data type	**Data media**	**Data recorder**	**Data recording timing**	**Data storage**
Test plan and report	Protected PDF files	Test responsible, DHI	When approved	Files and archives at DHI
Test details in laboratory and field	Log book and pre-prepared forms	Technician, DHI	During collection	Files and archives at DHI
Calculations	Excel files	Test responsible, DHI	During calculations	Files and archives at DHI
Analytical reports	Paper	Test responsible, DHI	When received	Files and archives at DHI

Forms for data recording are given in Appendix 6. Record will be made for tests at 15 and 30 minutes.

6 QUALITY ASSURANCE

The tests are performed under the quality management system of DHI which is ISO 9001 compliant /7/, but not certified. The DHI laboratories have ISO 17025 accreditations /5/ and OECD GLP approvals /8/ for a range of tests and ISO 17025 for sampling of drinking water. As part of the ISO 17025 and GLP inspections, the procedures for general laboratory processes, quality assurance and documentation/archiving are assessed.

6.1 Test plan review

The test plan will be subject to internal review by the verification responsible from DHI DANETV Water Centre: head of innovation Margrethe Winther-Nielsen. The test plan will also be subject to review by the Battelle Advanced Monitoring Systems Center Verification Test Coordinator and Quality Manager (Mary Schrock and Zachary Willenberg, respectively), as well as by the US EPA ETV AMS project officer and quality manager (John McKernan and Michelle Henderson, respectively). Furthermore, the test plan will be subject to review by ETV Canada by Director Technology Assessment and Quality Assurance Services Mona El-Hallak.

External review of the test plan will be done by the expert group assigned to this verification.

6.2 Performance control – reference test and analysis

Generally, our control of reference test and analysis is based on reference laboratories performing analyses under ISO 17025 accreditation /5/. Information on the laboratory quality assurance will be gathered. Physical inspection (audit) will only be performed if disagreements are suspected. Further for this verification the reference test and analyses are of minor extent and verification of the products will only to a minor extent be based on these results.

Performance control of ALontrol Microtox® tests will be performed by test of 3 replicates of a spiked water sample and a wastewater samples. 2 blank (pure MilliQ water) samples will be tested for control. Information of the laboratory quality assurance, method validation etc. will be gathered.

Performance control of Eurofins analysis will be performed by sending 2 blanks (2% NaCl MilliQ water) to analyses for each of the target compounds. Information of the laboratory quality assurance, proficiency test etc. will be gathered. Eurofins is including standard reference samples when they analyze. Details on their acceptance range and action if standard is out acceptance range are given in Table 6.1.

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Table 6.1 Eurofins reference standards and acceptance criteria.

Group	Compound	Method	Acceptance criteria	Action
Heavy metals	Cr	Use of NIST standard 1643d	1.79-2.42 µg/L	If control is not within acceptance criteria the series will be reanalyzed.
	Cu		1.89-2.55 µg/L	
	Zn		6.5-8.8 µg/L	
Organic pesticides	Flutriafol	Quality control is performed by spiking a sample and calculate retrieval.	70-120%	If retrieval is not with acceptance criteria sample and spiked sample is reanalyzed.
Organic pollutants	Triclosan	Use of standards prepared from pure chemicals from difference batches and suppliers. A standard concentration near LoD is included as well as a high standard concentration	The result for standard near LoD has to be convincing. Result of the samples has to be below concentration in the high standard.	Performance on the apparatus will be improved and the samples reanalyzed. Either reextraction with less sample material in use or the first extract will be diluted.
Industrial pollutants	Cyanide (CN^-)	Include standards of NaCN: 5 µg/l and 50 µg/l And K3(Fe(CN)6): 10 µg/l and 100 µg/l Replicate on every 20. Samples and minimum per series.	For NaCN: 4,45-5,55 µg/l and 44,5-55,5 µg/l For $K_3(Fe(CN)_6)$: > 9,0 µg/l and >90 µg/l Accepted difference < 18 %	If controls are not within acceptance criteria the series will be reanalyzed.
Surfactants	Nonylphenol ethoxylate	As for triclosan. As standards are used 4-nonylphenol of the following compounds: NP (technical mixture 4-nonylphenol) NPE1 (isomere mixture 4-nonylphenol) NPE2 (isomere mixture 4-nonylphenol).		

Table 6.2 Eurofins reference standards and acceptance criteria (cont.).

Group	Compound	Method	Acceptance criteria	Action
Surfactants (cont.)	SDS (sodium lauryl sulphate)	As for triclosan. As standards is used LAS-mix cas no. 69669-44-9 (Dodecylbenzene sulfonic acid, sodium salt). Further is also included quality control performed by spiking a sample and calculate retrieval.	The result for standard near LoD has to be convincing. Result of the samples has to be below concentration in the high standard. 70-120 %	Performance on the apparatus will be improved and the samples reanalyzed. Either reextraction with less sample material in use or the first extract will be diluted If retrieval is not with acceptance criteria sample and spiked sample is reanalyzed.
	CTAB (cetyl trimethyl ammonium bromide)	Include standards of Benzyl-dimethyltetra ammoniumchlorid dihydrat: 0,3 mg/l and 1,5 mg/l Replicate on every 20. Samples and minimum per series.	0,11-0,49 mg/l and 0,9-2,10 mg/L Accepted difference < 18 %	If controls are not within acceptance criteria the series will be reanalyzed.

At the moment Eurofins has not provided information included standard and acceptance criteria for cyanide, SDS and CTAB. The principles for these compounds are expected to be as for the compounds listed in Table 6.1.

Triclosan which is under implementation and Eurofins, and if one of the compounds cyanide, SDS and CTAB should lack acceptance criteria, performance evaluation (PE) audits will be considered at time of testing, depending on implementation progress for triclosan and the reaming quality control data from Eurofins.

6.3 Test system control

System control is planned to test the DHI DANETV Water Centre test system of the LUMIStox and ECLOX.

All stock solutions will be analyzed in duplicate to control the concentration of target compounds. Before testing with luminometers solid NaCl salt is added to the samples. Therefore, the samples sent for reference analysis will be stock solutions with added salt. This will give information about whether the salt addition causes precipitation of added compounds.

Luminescent bacteria tests of 2 blank samples will be performed at the reference laboratory to ensure that no sources of contamination are present in MilliQ water used for preparation of stock solutions. These blank samples are also used for control of the

reference laboratory Microtox® test. If test results indicate results of toxic water samples, both the water quality in DHI DANETV Water Centre and the test set-up at the reference laboratory will be inspected.

Luminescent bacteria test of blank samples (MilliQ water) are expected to produce very little or no inhibition. If the inhibition is different from what the vendor suggests for a blank sample, the analysis will be repeated once. For this re-analysis, a new blank sample will be prepared and clean vials/containers and fresh reagents will be used. If similar results persist, the vendor will be notified, but the verification test will proceed.

According to ISO 11348-3 three reference substances shall be tested for each batch of bacteria. These tests will be performed solely on the LUMIStox equipment at DHI. The testing of the batches is related to the bacteria and not to the equipment, therefore tests on one instrument are considered sufficient.

An overview of the reference performance control, described in Section 6.2, and the DHI DANETV Water Centre test system, described in this section, is given in Table 6.3.

Table 6.3 Summary of reference performance control and test system control.

Information/control type	**Reference laboratory**	**DHI Test laboratory**
Blank samples	Detection limit	Quality of MilliQ water
Reference test according to ISO 11348-3	-	Test of bacteria batches
Control, stock solutions	Precision	-
Wastewater	Precision	-
Quality control	Precision	-
Proficiency test	Trueness	-

6.4 Data integrity check procedures

All transfer of data from printed media to digital form will be checked. Transfer between digital media is checked by spot check of not less than 5% of the data. If errors are found in a spot check, all data from the transfer are checked.

6.5 Test system audits

An internal audit by DHI, following the GLP audit procedures by a trained auditor, will be performed (see the verification protocol for details).

The Battelle Quality Manager, Zachary Willenberg, will perform a technical systems audit (TSA) at least once during this verification and test. The purpose of this audit is to ensure that the verification test is performed in accordance with the AMS Center quality management plan /9/, this test plan, published reference methods and any methods used in the tests. In the TSA, the Battelle Quality Manager may review the reference methods used and compare actual test procedures to those specified or referenced in this plan. In the TSA, the Battelle Quality Manager will observe testing in progress, inspect documentation, and review technology-specific record books. He will also check standard certifications and may confer with other Battelle staff. A TSA report will be prepared, including a statement of findings and the corrective actions

taken. The AMS Center Quality Manager, the U.S. EPA Quality Manger and the DHI DANETV Water Centre Verification Responsible will receive a copy of Battelle's TSA report. The TSA findings will be communicated to technical staff at the time of the audit and documented in a TSA report.

The Battelle Quality Manager will perform an audit of data quality (ADQ). This will be a review of data acquisition and handling procedures and an audit of at least 10% of the data acquired in the test and verification. The Battelle Quality Manager will trace the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All calculations performed on the data undergoing the audit will be checked.

ETV Canada is not planning for a physical audit of the test operation.

6.6 Test report review

The test report will be subject to internal review by the verification responsible from DHI DANETV Water Centre: head of innovation Margrethe Winther-Nielsen.

U.S. EPA staff, John McKernan (JMK) and Michelle Henderson (MH), and Mona El-Hallak (MEH) from ETV Canada will review the test report.

External review of the test report will be done by the expert group together with the review of the verification report.

7 TEST REPORT

The test report will follow the template of the DHI DANETV verification center quality manual /10/. The test report will contain the test plan, except for this Chapter 7 on test report format, with the data and records from the tests to be inserted as new Chapter 7. For this joint verification, the principles (contents) of the U.S. EPA ETV and ETV Canada format will be complied with as well.

One joint test report will be prepared for LUMIStox and ECLOX.

7.1 Test site report

No tests will be performed in the field. Outdoor testing will be performed at DHI laboratory location in Hørsholm.

7.2 Test data report

The test data will include all data recorded during the test and the data reported by the analytical laboratories, see Appendix 6 for data forms.

7.3 Amendment report

The report section on deviations will compile all changes of this test plan occurring before testing with justification of deviations and evaluation of any consequences for the test data quality. A template for an amendment report is included in Appendix 8.

7.4 Deviations report

The report section on deviations will compile all deviations from this test plan occurring during testing with justification of deviations and evaluation of any consequences for the test data quality. A template for the deviation report is included in Appendix 8.

A P P E N D I X 1

Terms and definitions used in the test plan

The abbreviations and definitions used in the verification test plan are summarized below. Where discrepancies exist between DANETV and US EPA ETV terminology, definitions from both schemes are given.

Word	NOWATECH	US ETV
ADQ	Audit of data quality: An examination of a set of data after is has been collected and 100% verified by project personnel, consisting of tracing at least 10% of the test data from original recording through transferring, calculating, summarizing and reporting.	
Agreement with accepted values	Here defined as the % agreement between literature values and test results	
AMS Center	Advanced Monitoring Systems Center at Battelle	
Analytical laboratory	Independent analytical laboratory used to analyze reference samples	
Application	The use of a product specified with respect to matrix, target, effect and limitations	
BOD_5	Five-day biological oxygen demand	
CD	Criterion of detection	
CTAB	Cetyl trimethyl ammonium bromide	
DANAK	The Danish Accreditation and Metrology Fund	
DANETV ETV	The Danish Centre for Verification of Climate and Environmental Technologies	
DS	Danish Standard	
Effect	The way the target is affected	
EN	European standard	
ETV	Environmental technology verification (ETV) is an independent (third party) assessment of the performance of a technology or a product for a specified application, under defined conditions and adequate quality assurance.	EPA program that develops generic verification protocols and verifies the performance of innovative environmental technologies that have the potential to improve protection of human health and the environment
EU	European Union	
Evaluation	Evaluation of test data for a technology product for performance and data quality	An examination of the efficiency of a technology
Experts	Independent persons qualified on a technology in verification or on verification as a process	Peer reviewers appointed for a verification
GC	Gas chromatography	
GLP	Good laboratory practice	
ICP	Inductively coupled plasma	
ISO	International Standardization Organization	
LC	Liquid chromatography	
LID	Lowest ineffective dilution. Often seen as the dilution in a dilution series causing less than 20 % inhibition.	
Limit of detection LoD	Calculated from the standard deviation of replicate measurements at less than 5 times the detection limit evaluated. Corresponding to less than 5% risk of false blanks.	
LUMISsoft4	PC software from HACH-LANGE , produced for LUMIStox	

Word	NOWATECH	US ETV
LUMIStherm	Thermostat from HACH-LANGE , produced for LUMIStox	
LUMIStox	LUMIStox 300 bench top luminometer from HACH-LANGE	
Matrix	The type of material that the product is intended for	
Method	Generic document that provides rules, guidelines or characteristics for tests or analysis	
MS	Mass spectroscopy	
OECD	Organisation for Economic Co-operation and Development	
PE	Performance evaluation	
Performance claim	The effects foreseen by the vendor on the target (s) in the matrix of intended use	
Performance parameters	Parameters that can be documented quantitatively in tests and that provide the relevant information on the performance of an environmental technology product	
Precision	The relative standard deviation obtained from replicate measurements, here measured under repeatability or reproducibility conditions.	
(Environmental) product	Ready to market or prototype stage product, process, system or service based upon an environmental technology	(Environmental) technology
QA	Quality assurance	
Range of application	Generally: the range from the LoD to the highest concentration with linear response. For this verification the range is based on range of dilution of a test sample.	
Reference analyses	Analysis of content of compounds in stock solutions by a specified reference methods in an accredited (ISO 17025) laboratory.	
Reference test	Luminescence bacteria test performed according to ISO 11348-3 by an accredited (ISO 17025) laboratory.	
Repeatability	The precision obtained under repeatability conditions, that is with the same measurement procedure, same operators, same measuring system, same operating conditions, and same location and system, and replicate measurements on the same or similar objects over a short period of time.	
Reproducibility	The precision obtained under reproducibility conditions, that is with measurements that include different locations, operators, measuring systems, and replicate measurements on the same or similar objects	
Robustness	% variation in measurements resulting from defined changes in matrix properties.	
RSD	Relative standard deviation in %.	
SM	Standard method	
SS	Suspended solids	
Standard	Generic document established by consensus and approved by a recognized standardization body that provides rules, guidelines or	

Word	NOWATECH	US ETV
	characteristics for tests or analysis	
SWEDAC	Swedish Board for Accreditation and Conformity Assessment	
Target	The measurable property that is affected by the product.	
(Environmental) technology	The practical application of knowledge in the environmental area	An all-inclusive term used to describe pollution control devices and systems, waste treatment processes and storage facilities, and site remediation technologies and their components that may be utilized to remove pollutants or contaminants from, or to prevent them from entering, the environment.
Test/testing	Determination of the performance of a product by parameters defined for the application	
TOC	Total organic carbon	
Trueness	The % recovery of true value obtained either from knowledge on the preparation of test solutions or from measurements with reference methods.	
TSA	Technical system audit	
U.S. EPA	United States Environmental Protection Agency	
Vendor	The party delivering the product or service to the customer	The technology developer, owner, or licensee seeking verification
Verification	Evaluation of product performance parameters for a specified application under defined conditions and adequate quality assurance	Establishing or proving the truth of the performance of a technology under specific, predetermined criteria, test plans and adequate data QA procedures
Vibrio fischeri	Light producing bacteria used in luminescent bacteria test	
VKI	Former Danish Water Quality Institute, today DHI	

A P P E N D I X 2

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A P P E N D I X 3

Reference methods

Reference test and reference analyses are described in the test plan Section 4.3 Methods of test and analysis.

Conductivity and salinity measurement methods are provided with the instrument.

A cyanide test kit will be used according to the method description included in the kit.

A P P E N D I X 4

In-house test methods

Laboratory protocol for verification of LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer

1. Objective

The objective of this protocol is to describe in detail the work to be carried out for the verification of LUMIStox 300 Bench Top Luminometer and ECLOX Handheld Luminometer in accordance with the verification protocol /1/ and test plan /2/.

2. Identification

Project No.: 11800378-2

3. Vendor

HACH-LANGE GmbH,
Willstätterstrasse 11, 40549 Düsseldorf,
Germany,
phone +49 211 5288 0.
Contact
Dr. Elmar Grabert
email: elmar.grabert@hach-lange.de,
phone +49 211 5288 241.

4. Test facility

DHI
Agern Allé 5
DK-2970 Hørsholm
Denmark

5. Personnel responsible for the test

Test responsible:	Claus Jørgensen
Technicians:	Connie Seierø
	Jane Bergstrøm

6. Instruments to be tested

The test covers two instruments from the same vendor, both instruments determine acute toxicity with luminescent bacteria. The instruments are LUMIStox 300 bench top luminometer and ECLOX handheld luminometer. Both will be operated in connection with a LUMIStherm thermostat and the PC software LUMISsoft4 ver 1.001 /8/ except for test series E where the ECLOX will be operated with the firm ware.

7. Safety handling

The test compounds will be handled in accordance with the MSDSs which are available to the technicians.

8. Test principle

To verify the instruments the following performance parameters will be analysed:

The criterion of detection (CD)
The range of application
Precision
- repeatability
- reproducibility

Agreement with accepted values
Robustness

The tests will be performed in a series of experiments according to the test plan /2/:

Test series.	Performance parameters	Equipment			Matrix	
		LUMIStox	ECLOX incl. thermostat and software	ECLOX incl. firm-ware	2% NaCl in MQ water	Wastewater
A	Range, Repeatability, Agreement with accepted values	x	x		x	
B	Criterion of detection	x	x		x	
C	Robustness, effect of start conc. on repeatability	x	x		x	
D	Reproducibility	x	x		x	
E	Robustness, sample temperature at field use			x	x	
F	Robustness, sample temperature at laboratory use	x	x		x	
G	Robustness, pH	x	x		x	
H	Robustness, color	x	x		x	
I	Robustness, turbidity	x	x		x	
J+K	Robustness, matrix	x	x			x
L	Robustness, cuvettes	x			x	

9. Procedure

9.1 Start up procedure for LUMIStox 300.

Follow the procedure in the LUMIStox 300 operation manual /3/ page 6.

Perform daily temperature control of the LUMIStherm heating block(s) (se section 0).

Adjust the measuring shaft temperature according to section 3.7 of the operating manual /3/.

9.2 Start up procedure for ECLOX

Follow the procedure in the ECLOX user manual /4/ page 7.

Ensure that the temperature of the LUMIStherm heating block(s) is set to 15 °C.

9.3 Temperature control of LUMIStherm

9.3.1 Initial temperature control

The three LUMIStherm thermo blocks will initially be tested for temperature variation at 15 °C in all wells. A high quality traceable calibrated thermo sensor will be used with a precision of 0.1 °C.

1. Mark the three LUMIStherms A, B, and C respectively.
2. Switch on the LUMIStherms
3. Insert plastic vials (0) in all small wells (A1 to C10) and add 1 mL of sodium chloride solution (0). Insert reaction vials (0) in the two large right hand side wells and add 5 mL of sodium chloride solution (0). Wait 15 minutes to allow for temperature equilibration.
4. Temperature equilibrate the thermo sensor in one of the wells.
5. Measure the temperature in all wells and record in a spread sheet.
6. Determine the $T_{average}$, T_{max}, T_{min} and T_{median} temperature of the small wells for each LUMIStherm. The temperature will be accepted if all wells are within 15 °C ± 0.8 °C. Determine the temperature interval between max temperature and 16.0 °C (ΔT_{max}), and the temperature interval between min temperature and 14 °C (ΔT_{min})
7. Identify the small well with the median temperature.
8. Determine the temperature variation in well 5B over a period of 1.5 hours. A variation of ± 0.3 °C is acceptable.

9.3.2 Daily temperature control

Determine and record on each day of operation, the temperature in the median temperature well. The temperature will be accepted if the temperature is within the range between $T_{median} + \Delta T_{max}$ and $T_{median} - \Delta T_{min}$.

9.4 Storage and preparation of suspensions of luminescent bacteria (*Vibrio fischeri* NRRL-B-11177).

9.4.1 Storage

The freeze-dried bacteria can be stored at -18 °C until the date shown on the package. Reactivated bacteria should be used within 4 hours when possible. However longer storage time is acceptable as long as the validity criteria stated in clause 11 of EN/ISO 11348-3 /6/ are met. Reactivated bacteria should only be placed in temporary storage under undiluted condition. Tubes containing thawed but not reactivated freeze-dried bacteria can be refrozen. /5/.

9.4.2 Preparation of stock suspension

(According to EN/ISO 11348-3: 2007 /6/.)

Remove the vial of the freeze-dried culture from the freezer immediately before reconstitution in water. For the reconstitution, cool 1.2 mL of reconstitution solution LCX 047 (0) in a glass test tube to 4 °C ± 3 °C.

Pour this volume of cooled water all at once into the lyophilized bacteria in the vial, thereby minimizing cell damage during the rehydration process.

It is important that the water be added quickly to allow the bacteria to come into contact with the water at once, thus avoiding clumping and loss of activity. Therefore do not use a pipette. The exact volume of water is not critical.

The reconstituted luminescent bacteria suspension serves as a stock suspension; store at 4 °C ± 3 °C.

9.4.3 Preparation of test suspension

(According to EN/ISO 11348-3:2007, variant B /6/.)

The test suspension will be prepared outside the test tubes in a conical flask (volume e.g. 250 mL).

Ad 1 volume of stock suspension (0) to 50 volumes of the solution for freeze-dried bacteria (0) maintained at 4 °C ± 3 °C and mix the resultant suspension thoroughly.

9.4.4 Quality control of test bacteria

All batches of bacteria must be controlled according to clause 11 of EN/ISO 11348-3 /6/. The tests will be carried out on the first day of use of the specific bacterial batch.

Each stock suspension will be controlled as described in clause 11 of EN/ISO 11348-3 /6/. The reference substance will be selected on the basis of preliminary test results.

9.5 Sample preparation

Samples made by adding test chemicals to sodium chloride solution (0) are called “artificial samples” in this protocol.

Measure the oxygen concentration in all samples. A concentration > 3 mg/L will be accepted. /6/. Aerate if the concentration is < 3 mg/L

Measure the pH of all samples. If necessary adjust the pH with either HCl (0) or NaOH (0). Record the volumes used for pH adjustment. Restrict the volume added to no more than 5 % of the total volume /6/.

All artificial samples will be adjusted to pH 7.0 ± 0.2.

Waste water samples will be adjusted to be between pH 6.0 ± 0.2 and pH 8.5 ± 0.2 in agreement with EN/ISO 11348-3: 2007 /6/

The salt concentration of the sample will be increased to 2 % by adding solid NaCl. For example 2 g pr 100 mL of sample. /3/ If the salt concentration in the sample exceeds 20 g/L (guide value: conductivity of 35 mS/cm) do not add NaCl. The salt content should not exceed 50 g/L. /5/

9.6 Preparation of sample dilution rows

Dilution rows will be used in test series A, C, D, and K.

A dilution row will be produced in accordance with the standard dilution row described in the LUMIStox 300 Operation manual page 33 /3/. The principle is illustrated in figure 9.1.

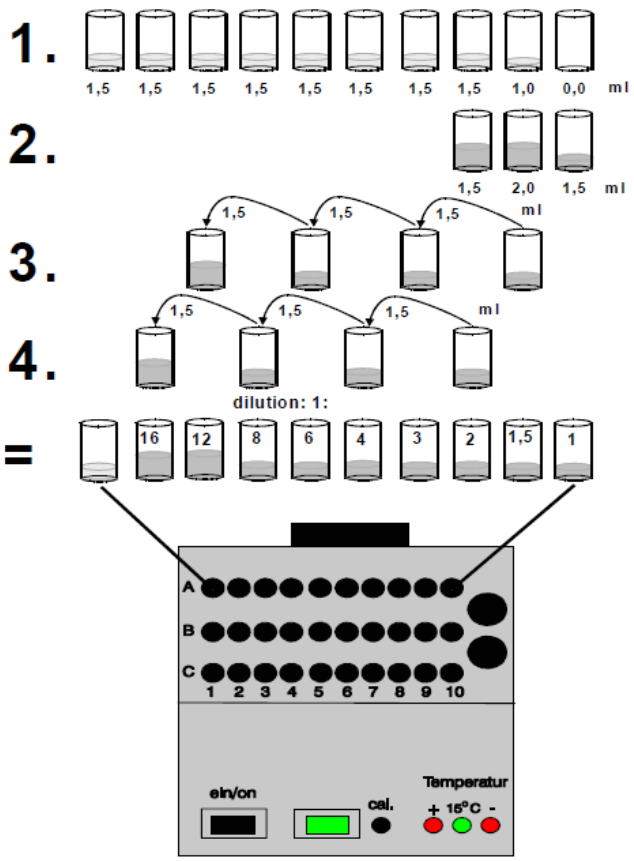


Figure 9.1: Principles of preparation of dilution rows from /3/.

1. Insert 10 vials into row A and pipet LCK 481 sodium chloride solution (0) into the vials according to figure 9.1, i.e. 1.5 mL in position A1 to A8 of the LUMIStherm thermo block and 1 mL in position A9.
2. Add 1.5 mL sample into the vial in position A10, 2 mL sample in the vial in position A9 and 1.5 mL sample in the vial in position A8.
3. Pipet 1.5 mL from the vial in position A9 to the vial in position A7 and mix thoroughly drawing the mixture into the pipette 3 times. Continue by pipetting 1.5 mL into the vials in the positions A5 and A3 as illustrated in figure 9.1.
4. Pipet 1.5 mL from the vial in position A8 to the vial in position A6 and mix thoroughly drawing the mixture into the pipette 3 times. Continue by pipetting 1.5 mL into the vials in the positions A4 and A2 as illustrated in figure 9.1.

Leave the dilutions in 15 minutes in the LUMIStherm thermo block to bring them to the correct temperature.

9.7 Test procedure

9.7.1 Determining inhibition under lab conditions

Connect the LUMIStox 300 and the ECLOX to the computers. Switch on the computers. Switch on the LUMIStox 300 and the ECLOX. Switch on the LUMIStherm thermo block(s). Allow 30 minutes for equilibration.

Prepare the dilution row as described in (0) or samples as described in (0). Prepare the test suspension as described in (0).

Use plastic measuring tubes (0) except in test series L, where both plastic tubes and glass tubes 0 will be used.

1. Insert the appropriate number of plastic measuring tubes (0) in rows B and C.
2. Pipette 0.5 mL bacteria test suspension (0) into the measuring tubes and leave 15 minutes to acquire the correct temperature.
3. Open the LUMISsoft software and enter information on the samples to be analysed according to the LUMISsoft manual p. 16 – 27 /8/.
4. Measure the initial luminescence in the vial in position B1 first on LUMIStox 300 then on ECLOX.
5. Measure the initial luminescence in the vial in position C1 first on LUMIStox 300 then on ECLOX. During the measurement of vial C1 add 0.5 mL of diluted sample from position A1 into the measuring vial in position B1 and mix 3 times with the pipette.
6. Measure the initial luminescence in the vial in position B2 first on LUMIStox 300 then on ECLOX. During the measurement of vial B2 add 0.5 mL of diluted sample from position A1 into measuring tube C1 and mix 3 times with the pipette. Continue until all measuring tubes have been measured and added sample. There is no need for changing pipette tips except for the control.
7. After 15 minutes calculated from the time of the first reading, determine the luminescence in the measuring tube B1first on the LUMIStox 300 then on the ECLOX. Measure the luminescence in the measuring tube C1 after the selected time interval ($T_{between}$). Continue to measure the luminescence in the remaining measuring tubes.
8. Repeat 7 after 30 minutes after the first reading.

9.7.2 Determining inhibition under field conditions

Follow the instructions in the ECLOX user manual pages 19 - 21.

10. Reagents and test tubes

10.1 Sodium chloride solution.

Dissolve 20 g of sodium chloride (NaCl) in MQ-water and make up to 1 L with MQ-water. Store at 4 °C to 8 °C.

10.2 Hach-Lange sodium chloride solution (LCK 481)

Sodium choride solution (2 %) delivered by Hach-Lange. no. 10159, exp. date 10.2010. Store at 4 °C to 8 °C.

10.3 Hach-Lange reconstitution solution (LCX 047)

Reconstitution solution after EN/ISO 11348-3 delivered by Hach-Lange. No. 04179, exp. date 10.2010. Store at 4 °C to 8 °C.

10.4 Sodium hydroxide solution

NaOH in MQ-water, 1 mol/L or another suitable concentration.

10.5 Hydrochloric acid

HCl in MQ-water, 1 mol/L or another suitable concentration.

10.6 Hach-Lange test suspension solution for freeze-dried bacteria (LCX 048)

Diluent after EN/ISO 11348-3 delivered by Hach-Lange. No. 10309, exp. date 10.2010. Store at 4 °C to 8 °C.

10.7 Reference substances

Do <u>not</u> adjust pH of the reference substance solutions.

10.7.1 Zinc sulphate heptahydrate

19.34 mg/L $ZnSO_4 \cdot 7\ H_2O$ in 2 % sodium chloride solution (0) .

10.7.2 3,5 – dichlorophenol

6.8 mg/L 3,5 – dichlorophenol (Purity > 99%) in 2 % sodium chloride solution (0) .

Potasium dichromate

105.8 mg/L $K_2Cr_2O_7$ in 2 % sodium chloride solution (0).

10.8 Test tubes

10.8.1 Plastic test tubes

Sarstedt tubes 3.5 mL, 55 x 12 mm, PS. ref no. 55.485. Delivered by Hach Lange

10.8.2 Glas test tubes

LZP 187 Glasküvetten für LUMIStox AR-Klar. 50.0 X 12.0/0.60 mm. Delivered by Hach Lange.

10.8.3 Reaction vials with cap.

LZP 065 Reaktionsgläser mit verschluss, delivered by Hach-Lange.

11. Test setup

Generally, tests will be run in triplicate, i.e. three rows of dilution will be prepared from the same artificial sample and tested in separate test runs. Each test run will be performed in duplicate (i.e. row B and C). All test runs will include a blank consisting of 0.5 mL of test suspension (0) and 0.5 mL of sodium chloride solution (0).

Readings will be done after 15 minutes and 30 minutes.

If there is a visible colour at the EC_{20} concentration, colour correction will be applied.

11.1 Test series A

11.1.1 Purpose

To analyse range, repeatability and agreement with accepted values of EC_{20} and EC_{50}.

11.1.2 Tests to be performed

EC_{20} and EC_{50} will be determined on artificial samples made in sodium chloride solution (0) with the compounds shown in table 11.1.

The test will be performed on LUMIStox 300 and ECLOX incl. thermostat and software.

Table 11.1: Compounds to be tested in test series A

CAS no.	Compound	Expected EC_{50} (mg/L)
7758-99-8	$CuSO_4,5H_2O$	7.1 as Cu^{2+}
7778-50-9	$K_2Cr_2O_7$	18.7 as Cr^{+6}
7446-20-0	$ZnSO_4,7H_2O$	2.2 as Zn^{2+}
76674-21-0	Flutriafol	unknown
3380-34-5	Triclosan	0.28
151-50-8	KCN	4 as CN^-
151-21-3	SDS	2.09
57-09-0	CTAB	0.97
104-35-8	4-NPE	unknown

For KCN, a pre-experiment will be performed to examine evaporation of HCN. An artificial KCN sample will be carried through the test procedure where the test suspension will be exchanged with the solution for freeze-dried bacteria (0) and without performing measurements of luminescence. Instead the CN^- concentration will be measured using a Hach-Lange test (LCK 315). Test row B will be analysed at time 0 and test row C will be analysed after time 30 minutes. In addition, the concentration of the artificial KCN sample will be analysed. If the decrease in the average CN^- concentration from time 0 to time 30 is higher than 20%, then the KCN test will be aborted.

11.1.3 Sampling for chemical analyses

Samples for chemical analysis of $CuSO_4$, $K_2Cr_2O_7$, $ZnSO_4$, KCN, Flutriafol and Triclosan will be taken in duplicate from the prepared artificial samples, and shipped to the analytical laboratory.

SDS, CTAB and 4-NPE are expected to adsorb to the measurement tubes. Therefore samples for chemical analysis will be prepared by adding 1.5 mL of artificial sample and 1.5 mL of solution for freeze-dried bacteria (0) in each of 10 plastic tubes (0) at 15 °C and mixed three times with the pipette. The mixtures will then be poured to glass sample containers. Only one sample will be analysed pr. compound.

Performance control of Eurofins analysis will be performed by sending 2 blanks (MilliQ water) to analysis for each of the target compounds.

Samples will be transferred to sample containers delivered by Eurofins.

Sample labeling will be coded.

11.1.4 Sampling for toxicity analysis at AlControl

Artificial samples will be taken for all target compounds except KCN. One of the samples will be analyzed three times. KCN is exempted to avoid complications related to shipment and handling by AlControl.

Two samples of 2 % NaCl solution (0) will be taken to ensure non-toxicity.

The samples are frozen at - 20 °C ± 3 °C and send to Alcontrol after the last sample is taken.

Sample labeling will be coded.

11.2 Test series B

11.2.1 Purpose

To determine criterion of detection.

11.2.2 Tests to be performed

The test will be performed on LUMIStox 300 and ECLOX incl. thermostat and software.

A number (≥ 9) of test mixtures of 0.5 mL of 2 % NaCl (0) and 0.5 mL test suspension (0) will be measured in duplicate.

11.3 Test series C

11.3.1 Purpose

To determine robustness of determination of EC_{50} and EC_{20} in relation to the concentration.

11.3.2 Tests to be performed

The test will be performed on LUMIStox 300 and ECLOX incl. thermostat and software.

A test compound and the concentration ranges will be decided after completion of series A.

The first concentration range will have the highest test concentration at approximately EC_{60}.
The second concentration range will have the highest test concentration at approximately EC_{30}.

11.4 Test series D

11.4.1 Purpose

To determine reproducibility.

11.4.2 Tests to be performed

The test will be performed on LUMIStox 300 and ECLOX incl. thermostat and software.

One test compound and the concentration range will be selected after completion of series A.

The reproducibility parameters will be: different days, different technicians, and different batches of test bacteria according to Table 7-1.

Table 7-1: Variation of reproducibility parameters

Day	Bacterial batch	Technician
1	A	J
2	B	C
3	C	J
*4	D	C

*Will only be performed if a bacterial fourth batch is made available.

11.5 Test series E

11.5.1 Purpose

To determine robustness of the ECLOX instrument at different temperatures.

11.5.2 Tests to be performed

Tests will be performed on the ECLOX instrument with firmware according to procedure described in the ECLOX user manual /4/ pages 19 to 21.

Based on the results obtained in series A, two compounds will be selected for test: one metal and one organic compound. Each compound will be tested in triplicate and at three different

temperatures: One at room temperature, one in a climate room at approximately 15 °C and one at 0 °C to 6 °C, preferable outdoor, otherwise in cooler room.

The test setup is illustrated in Table 7-2. The concentration of the test compound in the test sample shall be twice the EC_{50}.

Table 7-2: Test setup for series E			
Tube	Test suspension (0) (mL)	2% NaCl (0) (mL)	Sample (mL)
1	0.2	0.8	none
2	0.2	0.6	0.2
3	0.2	0.3	0.5
4	0.2	none	0.8

Each of the two test compounds will be tested in triplicate.

The room temperature will be recorded.

11.6 Test series F

11.6.1 Purpose

To determine robustness at different sample temperatures.

11.6.2 Tests to be performed

The test will be performed on LUMIStox 300 and ECLOX incl. thermostat and software.

The test compound will be selected based on results obtained in previous tests.

Adjust two of LUMIStherm thermo blocks to approximately 14 °C and 16 °C, respectively, after the procedure described in section 0. Adjustment of the temperature is done by turning the “Cal.” screw. It may not be possible to reach 14 °C and 16 °C. In this case maximum and minimum temperature adjustments will be selected.

The tests will be run at 14 °C, 15 °C and 16 °C at EC_{20} in triplicate.

The test will be performed as the last test to avoid temperature variations over the test series.

11.7 Test series G

11.7.1 Purpose

To determine robustness at different pH.

11.7.2 Tests to be performed

The test will be performed on LUMIStox 300 and ECLOX incl. thermostat and software.

The test compound will be selected based on results obtained in previous tests.

The test will be performed at EC_{20} in triplicate.

A stock solution of the test compound at a concentration corresponding to twice the EC_{20} will be prepared and separated into three separate artificial samples, which will be adjusted to pH 6.0 ± 0.2, 7.0 ± 0.2, or 8.5 ± 0.2 respectively with either HCl (0) or NaOH (0) and tested.

11.8 Test series H

11.8.1 Purpose

To determine robustness in relation to colour.

11.8.2 Tests to be performed

11.8.2.1 Screening of toxicity of dyes to determine dye test concentrations.

An artificial sample will be made in sodium chloride solution (0) as a mixture of 20 mg/L of Ponceau 4R (E124), 20 mg/L of Green S (E142) and 20 mg/L of Yellow no.5 (E102). Alternative concentrations may be used if appropriate.

EC_{50} on this sample will be determined on the LUMIStox 300 with the colour correction feature switched on. See page 23 of the LUMIStox user manual /3/ and pages 70 to 77 of the LUMISsoft 4 manual /8/.

The test data will be analysed with and without colour correction. A range of concentrations with colour correction and without toxicity will be determined and used to define the dye concentrations to be used in the subsequent test.

11.8.2.2 Colour robustness on LUMIStox 300.

11.8.2.2.1 Preparation of test samples

A stock solution in sodium chloride (0) with an appropriate concentration of the selected test compound will be made. An appropriate volume of the stock solution will be added to each of three 100 mL measuring flasks to achieve a concentration of the test compound corresponding to twice the EC_{20} in the final test sample. Varying volumes of sodium chloride solution (0) and the dye solution described in section 0 will be added to achieve the dye concentrations determined in the screening test in section 0.

11.8.2.2.2 Preparation of dye control samples

The dye control samples will be made as the test samples (0) except that the stock solution will be left out and replaced by sodium chloride solution (0).

11.8.2.2.3 Preparation of test compound control samples

The test compound control samples will be made as the test samples (0) except that the dye solution will be left out and replaced by sodium chloride solution (0).

11.8.2.2.4 Test setup

The test will be performed on LUMIStox 300 incl. thermostat and software.

The test will be performed in triplicate each with a blank consisting of 0.5 mL of test suspension (0) and 0.5 mL of sodium chloride solution (0), a dye control (0) and a test compound control (0) . Each triplicate in will be analysed in duplicate.

11.8.2.3 Colour robustness on ECLOX.

Tests will be performed on ECLOX incl. thermostat and software with colour correction according to EN/ISO 11348-3.

The tests will be performed on the same samples as used for the LUMIStox 300 (0).

11.9 Test series I

11.9.1 Purpose

To determine robustness in relation to turbidity.

11.9.2 Screening of toxicity of $BaSO_4$.

This screening test will be run to ensure that $BaSO_4$ is non-toxic.

A volume of 10 mL of a 0.2 g/L of $BaSO_4$ in sodium chloride solution (0) will be centrifuged 10 minutes at approx. 5000 g.

The inhibitory effect of the supernatant will be determined in 5 duplicate tests with 5 blanks run in the same rows. Readings after 15 minutes and 30 minutes.

If the average inhibition is significant higher than the CD determined in section 0 an alternative turbidity sample will be selected. If the alternative also shows inhibition, then the test for turbidity robustness will not be carried out.

11.9.3 Tests to be performed

11.9.3.1 Turbidity robustness on LUMIStox 300.

11.9.3.1.1 Preparation of test samples

A stock solution in sodium chloride (0) with an appropriate concentration of the selected test compound will be made. An appropriate volume of the stock solution will be added to each of three 100 mL measuring flasks to achieve a concentration of the test compound corresponding to twice the EC_{20} in the final test sample. Varying volumes of sodium chloride solution (0) and a 1 g/L $BaSO_4$ in sodium chloride solution (0) will be added to achieve final $BaSO_4$ concentrations of 0.2 mg/L, 0.1 mg/L and 0.05 mg/L.

11.9.3.1.2 Preparation of turbidity control samples

The turbidity control samples will be made as the test samples (0) except that the stock solution will be left out and replaced by sodium chloride solution (0).

11.9.3.1.3 Preparation of test compound control samples

The test compound control samples will be made as the test samples (0) except that the $BaSO_4$ suspension will be left out and replaced by sodium chloride solution (0).

11.9.3.1.4 Test setup

The test will be performed on LUMIStox 300 incl. thermostat and software.

The test will be performed in triplicate each with a blank consisting of 0.5 mL of test suspension (0) and 0.5 mL of sodium chloride solution (0), a turbidity control (0) and a test compound control (0) . Each triplicate in will be analysed in duplicate.

11.9.3.2 Turbidity robustness on ECLOX.

Tests will be performed on ECLOX incl. thermostat and software with colour correction according to EN/ISO 11348-3.

The tests will be performed on the same samples as used for the LUMIStox 300 (0).

11.10 Test series J

This test series will be performed after series K.

11.10.1 Purpose

To determine robustness in relation to the matrix.

11.10.2 Waste water samples

See section 0.

11.10.3 Preparation of test samples

11.10.3.1 Preparation of waste water test samples

If the waste water samples are found to be toxic, they will be diluted to non-toxicity level and otherwise handled as described in section 0.

Five test compounds will be selected based on results obtained in previous tests.

For each compound a stock solution in sodium chloride solution (0) with a concentration corresponding to 4 times the EC_{20} will be made. Waste water test samples will be made by mixing 1 part of waste water samples with 1 part of the 4 times EC_{20} solutions.

11.10.3.2 Preparation of test compound control samples

Test compound control samples will be made by mixing 1 part of sodium chloride solution (0) with 1 part of the 4 times EC_{20} solutions.

11.10.3.3 Preparation of waste water control samples

The waste water control samples will be made by mixing 1 part of sodium chloride solution (0) with 1 part of the waste water sample.

11.10.3.4 Sampling for toxicity analysis at AlControl

Samples of one spiked, non-inhibiting domestic wastewater and one spiked, non-inhibiting industrial wastewater. Three replicates will be performed for one of the wastewater samples.
The samples are frozen at - 20 °C ± 3 °C and send to Alcontrol after the last sample is taken.

Sample labeling will be coded.

11.10.4 Tests to be performed

The test will be performed on LUMIStox 300 and ECLOX incl. thermostat and software.

The test will be performed in triplicate each with a blank consisting of 0.5 mL of test suspension (0) and 0.5 mL of sodium chloride solution (0), a test compound control (0) and a waste water control (0). Each triplicate in will be analysed in duplicate.

11.11 Test series K

This test series will be performed before test series J.

11.11.1 Purpose

To determine robustness in relation to the matrix.

11.11.2 Waste water samples

Treated industrial waste water (2 times 5 L in pp-plastic containers) was received from Chemionova on December 7, 2009. The samples were cool upon arrival and stored at 4 °C ± 2 °C in the cooling room. The samples were marked with ØT-nr.: 09-0834, A and B respectively.

Treated domestic waste water will be obtained from Lundtofte Waste Water Treatment Plant.

11.11.2.1 Sampling for chemical analyses

Single samples for chemical analysis of the waste water will be taken prior to the test in the sample containers provided by Eurofins. The analytical parameters are shown in Table 4.1.

Table 7-3 Analytical parameters for waste water.	
Turbidity	COD
TOC	Suspended solids (SS)
Conductivity	Nitrogen (total)
Alkalinity	Phosphorus (total)
pH	BOD_5

11.11.3 Preparation of waste water samples

The samples will be handled as described in section 0.

11.11.4 Tests to be performed

The test will be performed on LUMIStox 300 incl. thermostat and software with the colour correction feature switched on.

Test will also be performed on ECLOX incl. thermostat and software. If a significant effect of colour correction is observed on the LUMIStox 300, colour correction according to ISO 11348-3 will be performed.

11.12 Test series L

11.12.1 Purpose

To determine robustness in relation to use of different measuring cuvettes

11.12.2 Tests to be performed

The test will be performed on LUMIStox 300 incl. thermostat and software.

Two test compounds will be selected based on results obtained in previous tests. One compound will be selected among the compounds expected to adsorb to the plastic cuvette (SDS, CTAB or 4-NPE) and one compound will be selected among the compounds not expected to adsorb to the plastic cuvette (Cu^{2+}, $Cr_2O_7^{2-}$ or Zn^{2+})

The test will be performed at EC_{20}, i.e. at a sample concentration corresponding to twice the EC_{20}. The test will be run in 3 glass test tubes (0) with samples and 3 corresponding blanks (0) in glass test tubes and in 3 plastic test tubes (0) with samples and 3 corresponding blanks (0) in plastic tubes. The test will be performed three times in duplicate.

12. Data to be recorded

All measurements of luminescence will be recorded electronically on the PCs connected to the instruments. At the end of a test day a copy of the data will be placed on the DHI server at \\Dkstor\11800378_DAN_ETV\DHI delcenter\Verifikationer\HachLange\DHI laboratory\results in separate folders named by the date (YYYY-MM-DD). In the test series E, data will be retrieved and stored electronically in a folder named “series E”.

The format of hard copies of the raw data will be decided at a later stage.

Data from initial and daily temperature control including will be recorded.

For each toxicity test, the following information will be recorded when relevant:

Date and time,
Test series, samples including controls and concentrations of test compounds,
Initials of the performing technician,
Bacterial batch, date and time of reconstitution and related quality control data,
Pipettes used,
pH of sample, pH meter used, pH adjustment,
Salinity, conductivity meter used,
Oxygen saturation, oxygen electrode used,
Stock solutions used.

13. Time schedule

Tests will be started 2010.01.13 and will go on for 4 weeks.

The planned sequence of the tests and expected days required is shown in Table 7-4. It is anticipated that approximately half of the time requires two technicians.

Table 7-4: Planned sequence of testing and expected time required	
Series L	1
Series B	0.1
Series A	3
Series G+H+I	1
Series D	1
Series K	1
Series D	1
Series J	1
Series D	1
Series C	1
Series E	1
Series F	1
	13

14. Quality Assurance

The quality assurance will be performed in accordance with the joint verification protocol /1/

15. Reports

Reporting will be performed in accordance with the joint test plan /2/

16. Archives

All data generated and all other records and information relevant to the quality and integrity of the study will be retained. They will be filed in the archives of DHI after termination of the study and retained for a period of 10 years after issue of the final report.

17. Deviations and protocol amendments

Deviations and amendments will be handled in accordance with the joint test plan /2/

18. References

/1/ LUMIStox 300 Bench Top Luminometer ECLOX Handheld Luminometer. Joint verification protocol. Luminescent bacteria test for use in wastewater. December 2009. Mette Tjener Andersson. DHI. Project no. 11800378

/2/ LUMIStox 300 Bench Top Luminometer ECLOX Handheld Luminometer Joint test plan. Luminescent bacteria test for use in wastewater. Claus Jørgensen and Mette Tjener Andersson. Project no. 11800378.

/3/ LUMIStox 300. Manual. Hach Lange. January 2008. Version 3.02 and above. BDA 356.

/4/ Luminiscent bacteria test using the ECLOX Instrument. User manual. September 2009, Edition beta 2. Hach Company.

/5/ Luminiscent bacteria test with freeze-dried bacteria according to EN/ISO 11348-3. Dr. Lange. Luminiscent bacteria test LCK 491.

/6/ EN/ISO 11348-3:2007(E). Water Quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischri* (Luminiscent bacteria test). Part 3: Method using freeze-dried bacteria.

/7/ Luminiscent bacteria test with freeze-dried bacteria according to EN/ISO 11348-3. Dr. Lange. Luminiscent bacteria test LCK 491.

/8/ Dr. Lange LUMISsoft 4 Manual LZV 093, ver 1.001.

PROTOCOL APPROVAL

Issued by _____ Date:

Claus Jørgensen
Test Responsible

Concurred by _____ Date:

Bodil Mose Pedersen
Quality Assurance Unit

Protocol copy no. of 3

A P P E N D I X 5

In-house analytical methods

None

A P P E N D I X 6

Data reporting forms

Test A

Test time:	15 / 30 minutes

LUMIStox

Compound		Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)
Cr(VI)	1	1			
		2			
		3			
Cu	2	1			
		2			
		3			
Zn	3	1			
		2			
		3			
Pesticide	4	1			
		2			
		3			
Triclosan	5	1			
		2			
		3			
Cyanide	6	1			
		2			
		3			
Nonylphenol ethoxylate	7	1			
		2			
		3			
SDS	8	1			
		2			
		3			
CTAB	9	1			
		2			
		3			

Test A

Test time:	15 / 30 minutes

ECLOX

Compound		Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)
Cr(VI)	1	1			
		2			
		3			
Cu	2	1			
		2			
		3			
Zn	3	1			
		2			
		3			
Pesticide	4	1			
		2			
		3			
Triclosan	5	1			
		2			
		3			
Cyanide	6	1			
		2			
		3			
Nonylphenol	7	1			
		2			
		3			
SDS	8	1			
		2			
		3			
CTAB	9	1			
		2			
		3			

Test B

Batch No.	

Test time: 15 / 30 minutes

LUMIStox

No.	Mesurement	% inhibition
1		
2		
3		
4		
5		
6		
7		
8		
9		

ECLOX

No.	Mesurement	% inhibition
1		
2		
3		
4		
5		
6		
7		
8		
9		

Test C

Test time:	15 / 30 minutes

LUMIStox

Start concentration	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)
~EC 60	1			
Actual start conc:	2			
_____ mg/L	3			
~EC 30	1			
Actual start conc:	2			
_____ mg/L	3			

ECLOX

Start concentration	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)
~EC 60	1			
Actual start conc:	2			
_____ mg/L	3			
~EC 30	1			
Actual start conc:	2			
_____ mg/L	3			

Test D

Test time:	15 / 30 minutes

LUMIStox

Day No.	Date	Replicates	Batch no.	EC20 (mg/L)	EC50 (mg/L)
1		1		Results from test A	
		2			
		3			
2		1			
		2			
		3			
3		1			
		2			
		3			
4		1			
		2			
		3			

ECLOX

Day No.	Date	Replicates	Batch no.	EC20 (mg/L)	EC50 (mg/L)
1		1		Results from test A	
		2			
		3			
2		1			
		2			
		3			
3		1			
		2			
		3			
4		1			
		2			
		3			

Test E

Test time 15 minutes

ECLOX

Compound 1:	Batch No.:

1. replicate

Tube	Bacteria susp. (mL)	2% NaCl (mL)	Sample (mL)	% inhibition	Sample conc.	App. sample conc.
1	0.2	0.8	no	n.a.	non-toxic ref.	n.a.
2	0.2	0.6	0.2			20%
3	0.2	0.3	0.5			50%
4	0.2	no	0.8			80%

2. replicate

Tube	Bacteria susp. (mL)	2% NaCl (mL)	Sample (mL)	% inhibition	Sample conc.	App. sample conc.
1	0.2	0.8	no	n.a.	non-toxic ref.	n.a.
2	0.2	0.6	0.2			20%
3	0.2	0.3	0.5			50%
4	0.2	no	0.8			80%

3. replicate

Tube	Bacteria susp. (mL)	2% NaCl (mL)	Sample (mL)	% inhibition	Sample conc.	App. sample conc.
1	0.2	0.8	no	n.a.	non-toxic ref.	n.a.
2	0.2	0.6	0.2			20%
3	0.2	0.3	0.5			50%
4	0.2	no	0.8			80%

Compound 2:	Batch No.:

1. replicate

Tube	Bacteria susp. (mL)	2% NaCl (mL)	Sample (mL)	% inhibition	Sample conc.	App. sample conc.
1	0.2	0.8	no	n.a.	non-toxic ref.	n.a.
2	0.2	0.6	0.2			20%
3	0.2	0.3	0.5			50%
4	0.2	no	0.8			80%

2. replicate

Tube	Bacteria susp. (mL)	2% NaCl (mL)	Sample (mL)	% inhibition	Sample conc.	App. sample conc.
1	0.2	0.8	no	n.a.	non-toxic ref.	n.a.
2	0.2	0.6	0.2			20%
3	0.2	0.3	0.5			50%
4	0.2	no	0.8			80%

3. replicate

Tube	Bacteria susp. (mL)	2% NaCl (mL)	Sample (mL)	% inhibition	Sample conc.	App. sample conc.
1	0.2	0.8	no	n.a.	non-toxic ref.	n.a.
2	0.2	0.6	0.2			20%
3	0.2	0.3	0.5			50%
4	0.2	no	0.8			80%

Test F

		Test time: 15 / 30 minutes
	Test compound:	
Conc ~EC 20	Actual start conc:	

LUMIStox

Temp. (°C)	Replicate	Batch No.	% inhibition
	1		
	2		
	3		
15	1		
	2		
	3		
	1		
	2		
	3		

ECLOX

Temp. (°C)	Replicate	Batch No.	% inhibition
	1		
	2		
	3		
15	1		
	2		
	3		
	1		
	2		
	3		

Test G

	Test time: 15 / 30 minutes
	Test compound:
Conc ~EC 20	Actual start conc:

LUMIStox

pH	Replicate	Batch No.	% inhibition
6.0	1		
	2		
	3		
7.0	1		
	2		
	3		
8.5	1		
	2		
	3		

ECLOX

pH	Replicate	Batch No.	% inhibition
6.0	1		
	2		
	3		
7.0	1		
	2		
	3		
8.5	1		
	2		
	3		

Test H

	Test time: 15 / 30 minutes
	Test compound:
Conc ~EC 20	Actual start conc:

LUMIStox

Color conc.	Intensity (nm)	Replicate	Batch No.	% inhibition
No		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
Blind (no sample)		1		
Medium color		2		
		3		

ECLOX

Color conc.	Intensity (nm)	Replicate	Batch No.	% inhibition
No		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
Blind (no sample)		1		
Medium color		2		
		3		

Test I

	Test time: 15 / 30 minutes
	Test compound:
Conc ~EC 20	Actual start conc:

LUMIStox

Turbide conc.	Turbidity	Replicate	Batch No.	% inhibition
No		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
Blind (no sample)		1		
Medium turbidity		2		
		3		

ECLOX

Turbide conc.	Turbidity	Replicate	Batch No.	% inhibition
No		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
Blind (no sample)		1		
Medium turbidity		2		
		3		

Test J

Bacteria batches as in Test A

Conc ~EC 20

LUMIStox

Test time: 15 / 30 minutes

Domestic wastewater

Compound	Conc (mg/L)	Replicate	Batch No.	% inhibition
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		

Industrial wastewater

Compound	Conc (mg/L)	Replicate	Batch No.	% inhibition
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		

Test J

Bacteria batches as in Test A

Conc ~EC 20

ECLOX

Test time: 15 / 30 minutes

Domestic wastewater

Compound	Conc (mg/L)	Replicate	Batch No.	% inhibition
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		

Industrial wastewater

Compound	Conc (mg/L)	Replicate	Batch No.	% inhibition
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		
		1		
		2		
		3		

Test K

Bacteria batches as in Test J

Mark test time:	15 / 30 minutes

LUMIStox

Wastewater	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)
Domestic	1			
	2			
	3			
Industrial	1			
	2			
	3			

ECLOX

Wastewater	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)
Domestic	1			
	2			
	3			
Industrial	1			
	2			
	3			

Test L

Compound 1	Mark test time:	15 / 30 minutes
	Test compound 1:	
Conc ~EC 20	Actual start conc:	

LUMIStox

Cuvette	Replicate	Batch No.	% inhibition
Glass	1		
	2		
	3		
Plastic	1		
	2		
	3		

Compound 2	Mark test time:	15 / 30 minutes
	Test compound 2:	
Conc ~EC 20	Actual start conc:	

LUMIStox

Cuvette	Replicate	Batch No.	% inhibition
Glass	1		
	2		
	3		
Plastic	1		
	2		
	3		

A P P E N D I X 7

Data management

In general, the data filing and archiving procedures of the DHI Quality Management System will be followed.

All data recording and reporting is done in English, communication with Danish external and internal can be in Danish.

Data storage, transfer and control
The data to be compiled and stored are summarized in Table 5.1, in Section 5.1 Data storage, transfer and control.

Analytical raw data will be filed and archived according to the specifications of the laboratories' quality management systems under their ISO 17025 accreditation and are thus not the concern of DHI staff.

Implementation
All e-mail communication is filed in the Outlook Exchange folders, see below structure.

The DHI person receiving an e-mail (to field, not cc field) will file the e-mail. The DHI person sending an e-mail will use the "send and file" option and thereby ensure prompt filing of all e-mails sent. There is generally no need to widespread cc when sending e-mails, unless specific action or communication is required.

All paper communication is immediately filed in the binder established by Mette Tjener Andersson (MTA) and available in her office. The title page of the binder will resemble the folder structure in Outlook Exchange, see below.

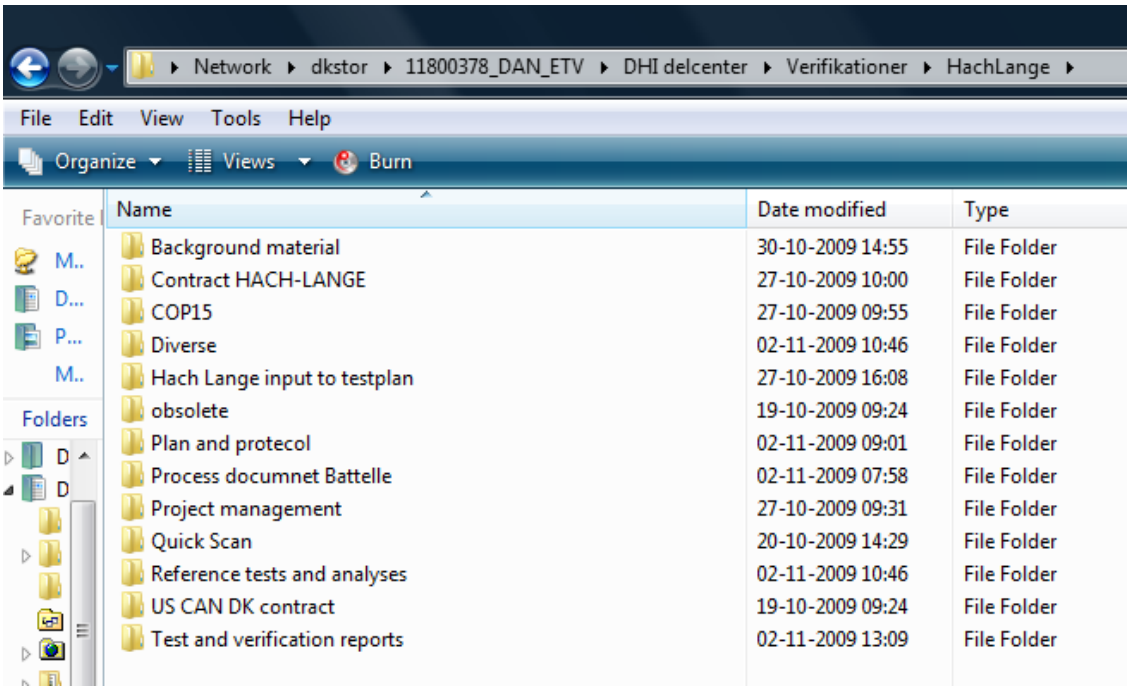
All recordings during testing in the laboratory or outdoors are done in water proof writing in hardback log-books with all pages numbered page/total page number. The log books are filed with the staff member using them until the testing is completed, then with Claus Jørgensen (CLJ), and will be available at his office.

All data needed for the tests are recorded in the data sheets available from Appendix 6 of the Test Plan. The format can be Word tables, Excel worksheets or paper sheets as decided by CLJ as test responsible. The outline and format are mandatory and can only be deviated from by recording a deviation with justification, see the Test Plan.

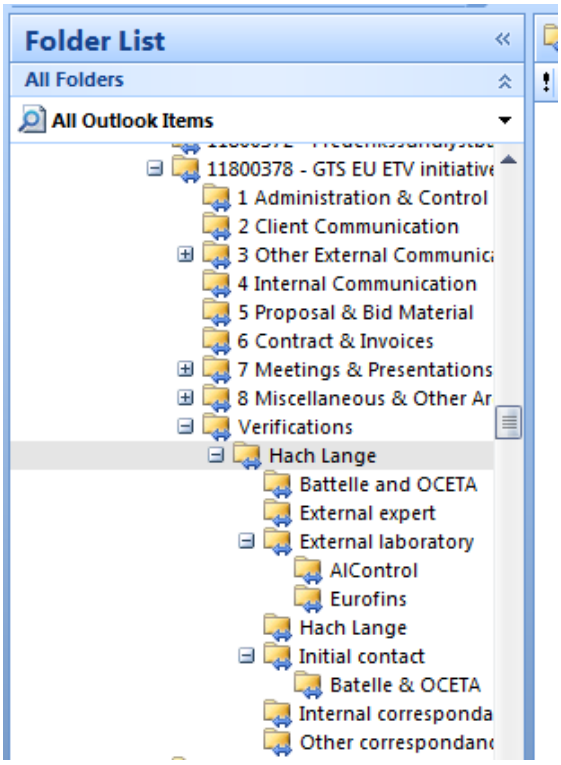
All calculations are done using Excel spreadsheets with names identifying the contents and with headings and notes explaining the calculations.

All electronic files are stored at dkstor in the folder structure shown below. File names are constructed to identify the contents. Subfolders can be established as found convenient, while again constructing folder names that identify the contents. When working away from a network connection (offline), copies of files can be used on personal PCs, but the server version is updated and the offline version deleted immediately after returning to the network connection.

dk stor:



Outlook Exchange:



A P P E N D I X 8

Deviations and amendments

Deviation reports

The test plan version approved must be followed. If (or rather when) deviations are needed during testing, the deviations are noted and justified in the format:

Deviation number	Experiment label Test Plan	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature test or field responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada

The verification protocol version approved must be followed. If deviations are needed during testing, the deviations are noted and justified in the format:

Deviation number	Verification protocol Chapter	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature verification responsible	Date	Signature internal auditor	Date	Signature Battelle AMS QM	Date	Signature ETV Canada

Deviation reports are continuously filled in and filed in the appropriate folder at dkstor, see Appendix 7.

Amendment reports

All changes in the protocol and test plan done in advance of verification and testing must be done by the document owner (protocol Mette Tjener Andersson (MTA), plan Claus Jørgensen (CLJ)) and approved by the verification responsible and the internal auditor. Amendments shall be made available for all involved.

The amendments will mostly have the form of a revised section or chapter of the protocol or plan, with the front page given below.

AMENDMENT

TESTING DOCUMENT TITLE AND DATE:

AMENDMENT NUMBER: __

DATE OF REVISED PART: __

PART TO BE CHANGED/REVISED:

CHANGE/REVISION:

Reference to revised part

REASON FOR CHANGE:

ORIGINATED BY:

_____

DHI DANETV Water Centre Verification or Test Responsible

_____

DATE

APPROVED BY:

_____

DHI DANETV Water Centre Internal Auditor

_____

DATE

_____

Battelle AMS Center Quality Manager

_____

DATE

_____

ETV Canada Quality Manager

_____

DATE