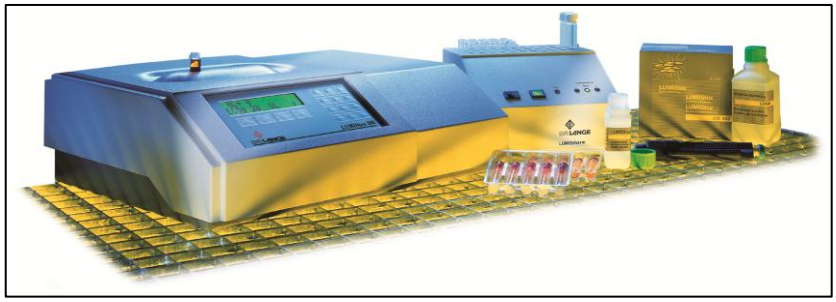
LUMIStox 300 Bench Top Luminometer
ECLOX Handheld Luminometer

Joint test report

Luminescent bacteria test for use in wastewater

Handheld ECLOX



LUMIStox 300

August 2011

Final version

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ECLOX Handheld Luminometer

Joint test report

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Project	Project No
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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 was to evaluate the performance of a wastewater rapid toxicity technology that can be used to monitor industrial or domestic wastewater.

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

2.1 Verification protocol reference

This test report is prepared in response to the test design established in the LUMIStox and ECLOX, test plan, for luminescent bacteria test for use in wastewater, 2009 /16/.

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 planning documents 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).

This test report has been reviewed by Associate Professor Kresten Ole Kusk.

3 TEST DESIGN

Test compounds were selected as described in the joint verification protocol Appendix 3 /1/. Due to problems dissolving three of the test compounds to toxic concentrations, the total number of compounds in the test was reduced from nine to six. The final test compounds are shown in Table 3.1. The compounds copper (heavy metal), flutriafol (pesticide), and nonylphenol ethoxylate (surfactant) were left out of the original test set-up for the reasons stated above. See details in deviations included in Appendix 7.

Table 3.1 Test compounds.

Group	CAS no.	Compound
Heavy metals	7733-02-0 7778-50-9	Zn^{2+} as $ZnSO_4+7H_2O$ $Cr_2O_7^{2-}$ as $K_2Cr_2O_7$
Organic pollutants	3380-34-5	Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether)
Industrial pollutants	57-12-5	CN^- (cyanide) as KCN
Surfactants	151-21-3 57-09-0	SDS (sodium lauryl sulphate) CTAB (cetyl trimethyl ammonium bromide)

The test design as performed is summarized in Table 3.2. Acronyms are explained in Appendix 1.

Test results are EC_{20} and EC_{50} values, representing the concentration causing respectively 20 % and 50 % inhibition of the luminescence of the *Vibrio fischeri* population. For tests where the luminometer was not connected to a PC with LUMISsoft4 software, results are given as % inhibition. This is the case for one test performed with the ECLOX (Test E).

All tests were performed in plastic cuvettes except for test L where LUMIStox robustness towards cuvettes (glass or plastic) was evaluated.

Table 3.2 Test design.

Test.		Equipment 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 six compounds. 3 test replicates (includes 2 measurement replicates each). Optimal concentrations 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 initial 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 3 different days with 3 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: 5°C, 16°C and 23°C. Measurement only after 15 minutes of inhibition			x	2 % NaCl MilliQ			x			Sample temperature at field use
F	Concentration ~ EC_{20} for 1 compound. For temperatures of 14°C, 15°C and 16°C. 3 test replicates (includes 2 measurement replicates each)	x	x		2 % NaCl MilliQ						Sample temperature at laboratory use

Test.		Equipment tested			Matrix	Criterion of detection (CD)	Range	Precision		Agreement with accepted values	Robustness
		LUMIStox	ECLOX incl. thermostat and software	ECLOX incl. firmware				Repeatability	Reproducibility		
G	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	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 blinds with each color concentration and no sample. LUMIStox with color correction, ECLOX with correction cuvettes according to ISO 11348-3	x	x		2 % NaCl MilliQ						Color
I	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 blinds with each turbid reagent/material concentration 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, performed in wastewater and in 2 % NaCl MilliQ as reference. Blind containing only wastewater. 3 test replicates (includes 2 measurement replicates each)	x	x		2% NaClwastewater						Matrix
K	Test of dilution series for undiluted and unspiked industrial and domestic wastewater. 2-3 test replicates (includes 2 measurement replicates each)	x	x		2% NaClwastewater						Matrix

Test.		Equipment tested			Matrix	Criterion of detection (CD)	Range	Precision		Agreement with accepted values	Robustness
		LUMIStox	ECLOX incl. thermostat and software	ECLOX incl. firmware				Repeatability	Reproducibility		
L	Concentration ~ EC_{20} for 2 compounds. 3 replicates. Repeated 3 times. Test of use of glass and plastic cuvettes	x			2 % NaCl MilliQ						Cuvettes

3.1 Test sites

The laboratory tests were conducted at DHI, Hørsholm, Denmark.

3.1.1 Types

A domestic wastewater sample for laboratory testing was obtained from the Lundtofte wastewater treatment plant. Industrial wastewater was obtained from an industrial wastewater treatment plant at Cheminova, a producer of pesticides in north-western Jutland, Denmark.

The wastewaters were collected by the personnel at the treatment plants. The sample from Cheminova was sent cold to DHI. The sample from Lundtofte wastewater treatment plant was picked up by DHI personnel immediately after sampling and brought to DHI. Both wastewater samples were stored at DHI at 5°C until use.

MilliQ water from the DHI laboratory with NaCl added to a concentration of 2 %, was used as diluent for the standard dilution series.

3.1.2 Addresses

Laboratory 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.

3.1.3 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™ 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.
- Three LUMIStherm heating blocks.

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/ As mentioned earlier, all tests were performed in plastic cuvettes except for test L where LUMIStox robustness towards cuvettes (glass or plastic) was evaluated.

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 Tests

The test program was 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 the instrument manuals (see section 3.1.3) 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, included 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 was 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 was tested to a less extent (only Test E).

The test was performed mainly in the laboratory, while two out of three parts of Test E on the ECLOX were performed in DHI climate chambers.

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 was incorporated in the manuals for LUMIStox and ECLOX. For ECLOX used in the field (Test E) a slightly modified method is used, main difference is no fixed temperature of samples and mixing of bacteria suspension with test sam-

ples is not 1:1 and in the ISO 11348-3 but instead 1:4. This is described in details in the manual for the ECLOX.

In test A dilution series of 9 dilutions were prepared for all selected compounds according to Annex B in ISO 11348-3. Each dilution was prepared and measured twice and the average was used as result. This was repeated 3 times. For all other tests except for test B and J, measurement was performed on compounds selected among the 6 target compounds at concentrations described in Table 3.2. The criterion of detection (Test B) and one of the robustness tests (Test J) analysis was performed on 2 % NaCl MilliQ water and on wastewater, respectively.

The determination of robustness against temperature at laboratory use (Test F), pH (Test G), color (Test H), turbidity (Test I) and type of cuvettes (Test L) was performed at one concentration (EC_{20}), while determination of robustness against initial concentration was performed at two concentrations (EC_{30} and EC_{60}) (Test C). The determination of robustness against temperature at field use (5, 16 and 23°C) (Test E) was performed at three concentrations (EC_{20}, EC_{50} and EC_{80}). The determination of robustness against the wastewater matrix with undiluted and unspiked industrial and domestic wastewater (Test K) was performed on dilutions series with 9 dilutions.

Stock solutions of each test compound were prepared in 2 % NaCl MilliQ water. Solid NaCl was added to wastewater used for Test J and K to obtain the salt concentration required for testing with the marine bacteria, *Vibrio fischeri*. Dilution series in Test K were prepared with dilution saltwater (2 % NaCl) provided from HACH-LANGE.

Color for Test H was prepared as a dark brown mixture of 50 mg/L Tartrazine, 50 mg/L New coccine and 6.7 mg/L Lissamine Green B, which are dyes representing the yellow, red and green color spectrum, respectively. The color mixture was tested to be non-toxic at concentration up to 25 % color mixture. Test H was performed with concentrations of color mixture of 0.2 (not visible), 6.25 and 12.5 % color mixture. Pictures of the colors in double concentration 0.4, 12.5 and 25 %[1] are shown in Appendix 6.

The turbid mixture used in Test L was prepared with $BaSO_4$. A $BaSO_4$ solution of 0.2 g/L was tested non-toxic. The turbidity robustness test was performed with concentrations of $BaSO_4$ of 0.05 g/L, 0.1 g/L and 0.2 g/L. Pictures of the turbid solutions in double concentration 0.1, 0.2 and 0.4 g/L are shown in Appendix 6. The toxicity of $BaSO_4$ was screened on samples centrifuged and filtered as prescribed in the ISO 11348-3.

The LUMISsoft4 PC software calculates EC_{50}-values as an overall result of the testing of a dilution series according to the ISO 11348-3. 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} per ISO11348-3. For compounds not previously tested it was therefore necessary to perform a range finding test to determine concentrations which would give inhibitions within the range of 10 % to 90 %. The software requires a minimum of three values between 10 % and 90 % to calculate the EC_{50}.

[1] When testing is performed bacteria suspension is added and concentrations are the half.

The principle of the dilution series in the thermostat is shown in Figure 3.1. The rows B and C contain the two measurement replicates included in all tests where the thermostat was used. When performing the test, the added bacterial suspension was doubling the volume. The final dilution series were therefore: control, 32, 24, 16, 12, 8, 6, 4, 3 and 2 times dilution.

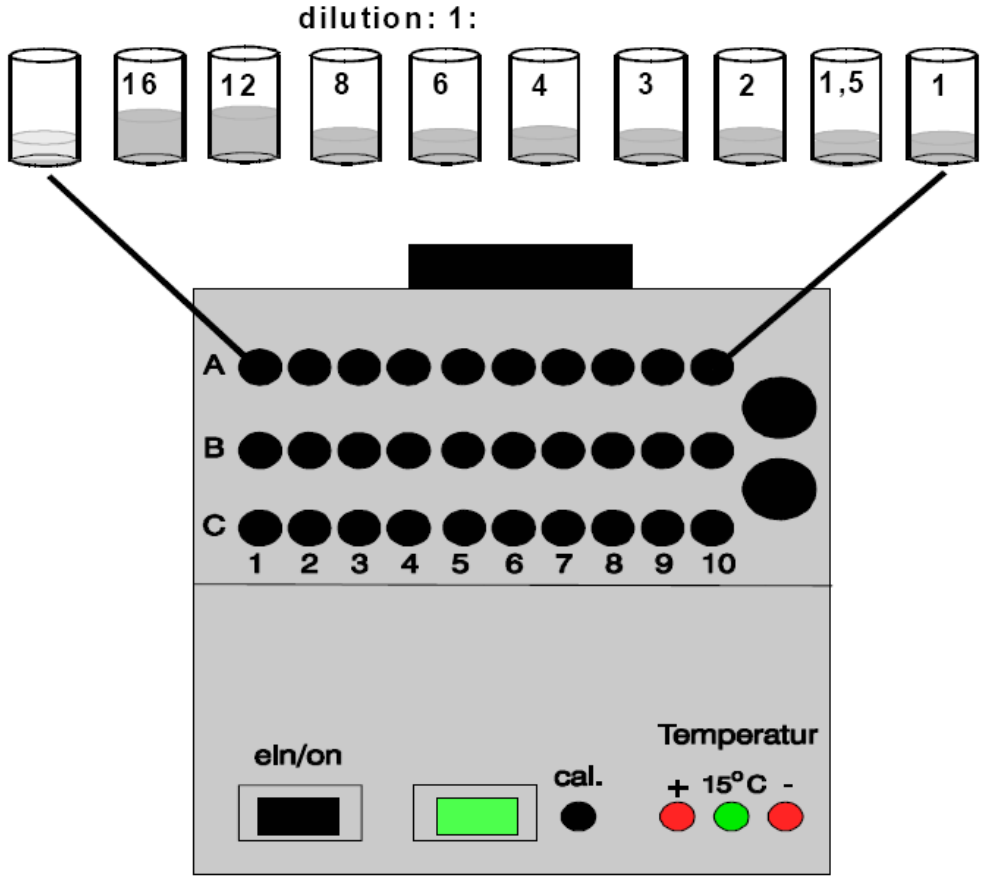


Figure 3.1 LUMIStherm thermostat and dilution series.

Test J and K were performed on effluent industrial and domestic wastewater. The toxicity (as indicated by inhibition) of types of wastewater was determined prior to their use. If the wastewater had been toxic, it would have been diluted with 2 % NaCl MilliQ water to a non-toxic concentration, and then spiked with the selected compounds. The two wastewater types, tested in Test K, were not toxic and they were, therefore, used without any dilution.

For field portability (Test E), the ECLOX without the LUMISsoft4 software was used (i.e., no PC is taken along). The firmware only shows readings of % inhibition. The goal of this test was to assess how stable individual inhibition measurements were at three different temperatures; a controlled 5°C, 15°C and ambient room temperature. Concentrations generating three inhibitions (20 %, 50 % and 80 %) were 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 was Claus Jørgensen, and the test technicians were Jane Bergstrøm and Connie Seierø.

3.2.3 Type and number of samples

The types and number of samples are summarized in Table 3.3.

Table 3.3 Number of measurements of samples and blanks.

Test No.	Performance parameters	Measurements of samples		Measurements of blanks and reference standards	
		LUMIStox	**ECLOX**	**LUMIStox**	**ECLOX**
A	Range Repeatability Agreement with accepted values	396	396	44	44
B	Criterion of detection			10	10
C	Robustness, initial concentration	96	96	12	12
D	Reproducibility	162	171	18	19
E	Repeatability, field		54		18
F	Robustness, sample temperature	18	18	18	18
G	Robustness, pH	18	18	6	6
H	Robustness, color	42	42	6	6
I	Robustness, turbidity	56	56	8	8
J	Robustness, matrix	132	132	12	12
K	Robustness, matrix	90	72	10	8
L	Robustness, cuvettes	36		12	
Total		**1046**	**1055**	**156**	**161**

3.2.4 Operation conditions

The operation conditions applied during the verification of the product were generally as required in ISO 11348-3. EC-values were determined for exposure times of 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 (Test E) on variation in sampling temperatures (indoor and outdoor during the Danish winter period) was included.

3.2.5 Operation measurements

During operation, the following conditions were recorded, when relevant:

- Conductivity/salinity of stock solutions.
- pH of stock solutions.
- Temperature in the thermostat was controlled daily.

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

The vendor has experienced that cyanide is difficult to handle. Therefore, a determination of the concentration of cyanide in the dilutions was performed with a test kit. An artificial cyanide sample was carried through the entire test procedure. Instead of adding bacteria solution 2 % NaCl was added. No measurements of luminescence were performed but the concentration of cyanide was analyzed using a HACH-LANGE test kit (LCK 315). Test row B was analyzed at time 0 and test row C was analyzed after

30 minutes. The LCK 315 from HACH-LANGE, uses a barbituric acid-pyridine method and has a range of measurement from 0.01-0.6 mg/L.

The results showed that cyanide was stable during that test i.e. the concentration after 30 minutes was within the acceptable range of 80-120 % of the initial concentrations. Cyanide was therefore included in the test program.

3.2.6 Product maintenance

The following storage information for the bacteria was 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 provided 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 provided 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.

3.2.7 Health, safety and wastes

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

Laboratory work during testing was performed 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 were handled carefully in accordance with material datasheets of the test substances. Wastewater was handled according to DHI's safety rules.

Chemicals and test solutions were 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 were not planned to be used as true values as seen in other verifications, but to be used to give an indication of the sensitivity of the test organisms and as help in identifying false negative tests performed with the LUMIStox or ECLOX equipment.

Luminescent bacteria reference tests were intended to be done under ISO 17025 accreditation, using the ISO 11348-3 Luminescent bacteria test method, with Microtox® equipment. The analytical laboratory chosen to perform the reference test turned out to not fully capable or equipped to fulfill the requirements of the ISO 11348-3, but instead used a modified method. It was investigated if the tests could be performed elsewhere fulfilling both the ISO 11348-3 and the accreditation requirement as well as operating different equipment than the LUMIStox (or ECLOX). A laboratory meeting these criteria could not be found, and it was decided to exclude further reference tests.

The originally plan was reference tests should be done by an external laboratory, ALcontrol, on the following selected samples:

- Test A: samples with the initial concentration for all target compounds tested (except 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.

After test of three samples: pure 2 % NaCl MilliQ water and 2 % NaCl MilliQ water spiked with SDS and zinc, respectively, it was noticed that the results obtained from the samples with zinc differed remarkable from the results of the Zn^{2+} reference standard listed in ISO 11348-3.

The laboratory, ALcontrol, was therefore asked to provide details of their test procedures. There were several deviations from the ISO 11348-3:

- Test reference standards used were from the bacteria supplier and were not in accordance with the ISO
- The ISO specifies that a sodium, magnesium and potassium solution shall be used for dissolving the bacteria. Instead was used a sodium solution with addition of magnesium.
- Dilution series were not prepared as stated in the ISO and replicate test of each cuvette, as specified in the ISO, were not performed.

When ISO reference standards were not tested as specified, it is not possible to determine if the test if valid according to section 11 of the ISO 11348-3.

The lack of use of the sodium, magnesium and potassium solution for dissolving the bacteria will affect the toxicity of chemicals. According to vendor a solution with only sodium will result in higher toxicity (lower EC_{50}-values) for zinc and lower toxicity (higher EC_{50}-values) for chromium.

Due to the above conditions only the results of the test with the 2 % NaCl MilliQ water were applied in this report.

With the aim to confirm test concentrations subsamples of test stock solutions were sent to an independent laboratory for chemical analysis under ISO 17025 accreditation /5/. The stock solutions were made in a 2 % NaCl solution to ensure same conditions as in the luminescent bacteria test. The stock solutions were prepared on the day of use and usually subsamples were shipped and received on the chemical laboratory on the same day. However $K_2Cr_2O_7$ samples were received on the laboratory the following day and $CuSO_4$ and $ZnSO_4$ samples, 5 and 6 days after preparation respectively. Samples of stock solution for analysis were taken at the start of the toxicity testing.

4.1 Analytical laboratory

Reference test of toxicity was 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 of stock solutions were performed 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 were tested with Microtox®, results were given as EC_{20}- and EC_{50}-values in % of solution.

All stock solutions were analyzed for the concentration of the added compound.

The wastewater samples were analyzed 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 was a Luminescent bacteria test method. The equipment from Microtox® was used. ALcontrol performs regular tests for zinc sulphate and phenol and compare them with specified intervals from the bacteria supplier. These test results were available for review. ALcontrol has also participated in one interlaboratory trial, from which results were available for review at the audit performed by Battelle.

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

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

Group	**Compound**	**Method**	**Limit of detection** µg/l	**Uncertainty** %
Heavy metals	Cr	ISO 17294m - ICP-MS	1.0	15 [1]
	Zn	ISO 17294m - ICP-MS	0.5	10 [1]
Organic pollutants	Triclosan [2]	-	-	-
Industrial pollutants	Cyanide (CN^-)	DS/EN ISO14403	1	10
Surfactants	SDS (sodium lauryl sulphate)	DS 237	25	15
	CTAB (cetyl trimethyl ammonium bromide) [3]	VKI method	100	20

[1] Eurofins states that salt content in samples can give higher RSD.

[2] The inclusion of triclosan in wastewater analyses is relatively new. Triclosan was set up by Eurofins in December 2009. This method is therefore not included under Eurofins accreditation.

[3] CTAB was analysed with a general method for cationic detergents. The method is calibrated with benzyl dimethyl tetradecyl ammonium chloride-dihydrate. The concentration of CTAB was calculated based on the mole weight of the two compounds.

Table 4.3 Method for parameters analyzed 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 have been available from Eurofins.

4.4 Analytical performance requirements

The analytical performance requirement for the reference test performed with Microtox® is 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, did not give information on the performance parameters.

Table 4.4 Required analytical performance.

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

4.5 Preservation and storage of reference samples

Samples for Microtox® testing were frozen until shipment according to instructions from the ALcontrol.

Stock solutions for chemical analyses were preserved according to instructions of Eurofins. Wastewater samples were stored at 5°C. The samples were shipped on ice and transported by a refrigerated van.

5 DATA MANAGEMENT

Data filing and archiving followed the procedures of the DHI Quality Management System, which specifies that all project material is to be filed after the project has been completed. The project material comprises all documents, calculations, analyses, results, etc. that will enable another DHI employee to scrutinize 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 types of data compiled and stored are summarized in Table 5.1.

Analytical raw data for stock solution verification and wastewater parameters from Eurofins were filed and archived according to the specifications of their laboratory 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

Data type	Data media	Data recorder	Data recording timing	Data storage
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 in the test plan /14/. Records were made for tests after 15 and 30 minutes of exposure.

6 QUALITY ASSURANCE

The tests were 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 has been subject to internal review by the verification responsible from DHI DANETV Water Centre: Head of Innovation Margrethe Winther-Nielsen. The test plan has also been 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 U.S. EPA ETV AMS project officer and quality manager (John McKernan and Michelle Henderson, respectively). Furthermore, the test plan has been subject to review by ETV Canada by Director Technology Assessment and Quality Assurance Services Mona El-Hallak.

External review of the test plan has been 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 has been based on laboratories performing analyses under ISO 17025 accreditation /5/. Information on the laboratory quality assurance has been gathered. Physical inspection (audit) was only planned to be performed if disagreements were suspected. This was not the case with regards to Eurofins and physical inspection there has not been performed by DHI. Battelle audited ALcontrol and differences in method noted. Upon examining of first test results the difference noticed at the audit led to further investigations and ended with eliminating the Microtox® at ALontrol as reference test.

Performance control of ALontrol Microtox® tests has not been performed due to decision on not perform test as planned. Information of the laboratory quality assurance,

method validation, etc. has been evaluated at the Battelle TSA audit. Further result from testing of standards done in addition to testing of samples has been gathered.

Performance control of Eurofins analysis was 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. has been gathered. Eurofins includes standard reference samples when they analyze. Details on their acceptance range and action if standard is out of acceptance range are given in Table 6.1.

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
	Zn		6.5-8.8 µg/L	
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 results for standard near LoD have 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	SDS (sodium lauryl sulphate)	As for triclosan. As standard is used SDS	The results for standard near LoD have 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 reana-lyzed. Either reextraction with less sample material in use or the first extract will be diluted. If retrieval is not with acceptance

Group	Compound	Method	Acceptance criteria	Action
		Further is also included quality control performed by spiking a sample and calculate retrieval	70-120 %	criteria sample and spiked sample are reanalyzed
Surfactants	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

Performance evaluation (PE) audits have been considered, but the gathered quality control data from Eurofins did not show any need for PE-audit.

6.3 Test system control

System control was used to test the DHI DANETV Water Centre test system of the LUMIStox and ECLOX.

All stock solutions were analyzed in duplicate to confirm the concentration of target compounds. Before testing with luminometers solid NaCl salt was added to the samples. Therefore, solid NaCL was also added to the samples sent for reference analysis.

Luminescent bacteria test of 1 blank sample was performed at ALcontrol to ensure that no toxic sources of contamination are present in MilliQ water used for preparation of stock solutions. The blank sample was also used for control of ALcontrol Microtox® test. The blank sample was found to be non-toxic.

According to ISO 11348-3 three reference substances shall be tested for each batch of bacteria. These tests were 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 were 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 6.2. The results of the test quality assurance are given in Section 7.3

Table 6.2 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 as well as manual transfer from one program to another was checked.

6.5 Test system audits

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

The Battelle Quality Manager, Zachary Willenberg, performed a technical systems audit (TSA) during this verification and test. The purpose of this audit was to ensure that the verification test was performed in accordance with the AMS Center quality management plan /9/, the test plan /14/, published reference methods and any methods used in the tests. In the TSA, the Battelle Quality Manager reviewed the reference methods used and compared actual test procedures to those specified or referenced in the plan. In the TSA, the Battelle Quality Manager was observing testing in progress, inspecting documentation, and reviewing technology-specific record books. He also checked standard certifications. A TSA report was prepared /14/, 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 received a copy of Battelle's TSA report. The TSA findings were communicated to technical staff at the time of the audit and documented in the TSA report.

The Battelle Quality Manager, or designee and the ETV Canada Quality Manager, performed an audit of data quality. This was 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 Quality Managers traced the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All calculations performed on the data undergoing the audit were checked.

6.6 Test report review

The test report has been subject to internal review by the verification responsible from DHI DANETV Water Centre: Head of Innovation Margrethe Winther-Nielsen.

Mona El-Hallak (MEH) from ETV Canada has reviewed the test report.

External review of the test report was done by Associate Professor Kresten Ole Kusk.

7 TEST RESULTS

This test report follows the template of the DHI DANETV verification center quality manual /10/.

For this joint verification, the principles (contents) of the U.S. EPA ETV and ETV Canada format have been complied with as well.

One joint test report is prepared for LUMIStox and ECLOX.

7.1 Test data summary

The test results are summarized below and presented according to the performance parameters investigated. For complete descriptions of the raw data collected and calculations used in reporting, the reader is referred to the test design in Section 3, the raw data in Appendix 6 and the calculations in Section 8.1 in the verification report /16/. The Excel sheets containing the actual calculations are archived at DHI.

7.1.1 Criterion of detection

The criterion of detection was calculated based on the results from Test B, where series of 9 samples including bacteria, but no toxic compounds, were tested. The criterion of detection for LUMIStox and ECLOX after 15 and 30 minutes exposure respectively, is given in Table 7.1.

Table 7.1 Criterion on detection (% inhibition). Number of replicates (n) is 3.

Test time	LUMIStox	ECLOX
15 minutes	6.7	7.5
30 minutes	5.8	5.5

7.1.2 Range of application

Range of application in this context means the concentration range where (pure water) samples can be tested without dilution or pre-concentration.

The range of application was based on the results from Test A, where EC_{50} values were determined for 6 target compounds.

To be able to determine the EC_{50}-value, an initial concentration just above 2* EC_{50} is needed, since the standard procedure is to dilute the sample to half the initial concentration before testing. Without extraordinary dilution of the sample, the EC_{50}-value has to be detected within the regular dilution series containing 9 dilutions (limitation by the thermoblock). The maximum concentration in the sample can therefore be just less than $32*EC_{50.}$ The compound specific ranges of application are listed in Table 7.2 and Table 7.3 together with the average EC_{50}-values.

Table 7.2 LUMIStox range of application in 2 % NaCl MilliQ water for target compounds (mg/L). Number of replicates (n) is 3 but 4 for cyanide.

LUMIStox	**15 minutes**			**30 minutes**		
Compound	**Average EC_{50}** (mg/L)	**Range of application** (mg/L)		**Average EC_{50}** (mg/L)	**Range of application** (mg/L)	
		Minimum	**Maximum**		**Minimum**	**Maximum**
Zn^{2+}	8.5	>17	<270	4.1	>8.3	<130
$Cr_2O_7^{2-}$	n.c. [1]	-	-	17	>35	<560
Triclosan [3]	0.40	>0.79	<13	0.53	>1.1	<17
Cyanide	24 [2]	>48	<70	24	>48	<780
SDS [3]	1.4	>2.8	<44	1.0	>2.0	<32
CTAB [3]	1.3	>2.7	<43	0.97	>1.9	<31

n.c.: Not calculated.

[1] EC_{50} for $Cr_2O_7^{2-}$ was not possible to calculate after 15 minutes. The requirement of one measurement above 50% inhibition was not fulfilled.

[2] EC_{50} for cyanide was only possible to calculate after 15 minutes for two out of four replicates. The requirement of one measurement above 50% inhibition was not fulfilled.

[3] The recovery of these compounds in mixed solutions was only 2-7 %. The listed EC-values are based on the added amount of compound. See details on recovery later in section 7.3.3.

Table 7.3 ECLOX range of application in 2 % NaCl MilliQ water for target compounds (mg/L). Number of replicates (n) is 3 but 4 for cyanide.

ECLOX	**15 minutes**			**30 minutes**		
Compound	**Average EC_{50}** (mg/L)	**Range of application** (mg/L)		**Average EC_{50}** (mg/L)	**Range of application** (mg/L)	
		Minimum	**Maximum**		**Minimum**	**Maximum**
Zn^{2+}	8.4	>17	<270	4.1	>8.2	<130
$Cr_2O_7^{2-}$	n.c. [1]	-	-	18	>37	<590
Triclosan [4]	0.39	>0.77	<12	0.53	>1.1	<17
Cyanide	23 [2]	>45	<730	18 [3]	>35	<570
SDS [4]	1.4	>2.8	<45	0.99	>2.0	<32
CTAB [4]	1.4	>2.9	<46	0.96	>1.9	<31

n.c.: Not calculated.

[1] EC_{50} for $Cr_2O_7^{2-}$ was not possible to calculate after 15 minutes. The requirement of one measurement above 50% inhibition was not fulfilled.

[2] EC_{50} for cyanide was only possible to calculate after 15 minutes for three out of four replicates. The requirement of one measurement above 50% inhibition was not fulfilled.

[3] EC_{50} for cyanide was only possible to calculate after 30 minutes for three out of four replicates. The requirement of one measurement above 50% inhibition was not fulfilled.

[4] The recovery of these compounds in mixed solutions was only 2-7 %. The listed EC-values are based on the added amount of compound. See details on recovery later in section 7.3.3.

7.1.3 Precision

The precision in terms of repeatability is presented in Table 7.4 and Table 7.5. The repeatability is calculated for the 6 target compounds based on the results from Test A.

Table 7.4 LUMIStox repeatability as relative standard deviation (RSD) in percent. For target compounds in 2 % NaCl MilliQ water. Number of replicates (n) is 3 and 4 for cyanide.

LUMIStox	**15 minutes**		**30 minutes**	
	EC_{20} RSD (%)	**EC_{50} RSD** (%)	**EC_{20} RSD** (%)	**EC_{50} RSD** (%)
Zn^{2+}	10	4.5	12	5.0
$Cr_2O_7^{2-}$	60	n.a.	55	29
Triclosan	13	7.4	13	5.5
Cyanide	15	18	73	24
SDS	33	29	44	33
CTAB	2.4	3.6	6.3	2.4

n.a.: Not applicable. EC_{50} could not be determined.

Table 7.5 ECLOX repeatability as relative standard deviation (RSD) in percent. For target compounds in 2 % NaCl MilliQ water. Number of replicates (n) is 3 and 4 for cyanide.

ECLOX	**15 minutes**		**30 minutes**	
	EC_{20} RSD (%)	**EC_{50} RSD** (%)	**EC_{20} RSD** (%)	**EC_{50} RSD** (%)
Zn^{2+}	9.3	2.7	14	4.9
$Cr_2O_7^{2-}$	34	n.a.	30	24
Triclosan	13	4.6	1.9	2.2
Cyanide	14	15	40	16
SDS	40	34	41	38
CTAB	11	6.3	7.2	1.2

n.a.: Not applicable. EC_{50} could not be determined.

The log-log linearity, used by the model for EC-calculation, was relatively low for cyanide, causing high relative standard deviations. More details on cyanide are found in Section 7.2.8.

The precision in terms of reproducibility is presented in Table 7.6. Reproducibility is based on the results from Test D, which was performed with Zn^{2+} as the target compound. EC_{50}-values are closely related to the activity of the bacteria, details on this are described in Section 7.2.7.

Table 7.6 LUMIStox and ECLOX reproducibility as relative standard deviation (RSD) in percent for Zn^{2+} in 2 % NaCl MilliQ water. Test was performed on three bacteria batches on three different days. Number of replicates (n) is 3, except for ECLOX, batch 02099 where 4 replicates were tested.

Zn^{2+}	**15 minutes**		**30 minutes**	
	EC_{20} RSD (%)	**EC_{50} RSD** (%)	**EC_{20} RSD** (%)	**EC_{50} RSD** (%)
LUMIStox	22	28	36	30
ECLOX	46	63	55	51

7.1.4 Agreement with accepted values

The agreement of the test result (EC_{50}) with an average accepted EC_{50}-value of substance i (A_i) has been calculated for each target compound and expressed in percentage of the accepted average value.

The LUMIStox and ECLOX EC_{50} values were obtained from Test A. The sources of accepted literature EC_{50} values obtained with the ISO 11348-3 method are listed with references in the Verification Protocol, Appendix 3 /1/. The average agreement was determined for all compounds which had literature values were it was known that the test was performed according to the ISO 11348-3.

Table 7.7 LUMIStox EC_{50} agreement with accepted values (A) in percent.

Compound	Accepted values			LUMIStox	
	EC_{50} (mg/L)	**Test time**	**According to ISO 11348-3**	**EC_{50}** (mg/L)	**A_i** (%)
Zn^{2+} ($ZnSO_4 \cdot 7H_2O$)	2.2 mg/l ± 23 %	30 min	Yes	4.1 ± 5.0 %	186
$Cr_2O_7^{2-}$ ($K_2Cr_2O_7$)	18.7 mg/L ±11 %	30 min	Yes	17 ± 29 %	91
Triclosan	0.28	15 min	Yes	0.40 ± 7.4 %	143
	0.28	30 min	Yes	0.53 ± 5.5 %	189
CTAB	0.97	30 min	Yes	0.97 ± 2.4 %	100

Table 7.8 ECLOX EC_{50} agreement with accepted values (A) in percent.

Compound	Accepted values			ECLOX	
	EC_{50} (mg/L)	**Test time**	**According to ISO 11348-3**	**EC_{50}** (mg/L)	**A_i** (%)
Zn^{2+} ($ZnSO_4 \cdot 7H_2O$)	2.2 mg/l ± 23 %	30 min	Yes	4.1 ± 4.9 %	186
$Cr_2O_7^{2-}$ ($K_2Cr_2O_7$)	18.7 mg/L ± 11 %	30 min	Yes	18 ± 24 %	96
Triclosan	0.28	15 min	Yes	0.39 ± 4.6 %	139
	0.28	30 min	Yes	0.53 ± 2.2 %	190
CTAB	0.97	30 min	Yes	0.96 ± 1.2 %	99

When evaluating the agreement with accepted values it should be taken into account that bacterial activity for some compounds affects the EC_{50}-values. It has been shown that a low bacterial sensitivity, indicated by a low inhibition by the Zn^{2+} standard, results in a higher EC_{50}. For Test A the activity of the bacteria caused an inhibition of approximately 25 % for the Zn^{2+} standard in a concentration which should equal EC_{50} according to the ISO 11348-3 method. The inhibition was therefore half of what could be expected from the EC_{50}-value, but still within the accepted range from 20-50 % inhibition which is the acceptable range in the ISO 11348-3 method. The concentration needed in Test A to obtain 50 % inhibition was, due to the low bacteria activity, a factor of two higher than the EC_{50}-value listed in the ISO 11348-3 and resulted in an agreement with accepted value ($A_{Zn^{2+}}$) of 186 %.

7.1.5 Robustness

Initial concentration, temperature, pH, color, turbidity and type of cuvettes

The robustness of the LUMIStox and ECLOX measurements was tested against differences in initial concentration, temperature, pH, color, turbidity and type of cuvettes. The robustness was calculated as the average measurement under conditions of the robustness test divided by average measurement under reference conditions, and reported as a percent.

The results of the robustness test are both EC-values (Test C) and % inhibition (all other robustness tests). The robustness under different test conditions is listed in Table 7.9 to Table 7.12. The tables specify the test name (Test C to L) as well as the target compound used in the test.

For the tests with color and turbidity (Test H and I) three different concentrations of each color or turbid $BaSO_4$-reagent were used. Pictures have been taken to show the difference in color and turbidity. They are included in Appendix 6 along with the test results.

Table 7.9 LUMIStox robustness (R) in percent. Test results are presented as EC-values. R values significantly different (95 % confidence level, two-sided t-test) from 100 % indicated in bold.

LUMIStox	**Test**	**Target compound**	**Condition**	**15 min**		**30 min**	
				EC_{20}	**EC_{50}**	**EC_{20}**	**EC_{50}**
				R (%)	**R** (%)	**R** (%)	**R** (%)
Initial concentration Ref. ~EC_{90}	C	SDS	Initial con-centration ~EC_{30}	103	n.a.	104	n.a.
			Initial con-centration ~EC_{60}	78	93	86	96

n.a.: Not applicable.

Table 7.10 LUMIStox robustness (R) in percent. Test results are presented as % inhibition. R values significantly different (95 % confidence level, two-sided t-test) from 100 % indicated in bold.

LUMIStox	**Test**	**Target compound**	**Condition**	**15 min R** (%)	**30 min R** (%)
Temperature, lab Ref.15.4°C	F	SDS	14.0°C	99	105
			16.1°C	69	71
pH Ref. 7.0	G	SDS	6.0	96	110
			8.5	101	107
Color Ref. no color	H	SDS	0.2 % dye, with c.c.	94	102
			0.2 % dye, without c.c.	98	105
			6.25 % dye, with c.c.	108	107
			6.25 % dye, without c.c.	**170**	**156**
			12.5 % dye, with c.c.	117	114
			12.5 % dye, without c.c.	**220**	**197**
Turbidity Ref. no turbidity	I	SDS	0.05 g $BaSO_4$/L, with c.c.	55	70
			0.05 g $BaSO_4$/L, without c.c.	112	106
			0.10 g $BaSO_4$/L, with c.c.	**8**	41
			0.10 g $BaSO_4$/L, without c.c.	105	97

LUMIStox	Test	Target compound	Condition	15 min R (%)	30 min R (%)
			0.20 g $BaSO_4$/L, with c.c.	**-90** [2]	**-20** [2]
			0.20 g $BaSO_4$/L, without c.c.	97	88
Cuvette material[1]	L	Zn^{2+}	Plastic	101 (99-160)	107 (106-117)
Ref. glass		SDS	Plastic	108 (93-108)	99 (90-101)

c.c.: Color correction.

[1] Test performed in triplicates (with 3 replicates in each test). Median and interval are given as result.

[2] Negative values occur when there inhibition is negative. Negative inhibition means that the solution tested gives better growth conditions for the bacteria than the control.

Table 7.11 ECLOX robustness (R) in percent. Test results are presented as EC-values. R values significantly different (95 % confidence level, two-sided t-test) from 100 % indicated in bold.

ECLOX	Test	Target compound	Condition	15 min		30 min	
				EC_{20} R (%)	EC_{50} R (%)	EC_{20} R (%)	EC_{50} R (%)
Initial concentration Ref. ~EC_{90}	C	SDS	Initial concentration ~EC_{30}	101	n.a.	125	n.a.
			Initial concentration ~EC_{60}	93	94	91	97

n.a.: Not applicable.

Table 7.12 ECLOX robustness (R) in percent. Test results are presented as % inhibition .R values significantly different (95 % confidence level, two-sided t-test) from 100 % indicated in bold. Number of replicates are 3, except for Test I on turbidity were the number of replicates are 4.

ECLOX	Test	Target compound	Condition	15 min R (%)	30 min R (%)
Temperature, field[1] Ref. 16°C	E	Zn^{2+}	5°C	**27 (11-35)**	n.a.
			23°C	**116 (108-171)**	n.a.
		SDS	5°C	100 (93-105)	n.a.
			23°C	**75 (73-75)**	n.a.
Temperature, lab Ref.15.4°C	F	SDS	14.0°C	88	100
			16.1°C	91	85
pH Ref. 7.0	G	SDS	6.0	111	113
			8.5	101	105
Color Ref. no color	H	SDS	0.2 % dye, with c.c.	124	124
			0.2 % dye, without c.c.	105	110
			6.25 % dye, with c.c.	107	112
			6.25 % dye, without c.c.	**155**	**148**

ECLOX	Test	Target compound	Condition	15 min R (%)	30 min R (%)
			12.5 % dye, with c.c.	128	115
			12.5 % dye, without c.c.	**220**	**180**
Turbidity Ref. no turbidity	I	SDS	0.05 g $BaSO_4$/L, with c.c.	**135**	111
			0.05 g $BaSO_4$/L, without c.c.	109	93
			0.10 g $BaSO_4$/L, with c.c.	**154**	130
			0.10 g $BaSO_4$/L, without c.c.	118	107
			0.20 g $BaSO_4$/L, with c.c.	115	101
			0.20 g $BaSO_4$/L, without c.c.	92	86

n.a.: Not applicable.

c.c.: Color correction.

[1] Performed at three different concentrations. Median and interval are given as result.

The results show that the use of color correction is essential when testing colored samples (Test H). With color correction robustness of 107-128 % were seen for colored samples (6.25 and 12.5 % dye), while without the color correction the robustness was significantly different from the reference and in the range from 148-220 %. For turbid $BaSO_4$ samples the use of color correction is unnecessary (Test I) (these sample were not settle or centrifuges as suggested in ISO 11348-3). Without color correction robustness of 86-118 % were seen for turbid samples, while with the color correction the robustness was significantly different from the reference and in the range from -90-154 %. However this may be different for other types of turbid samples.

The use of ECLOX under field temperatures (5°C and 23°C) gave very different results from the reference test at 16°C. The bacterial activity at 5°C was generally very low, resulting in high variation in the results. The test was performed for SDS and zinc. The robustness for the two compounds differs significantly, indicating that the robustness is compound specific.

Wastewater matrix

Adding compounds to a wastewater containing ions, particles etc. may cause some processes such as complexation and adsorbtion to proceed in the wastewater rendering the added compound less toxic (or in some cases more toxic) than the compound in 2 % NaCl MilliQ water.

The effect on difference in matrix has also been tested (Test J). Two non-toxic wastewater types, respectively an industrial and a domestic wastewater, were used as the matrix and compared to the 2 % NaCl MilliQ water used in other tests.

The baseline luminescence of the non-toxic wastewater differed slightly from the baseline of the 2 % NaCl MilliQ water, illustrated in Table 7.14. The domestic wastewater appears in this case to enhance the luminescence, which will cause negative inhibition.

Table 7.13 Wastewater luminescence baseline given as % inhibition. Number of replicates is 3.

Wastewater	LUMIStox		ECLOX	
	15 min % inhibition	**30 min** % inhibition	**15 min** % inhibition	**30 min** % inhibition
Industrial	1.2	1.5	-2.9	-3.3
Domestic	-8.1	-5.7	-6.6	-5.3

Table 7.14 and [1] Negative values occur when there inhibition is negative. Negative inhibition means that the solution tested gives better growth conditions for the bacteria than the control.

Table 7.15 show the robustness towards wastewater. The domestic wastewater is reported both with and without an adjustment to the baseline to account for the waste waters positive effect on the bacteria luminescence (negative inhibition from the wastewater, see Table 7.13).

Table 7.14 LUMIStox robustness (R) towards wastewater given in percent. R values significantly different (95 % confidence level, two-sided t-test) from 100 % indicated in bold.

LUMIStox	Target compound and concentration	Wastewater			Adjusted baseline	
			15 min Inhibition R (%)	**30 min Inhibition R** (%)	**15 min Inhibition R** (%)	**30 min Inhibition R** (%)
Matrix Ref. 2 % NaCl MilliQ water	Zn^{2+} 4.00 mg/L	Industrial	77	43		
		Domestic	**31**	84	127	**123**
	$Cr_2O_7^{2-}$ 2.80 mg/L	Industrial	31	**0**		
		Domestic	**-50** [1]	**-10** [1]	15	**22**
	Triclosan 0.60 mg/L	Industrial	114	141		
		Domestic	84	57	105	96
	SDS 0.80 mg/L	Industrial	68	**28**		
		Domestic	66	**64**	107	96
	CTAB 1.20 mg/L	Industrial	102	68		
		Domestic	75	**52**	118	78

[1] Negative values occur when there inhibition is negative. Negative inhibition means that the solution tested gives better growth conditions for the bacteria than the control.

Table 7.15 ECLOX robustness (R) towards wastewater given in percent. R values significantly different (95 % confidence level, two-sided t-test) from 100 % indicated in bold.

ECLOX	Target compound	Wastewater			Adjusted baseline	
			15 min Inhibition R (%)	**30 min Inhibition R** (%)	**15 min Inhibition R** (%)	**30 min Inhibition R** (%)
Matrix Ref. 2 % NaCl MilliQ water	Zn^{2+} 4.00 mg/L	Industrial	**56**	**22**		
		Domestic	37	85	132	**125**
	$Cr_2O_7^{2-}$ 2.80 mg/L	Industrial	**12**	**-10** [1]		
		Domestic	**-60** [1]	**-20** [1]	**14**	**13**
	Triclosan 0.60 mg/L	Industrial	116	141		
		Domestic	89	62	110	101
	SDS 0.80 mg/L	Industrial	68	**35**		
		Domestic	**71**	**67**	111	101
	CTAB 1.20 mg/L	Industrial	99	61		
		Domestic	**64**	**49**	101	**73**

[1] Negative values occur when there inhibition is negative. Negative inhibition means that the solution tested gives better growth conditions for the bacteria than the control.

Especially chromium shows a change in toxicity when added to the wastewater, but effects are also seen in some cases for zinc, SDS and CTAB.

7.2 Test performance observation

7.2.1 Color correction

Of the tested target compounds only chromium was slightly colored. For the three samples of chromium tested in Test A applying Lumistox with the color correction function in the software, only one of the three replicates changed the measured values and the change in values was very limited.

It is not possible directly to perform color correction on the ECLOX measurements. Instead an additional test with special color correction cuvettes is needed. Due to the very limited color in the test samples it has been chosen not to use color correction for any tests except for the test H and I, were color and turbid material respectively was added to the samples.

7.2.2 Requirements to reference standards and controls

The ISO 11348-3 requests test of reference standards and acceptance of control. Each delivered batch must be checked with three reference substances. This was carried out and results are shown in Table 7.22. Since the references were close to the requirement limits in the ISO one reference (Zn^{2+}) was included in all test runs, instead of as required for each reconstitution. For a few of the Zn-reference standards test results (approximately 10% for measured in the LUMIStox) of did not fulfill the ISO requirement. However, all results have been included in the evaluation, since the general check (reported in Table 7.22) was fulfilling the ISO requirements.

In some cases a low bacterial activity was reflected in the test results (see Section 7.2 for further details). Slightly higher standard deviations were observed and less significantly differences between e.g. robustness parameters.

The ISO also sets limits to the deviation between replicate control samples. Thus parallel determination may not deviate from their mean by more than 3%. HACH-LANGE has informed DHI that for the ECLOX, this can be difficult to fulfill. It does not seem to affect the test results that the controls did not fulfilled the validation criteria of the ISO and no data has been excluded for this reason.

7.2.3 Lifetime of bacteria

HACH-LANGE has specified that the lifetime of the bacteria is four hours after rehydration and the ISO 11348 criteria of validity are met at every time. In few cases older bacteria has been used as specified in the results included in Appendix 6.

The activity of the bacteria decreased over time in some of the tests (e.g. Test G on change in pH). The requirements for the Zn^{2+} reference standard could not be fulfilled at the end of the test, even though the bacteria were not more than four hours old. The opposite was also seen (e.g. Test D). Here the requirement to the Zn^{2+} reference standard was not fulfilled during the tests performed within the four hours time frame, but it was fulfilled for an additional replicate performed on bacteria older than four hours.

7.2.4 Software

There have been a few incidents were the software has caused a difficulties during the testing.

In one incident the software was operating during the test, but it did not save the data. The actual test i.e. three replicates had to be repeated another day.

The software works well for estimation of EC-values according to the ISO-standard, but when applying different set-up and dilutions others than the standards, problems sometimes arrose. As example, it was not possible to include more than one control and it was not possible to include color correction information obtained with special color correction cuvettes (ECLOX) when there were not enough samples to calculate an EC_{50}-value. The ability to make such changes would have been useful for Test H (color robustness) and Test I (turbidity robustness), where only one test concentration was used.

7.2.5 Target compounds

The original plan was to include 9 target compounds. Three compounds had to be excluded from the test: flutriafol, nonyl phenol ethoxylate (NPE) and copper.

The pesticide flutriafol and the detergent NPE had limited solubility and were not toxic in a saturated solution. That made them not useable in the test system.

Copper, added as $CuSO_4 \cdot 5H_2O$, also had low toxicity in the highest soluable concentration. The chemistry of copper, and thereby also the toxicity, is easily changed in the chosen test system. On one occasion the pH adjustment from a initial pH 6.1 to pH ~7.0 failed. The pH went from 9.3 to 5.2 during the adjustment. The final pH was 6.8. This made the sample very toxic compared to the other replicates. Therefore, copper was not useable in the test system

CTAB was difficult to dissolve and was only used in a limited number of tests. Triclosan had to first be dissolved in ethanol and thereafter in water. Ethanol concentration in the artificial sample and the control was 100 µL/L, and found not to be toxic.

7.2.6 Bacteria activity and quantities of bacteria batches

The activity of the batch 02099 did not fulfill the requirements of the ISO standard for % inhibition on Zn^{2+} and $Cr_2O_7^{2-}$. The batch was therefore only used in a single test (Test D on reproducibility).

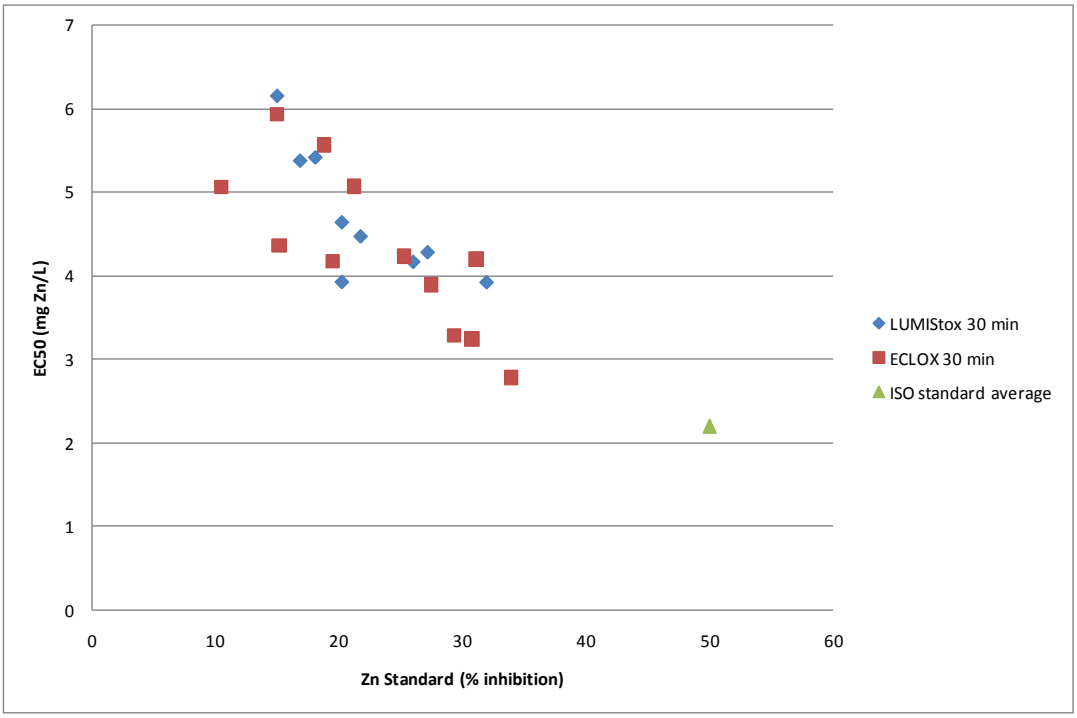
Other batches were not immediately available from the vendor. For the same reason the number of tested batches in Test D on reproducibility was three instead of four as originally described in the Test Plan /14/.

7.2.7 Bacteria activity and relation to EC-values

According to the ISO 11348-3, a reference standard must be tested for each reconstitution of a bacteria batch to ensure an acceptable sensitivity of the bacteria. This needs to be done only once for each reconstitution; however, for this evaluation the reference standard was included in each test measurement. A 2.2 mg/l Zn^{2+} reference was used, which must produce an inhibition between 20% and 80%. This requirement was in general met for each reconstitution; however, there was variability in the results of multiple analyses, including some which were below the 20% threshold. The meas-

ured EC_{50} appeared to be correlated with the result of the reference standard. This has been analyzed in Figure 7.1, which shows the relationship for the target compound zinc and the Zn^{2+} reference. It is seen that a low inhibition by the Zn^{2+} reference indicates a low sensitivity of the bacteria and consequently gives a higher EC_{50}.

Figure 7.1 *Relation between EC_{50}-values for zinc and the activity of the bacteria expressed as % inhibition of the Zn^{2+} reference standard of 2.2 mg/L. Data are from Test D, performed on batches 10129, 11169 and 02099 as well as the average from the ISO-standard.*



For chromium, which also is included as a reference standard in the ISO method but was not the choice for continuing evaluation of reconstituted bacteria batches for this evaluation, the results for testing are summarized in Table 7.16. The activity of the used bacteria batch from its initial evaluation with chromium as one of the three reference standards is a factor of 1.2 higher than the average in the ISO, while the EC_{50}-values are more or less identical. Since a factor of only 1.2 is not sufficient to state whether there is a difference in activity, it is not possible to determine, whether EC_{50}-values for chromium are related to bacteria activity or not.

Table 7.16 *Relation between EC_{50}-values for chromium and the activity of the bacteria expressed as % inhibition of the Cr^{6+} reference standard of 18.7 mg/L. Data are from Test A, performed on batch 10129 and the ISO-standard.*

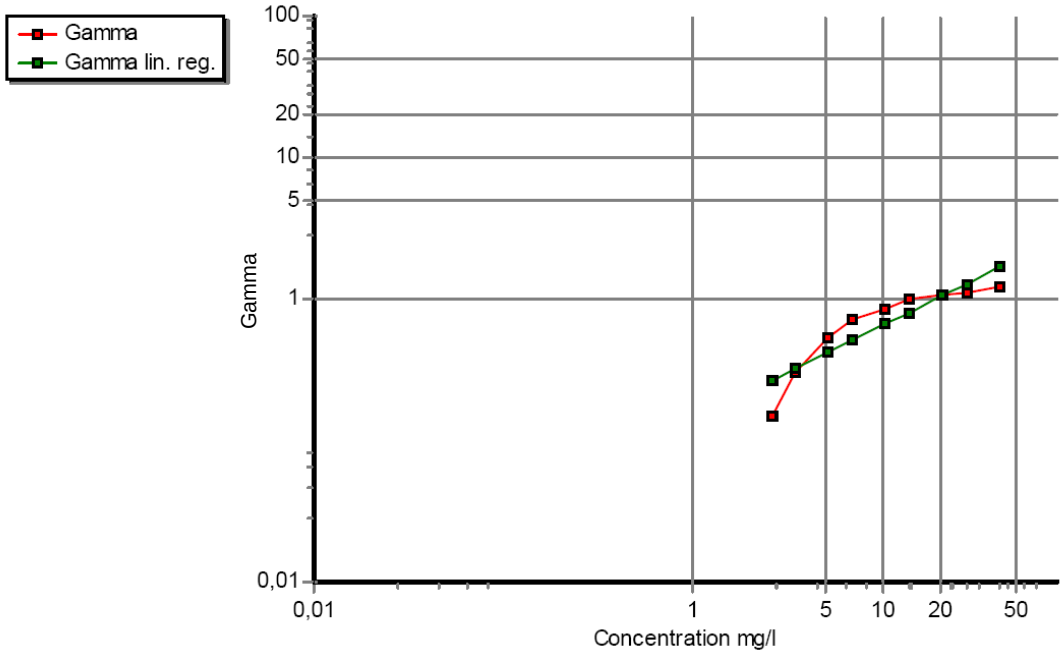
	EC_{50} 30 min mg/L	**Inhibition of Cr^{6+} reference standard** %
ISO standard	18.7 ±11%	50
LUMIStox	17	60 ± 0.12
ECLOX	18	

The examples of zinc and chromium show that the bacterial activity and its impact on toxicity measurements is compound-related. This relationship cannot be determined for the other compounds used in this evaluation without more intensive testing.

7.2.8 *Log-log linearity*

The model used for calculation of EC-values is based on log-log linearity between inhibition expressed as Gamma (see equation 5 in ISO 11348-3) and the concentration. Gamma is 1 at 50 % inhibition. The measurements generally matched the log-log linearity model, but for cyanide this model was often seen not to match. An example is shown in Figure 7.2. In the shown example the model (Gamma lin. reg.) does not fit the measurements (Gamma) at any concentration. The EC_{50} is estimated by the model to be 19 mg/L, while the measurements indicate the EC_{50} should be 14 mg/L.

Figure 7.2 Cyanide log-log curve. Test results from Test A on ECLOX, replicate 4 after 15 minutes incubation time.



7.3 *Test quality assurance summary*

7.3.1 *Reference analysis performance data*

Control data for the reference analysis obtained from Eurofins are summarized in Table 7.17.

Table 7.17 Performance parameters for reference analysis control data.

Target compound	Limit of detection µg/L	Precision (RSD) %	Trueness %
Zn^{2+}	0.50	15	98-99
$Cr_2O_7^{2-}$	0.50	15	103
Triclosan	0.10	Not specified	103
Cyanide (CN^-)	1	10	99
SDS (anionic surfactants[1])	25	15	101
CTAB (cationic surfactants[2])	100	20	95

[1] Reference compound is SDS.

[2] Reference compound is Benzyl-dimethyltetra ammoniumchlorid dihydrat, molar weight 404,00 g/mol.

Eurofins has participated in proficiency tests for most of the tested compounds. The results of the latest proficiency tests are shown in Table 7.18.

Table 7.18 Results of Eurofins proficiency tests.

Parameter	Nominal value	Zeta-score	Supplier
Zinc	614 µg/L	0.316	APG, November 2009 WS, 1. round
Chromium	83.1 µg/L	0.157	FAPAS (LEAP), Waste Water, G20+G21
Triclosan	Eurofins has not participated in proficiency test, since triclosan is a new parameter for them and is not covered by their accreditation		
Cyanide	7.00-11.27 µg/L	0.377	KIWA, drinking water, 09-03
Anionic surfactants	50.0-119.6 µg/L	-0.464	KIWA, drinking water, 09-03
Cationic surfactants	Eurofins is not aware of supplier of proficiency test for cationic surfactants within the measuring area		

7.3.2 Reference test performance data

ALcontrol uses zinc sulfate and phenol as reference standards. Results of the measurements from the day of testing samples from this project are within the specification of the bacteria supplier, though the control chart for zinc shows that over the period the references have been at a low level, around 70% of the expected average.

ALcontrol participates in an annual proficiency test with the Microtox. The results were audited by Battelle as part of the technical systems audit (TSA) at ALcontrol and found to be within the acceptance criteria.

7.3.3 Test system control data

Blank samples

The 2% NaCl MilliQ water used to prepare stock solutions of test compounds was analyzed for background levels of these target compounds. The results are shown in Table 7.19. These results also served as a test of the detections limits of the Eurofins laboratory.

The results showed that the 2% NaCl MilliQ water did not contain any of the target compounds in significant concentrations.

Table 7.19 Concentrations of target compounds in 2% NaCl MilliQ water (blank) samples.

Target compound	Concentration µg/L	
	Replicate 1	Replicate 2
Zn^{2+}	<0.50	<0.50
$Cr_2O_7^{2-}$	0.5	0.6
Triclosan	<0.10	0.19
Cyanide (CN^-)	<1	<1
SDS (anionic surfactants[1])	<25	<25
CTAB (cationic surfactants[2])	<100	<100

[1] Reference compound is SDS.

[2] Reference compound is Benzyl-dimethyltetra ammoniumchlorid dihydrat, molar weight 404,00 g/mol.

The 2% NaCl MilliQ water was tested for toxicity at ALcontrol.

The results are shown in Table 7.20.

Table 7.20 Toxicity in percentage of sample volume of 2% NaCl MilliQ water (blank) samples.

Time	EC-value	Concentration %
5 minutes	EC_{20}	78
	EC_{50}	>82
15 minutes	EC_{20}	>82
	EC_{50}	>82
30 minutes	EC_{20}	>82
	EC_{50}	>82

The results showed no significant toxicity of the 2% NaCl MilliQ water.

Control, stock solutions

The concentrations and the stability of the stock solutions were evaluated by sending subsamples of those solutions to Eurofins laboratory. Table 7.21 shows the results of the analysis and the recovery of the concentrations in the stock solutions.

The surfactants SDS and CTAB were expected to adhere to the cuvettes. In addition CTAB was difficult to dissolve. The stock solutions were therefore treated as the test samples (added to cuvettes and left for 30 minutes) before sending to Eurofins, details can be found in Appendix 4, section 11.1.3.

Table 7.21 Concentrations (average and relevant range (high/low value divided by average)) of target compounds in spiked 2 % NaCl MilliQ water stock solutions.

Target compound	Measured concentration		Prepared concentration	Recovery
	Average µg/L	Relevant range %	µg/L	%
Zn^{2+}	17,500	± 5.7	22,000	80
$Cr_2O_7^{2-}$	52,000	± 7.7	56,100	93
Triclosan	355	± 2.8	1,600	22
Cyanide (CN^-)	31,500	± 9.5	32,885	96
SDS (anionic surfactants[1])	2,550	± 12	35,950	7.1
CTAB (cationic surfactants[2])	725	± 32		
CTAB[3]	560	± 32	30,000	1.9

[1] Reference compound is SDS.

[2] Reference compound is Benzyl-dimethyltetra ammoniumchlorid dihydrat ($(C_6H_5CH_2)(CH_3)_2N(C_{12}{}^-C_{14}Alkyl)^+Cl^-$), molar weight 404.00 g/mol. Molar weight of cation 368.5 g/mol.

[3] Concentration of CTAB, molar weight 364.45 g/mol has been calculated based on CTAB (cationic surfactants) results. Molar weight of cation 284.5 g/mol.

SDS and CTAB were lost as expected during the testing. The triclosan stock solution also showed a significant loss, with a recovery of only 22 %. These losses were considered when evaluating the EC-values, and a note on this was included in Table 7.2 and Table 7.3. When compared to other EC-values (accepted values) it should be kept in mind, that facilities performing those tests may not have checked the compound recovery, especially not in a set-up as done here for SDS and CTAB.

A determination of the concentration of cyanide in the dilutions was performed with a test kit. An artificial cyanide sample was carried through the test procedure. Instead of adding bacteria solution, 2 % NaCl was added. No measurements of luminescence were performed. Instead the cyanide concentration was measured using a HACH-LANGE test kit (LCK 315). Test row B was analyzed at time 0 and test row C was analyzed after 30 minutes. The results showed that the recovery of cyanide after 30 minutes was from 80-120 % compared to the concentrations at 0 minutes. It was decided that if the loss of cyanide exceeded 20 % the results of the cyanide test would not be included in the verification. Since there was not a loss of more than 20 %, cyanide was included in the test program.

Reference tests according to ISO 11348-3

The bacterial batches used in the tests were tested for compliance with the requirements in the ISO 11348-3, Section 11. For all reference standards the criteria is 20-80 % inhibition. The results of the reference tests are shown in Table 7.22.

Table 7.22 Reference tests of bacteria batches performed in accordance with ISO 11348-3. Tests are performed on LUMIStox, number of replicates are two except for Batch 02099 with only one test.

Batch	**Zn^{2+} (2.2 mg/L)** %	**$Cr_2O_7^{2-}$ (18.7 mg/L)** %	**3,5-dichlorophenol (3.4 mg/L)** %
10129	22 ± 0.028	60 ± 0.12	20 ± 2.3
11169	36 ± 0.90	53 ± 1.4	28 ± 0.62
02099	15	96	39
ISO requirement	20-80		

For the batches 10129 and 11169 both Zn^{2+} and especially 3,5-dichlorophenol were close to the lower limit of 20 %, one tested sample was even below 20 % for 3,5-dichlorophenol. It was therefore decided to include the reference standard Zn^{2+} in all tests, to be able to follow the bacteria activity.

The bacteria batch 02099 was only used in one test (Test D), the results for this bacterial batch did not fulfill the requirements of performing replicate tests and meeting the % inhibition requirement of the ISO standard.

Wastewater

Various chemical wastewater parameters were analyzed. The results are shown in Table 7.23.

Table 7.23 Results of analytical parameters analyzed in wastewater.

Parameters	**Unit**	**Industrial wastewater**	**Domestic wastewater**
Turbidity	FTU	15	2.4
TOC	mg/L	39	10
Conductivity	mS/m	4300	140
Alkalinity	mmol/L	6.9	5.5
pH	-	7.7	7.5
COD	mg/L	110	28
Suspended solids (SS)	mg/L	83	4.9
Nitrogen (total)	mg/L	6.3	6.9

Parameters	Unit	Industrial wastewater	Domestic wastewater
Phosphorus (total)	mg/L	4.2	0.23
BOD_5	mg/L	3.4	5.2

The two types of wastewater mainly differs in conductivity and content of organic material (TOC, COD and SS).

7.4 Amendments to and deviations from test plan

There has been no amendment to the test plan.

There have been 10 deviations to the test plan, all deviations have been approved. The test report reflects these deviations. A list of the deviations is included in Appendix 7.

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 U.S. 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 it 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 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 were provided with the instrument.

A cyanide test kit, HACH-LANGE LCK 315, was 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) (see section 9.3.2).

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 (10.8.1) in all small wells (A1 to C10) and add 1 mL of sodium chloride solution (10.1). Insert reaction vials (10.8.3) in the two large right hand side wells and add 5 mL of sodium chloride solution (10.1). 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} + ΔT_{max} and T_{median} - ΔT_{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 (10.3) 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 (9.4.2) to 50 volumes of the solution for freeze-dried bacteria (10.6) 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 (10.1) 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 (10.5) or NaOH (10.4). 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.

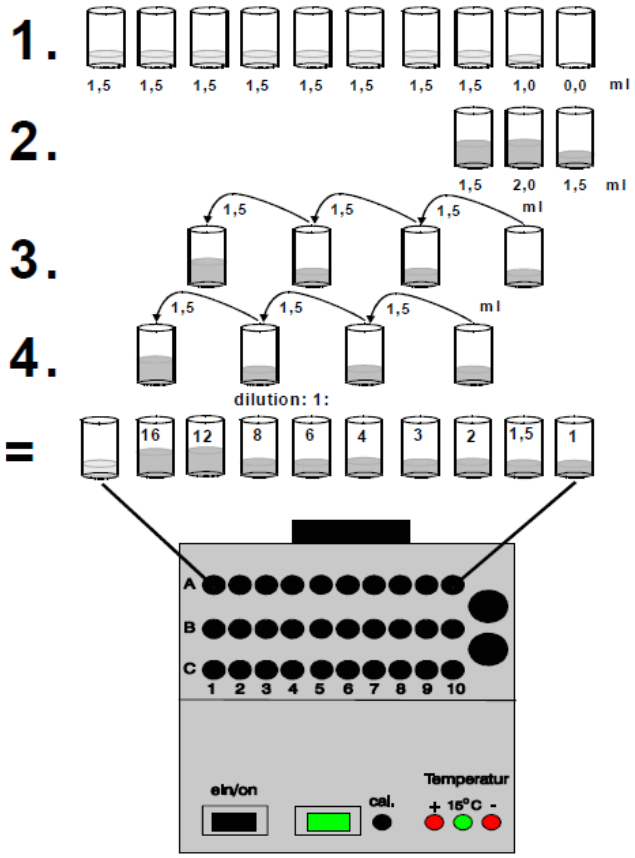


Figure 9.1: Principles of preparation of dilution rows from /3/.

1. Insert 10 vials into row A and pipet LCK 481 sodium chloride solution (10.2) 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 (9.6) or samples as described in (9.5). Prepare the test suspension as described in (9.4.3).

Use plastic measuring tubes (10.8.1) except in test series L, where both plastic tubes and glass tubes (10.8.2) will be used.

1. Insert the appropriate number of plastic measuring tubes (10.8.1) in rows B and C.
2. Pipette 0.5 mL bacteria test suspension (9.4.3) 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 (10.1) .

10.7.2 3,5 – dichlorophenol

6.8 mg/L 3,5 – dichlorophenol (Purity > 99%) in 2 % sodium chloride solution (10.1) .

10.7.3 Potasium dichromate

105.8 mg/L $K_2Cr_2O_7$ in 2 % sodium chloride solution (10.1).

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 (9.4.3) and 0.5 mL of sodium chloride solution (10.2).

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 (10.1) 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 (10.6) 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 (10.6) in each of 10 plastic tubes (10.8.1) 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 (10.2) 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 (10.1) and 0.5 mL test suspension (9.4.3) 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 7-24.

Table 7-24: 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, two in a climate rooms at respective approximately 5 and 15 °C.

The test setup is illustrated in Table 7-25. The concentration of the test compound in the test sample shall be twice the EC_{50}.

Table 7-25: Test setup for series E			
Tube	Test suspension (9.4.3) (mL)	2% NaCl (10.1) (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 9.3.1. 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 (10.5) or NaOH (10.4) 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 (10.1) 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 (10.1) 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 (10.1) and the dye solution described in section 11.8.2.1 will be added to achieve the dye concentrations determined in the screening test in section 11.8.2.1.

11.8.2.2.2 Preparation of dye control samples

The dye control samples will be made as the test samples (11.8.2.2.1) except that the stock solution will be left out and replaced by sodium chloride solution (10.1).

11.8.2.2.3 Preparation of test compound control samples

The test compound control samples will be made as the test samples (11.8.2.2.1) except that the dye solution will be left out and replaced by sodium chloride solution (10.1).

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 (9.4.3) and 0.5 mL of sodium chloride solution (10.2), a dye control (11.8.2.2.2) and a test compound control (11.8.2.2.3). 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 (11.8.2.2).

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 (10.1) 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 11.2 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 (10.1) 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 (10.1) and a 1 g/L $BaSO_4$ in sodium chloride solution (10.1) 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 (11.9.3.1.1) except that the stock solution will be left out and replaced by sodium chloride solution (10.1).

11.9.3.1.3 Preparation of test compound control samples

The test compound control samples will be made as the test samples (11.9.3.1.1) except that the $BaSO_4$ suspension will be left out and replaced by sodium chloride solution (10.1).

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 (9.4.3) and 0.5 mL of sodium chloride solution (10.1), a turbidity control (11.9.3.1.2) and a test compound control (11.9.3.1.3) . 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 (11.9.3.1).

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 011.11.2.

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 9.5.

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

For each compound a stock solution in sodium chloride solution (010.1) 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 (10.1) 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 (10.1) 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 (9.4.3) and 0.5 mL of sodium chloride solution (10.1), a test compound control (11.10.3.2) and a waste water control (11.10.3.3). 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 7-26 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 9.5.

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 (10.8.2) with samples and 3 corresponding blanks (10.1) in glass test tubes and in 3 plastic test tubes (10.8.1) with samples and 3 corresponding blanks (10.1) 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 7-27. It is anticipated that approximately half of the time requires two technicians.

Table 7-27: 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

Test data report

Test A **15 min testtid**

LUMIStox

Compound	Replicate	Control 15 min valid	Remarks	Batch No.	Without color correction EC20 (mg/L)	Without color correction EC50 (mg/L)	color correction	With color correction EC20 (mg/L)	With color correction EC50 (mg/L)	Standard **ZnSO4,7H2O** 9.67 mg/L
1	1	3.33		10129	3.614	8.837	N			27.12
ZnSO4,7H2O	2	2.33			3.686	8.581	N			25.95
as Zn	3	2.85			3.088	8.189	N			20.17
2	1	1.57		10129	0.003037	n.c	Y	n.c	n.c	31.90
CuSO4,5H2O	2	0.80			n.c	n.c	Y	n.c	n.c	29.06
as Cu	3	0.38			n.c	n.c	Y	n.c	n.c	-
3	1	1.47		11169	0.33	1.02	N			28.70
SDS	2	2.40			0.46	1.437	N			27.64
	3	2.61			0.59	1.69	N			30.20
4	1	3.44		10129	0.16	0.37	N			22.43
Triclosan [4]	2	1.61			0.20	0.40	N			24.16
	3	1.01			0.20	0.42	N			20.91
5	1	0.58		10129	4.413	n.c	Y	4.413	n.c	45.88
K2Cr2O7	2	0.43			1.374	n.c	Y	1.288	n.c	38.36
as Cr	3	0.34			5.074	n.c	Y	4.459	n.c	40.86
6	1	2.31		10129	0.73	1.308	N			28.98
CTAB	2	0.71			0.76	1.327	N			28.74
	3	1.35			0.75	1.389	N			28.75
7	1	2.60	[5]	10129	68.532	n.c	N			45.91
Flutriafol	2	3.03	normal procedure		n.c	n.c	N			23.87
	3									
8	1	1.89		10129	2.546	n.c	N			29.51
KCN	2	5.46			3.003	n.c	N			31.96
	3	0.29	[6]		2.546	28.504	N			38.14
	4	0.77	[6]		2.205	19.54	N			38.64

ECLOX

Compound	Replicate	Control 15 min valid	Remarks	Batch No.	Without color correction EC20 (mg/L)	Without color correction EC50 (mg/L)	Standard **ZnSO4,7H2O** 9.67 mg/L
1	1	2.35		10129	3.284	8.555	19.50
ZnSO4,7H2O	2	2.05			3.832	8.537	27.45
as Zn	3	1.91			3.297	8.177	25.21
2	1	0.45		10129	n.c	n.c	26.06
CuSO4,5H2O	2	0.34			n.c	n.c	22.64
as Cu	3	1.85			n.c	n.c	-
	4	3.68	[1]		0.4	1.887	28.98
	5	3.46	[2]		n.c	n.c	26.07
3	1	0.52	[3]	11169	0.33	1.16	28.45
SDS	2	0.25			0.40	1.086	32.38
	3	2.53			0.64	1.888	29.93
4	1	0.74		10129	0.16	0.37	26.81
Triclosan [4]	2	2.34			0.19	0.39	20.98
	3	1.40			0.20	0.40	24.04
5	1	0.34		10129	3.90	n.c	53.77
K2Cr2O7	2	0.29			2.194	n.c	39.31
as Cr	3	0.29			2.776	n.c	41.03
6	1	2.53		10129	0.91	1.505	28.77
CTAB	2	2.18			0.81	1.418	25.38
	3	3.95			0.76	1.354	27.21
7	1	0.83	[5]	10129	49.216	n.c	36.54
Flutriafol	2	1.9	normal procedure		n.c	n.c	26.82
	3						
8	1	0.27		10129	2.88	26.201	29.91
KCN	2	2.26			2.94	n.c	28.28
	3	4.28	[6]		2.19	23.07	30.81
	4	0.21	[6]		2.403	18.973	39.09

[1] pH adjustment from 6.1 failed and the pH was from 9.3 to 5.2 during the adjustment. The final pH was 6.8.

[2] The sample was kept frozen from 20.01. to 22.01. where is was defrosted and tested

[3] Manual entry of the measured values

[4] Triclosan dissolved in ethanol. Ethanol concentration in the artificial sample and the control was 100 µL/L.
The control with ethanol was used in the calculation of the EC values. The control in block B without ethanol, see the standard window.

[5] The test mixture contains 80% sample and 20% test suspension.

[6] Bacteria more than 4 hours old.

Test A **30 min testtid**

LUMIStox

Compound	Replicate	Control 30 min valid	Remarks	Batch No.	Without color correction EC20 (mg/L)	Without color correction EC50 (mg/L)	color correction	With color correction EC20 (mg/L)	With color correction EC50 (mg/L)	Standard **ZnSO4,7H2O** 9.67 mg/L
1	1	1.18		10129	2.09	4.28	N			27.12
ZnSO4,7H2O	2	1.11			1.98	4.17	N			25.95
as Zn	3	2.34			1.70	3.93	N			20.17
2	1	0.12		10129	0.56	n.c	Y	0.56	n.c	31.90
CuSO4,5H2O	2	0.86			0.72	n.c	Y	0.72	n.c	29.06
as Cu	3	2.73			0.67	n.c	Y	0.67	n.c	-
3	1	1.15		11169	0.24	0.72	N			28.70
SDS	2	1.35			0.33	1.00	N			27.64
	3	2.03			0.51	1.28	N			30.20
4	1	1.24		10129	0.26	0.51	N			22.43
Triclosan [4]	2	0.02			0.32	0.53	N			24.16
	3	0.52			0.27	0.56	N			20.91
5	1	0.21	[6], [7]	10129	1.413	20.73	Y	1.413	20.73	45.88
K2Cr2O7	2	1.70	[7]		0.45	12.1	Y	0.56	13.3	38.36
as Cr	3	1.41			1.49	19.2	Y	1.49	19.2	40.86
6	1	2.31		10129	0.56	0.99	N			28.98
CTAB	2	2.29			0.53	0.95	N			28.74
	3	2.11			0.59	0.96	N			28.75
7	1	3.50	[5]	10129	n.c	n.c	N			45.91
Flutriafol	2	2.75	normal procedure		n.c	n.c	N			23.87
	3									
8	1	6.07		10129	0.37	27.7	N			29.51
KCN	2	5.47			0.75	28.1	N			31.96
	3	0.80			1.60	25.1	N			38.14
	4	2.61			0.56	16.0	N			38.64

ECLOX

Compound	Replicate	Control 30 min valid	Remarks	Batch No.	Without color correction EC20 (mg/L)	Without color correction EC50 (mg/L)	Standard **ZnSO4,7H2O** 9.67 mg/L
1	1	2.41		10129	1.67	4.17	19.50
ZnSO4,7H2O	2	1.88			2.11	3.89	27.45
as Zn	3	0.55			1.89	4.23	25.21
2	1	0.45		10129	0.87	n.c	26.06
CuSO4,5H2O	2	0.89			1.14	n.c	22.64
as Cu	3	3.98			0.98	n.c	-
	4	2.06	[1]		0.24	0.52	28.98
	5	4.14	[2]		n.c	n.c	26.07
3	1	2.70	[3]	11169	0.26	0.84	28.45
SDS	2	2.38			0.29	0.75	32.38
	3	1.94			0.50	1.38	29.93
4	1	0.69		10129	0.31	0.52	26.81
Triclosan [4]	2	3.39			0.31	0.54	20.98
	3	3.13			0.30	0.52	24.04
5	1	1.29	[6], [7]	10129	1.098	13.711	53.77
K2Cr2O7	2	1.39	[7]		0.89	21.1	39.31
as Cr	3	1.05	[7]		0.65	20.1	41.03
6	1	2.86		10129	0.56	0.97	28.77
CTAB	2	0.05			0.55	0.96	25.38
	3	3.20			0.62	0.95	27.21
7	1	4.28	[5]	10129	n.c	n.c	36.54
Flutriafol	2	0.08	normal procedure		n.c	n.c	26.82
	3						
8	1	0.51		10129	0.94	20.1	29.91
KCN	2	1.15			0.66	n.c	28.28
	3	3.89			0.65	18.6	30.81
	4	2.23			0.39	14.4	39.09

[1] pH adjustment from 6.1 failed and the pH was from 9.3 to 5.2 during the adjustment. The final pH was 6.8.

[2] The sample was kept frozen from 20.01. to 22.01. where is was defrosted and tested

[3] Manual entry of the measured values

[4] Triclosan dissolved in ethanol. Ethanol concentration in the artificial sample and the control was 100 µL/L.
The control with ethanol was used in the calculation of the EC values. The control in block B without ethanol, see the standard window.

[5] The test mixture contains 80% sample and 20% test suspension.

[6] The control values gained with K2Cr2O7 was replaced with the values from testing the standard as one of the control tubes was lost during the test.

[7] No measurement below 20 % inhibition. Affects determination of EC20

Test B

Batch No.	10129

Test time:	15 minutes

LUMIStox

No.	Mesurement	% inhibition
1		0.48
2		-0.30
3		3.88
4		1.34
5		1.11
6		6.36
7		9.71
8		7.02
9		4.80

ECLOX

No.	Mesurement	% inhibition
1		-3.40
2		6.62
3		5.49
4		4.52
5		9.20
6		6.30
7		5.69
8		9.85
9		7.00

Test B

Batch No.	10129

Test time:	30 minutes

LUMIStox

No.	Mesurement	% inhibition
1		1.80
2		1.70
3		5.21
4		0.39
5		3.40
6		3.88
7		9.51
8		7.90
9		5.25

ECLOX

No.	Mesurement	% inhibition
1		-2.50
2		5.51
3		4.68
4		2.94
5		6.98
6		3.46
7		2.79
8		6.71
9		4.30

Standards for BL100204-67 and BE100204-68 are both valid
Control for BL100204-67 is 3.35 after 15 minutes, but is valid after 30 minutes,
control is valid for BE100204-68

Test C

Test time: 15 minutes

LUMIStox

Start concentration	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Validity check Standard	Validity check Control
~EC 60*2	1	10129	0.32	1.039		2.63
Actual start conc:	2		0.31	1.113		1.68
3,4 mgSDS/L	3		0.44	1.678		0.96
~EC 30*2	1	10129	0.35			1.95
Actual start conc:	2		0.36			1.27
1,2 mg SDS/L	3		0.71		*17.55*	2.29

Replicate no 3, bacteria batch more than 4 hours old

ECLOX

Start concentration	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Standard	Control
~EC 60*2	1	10129	0.37	1.154		0.37
Actual start conc:	2		0.35	1.036		1.58
3,4 mgSDS/L	3		0.55	1.741		2.31
~EC 30*2	1	10129	0.38			0.54
Actual start conc:	2		0.37			4.57
1,2 mg SDS/L	3		0.63		*18.25*	1.19

Replicate no 3, bacteria batch more than 4 hours old

Test C

Test time: 30 minutes

LUMIStox

Start concentration	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Validity check Standard	Validity check Control
~EC 60*2	1	10129	0.25	0.77	25.44	2.44
Actual start conc:	2		0.26	0.83	23.66	0.69
3,4 mgSDS/L	3		0.42	1.28	27.03	0.24
~EC 30*2	1	10129	0.30		26.62	1.56
Actual start conc:	2		0.27		20.52	1.26
1,2 mg SDS/L	3		0.55		17.55	4.36

Replicate no 3, bacteria batch more than 4 hours old

ECLOX

Start concentration	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Standard	Control
~EC 60*2	1	10129	0.24	0.84	27.25	4.39
Actual start conc:	2		0.32	0.80	23.87	2.08
3,4 mgSDS/L	3		0.40	1.23	28.51	2.48
~EC 30*2	1	10129	0.31		28.91	0.25
Actual start conc:	2		0.30		20.65	3.89
1,2 mg SDS/L	3		0.70		18.25	2.30

Replicate no 3, bacteria batch more than 4 hours old

Test D

Test time:	15 minutes
Test compound:	ZnSO4,7H2O as Zn

LUMIStox

Day No.	Date	Replicates	Batch no.	EC20 (mg/L)	EC50 (mg/L)	Validity check	
						Standard	Control
1	19-01-2010 (Test A)	1	10219	3.614	8.837		3.33
		2		3.686	8.581		2.33
		3		3.088	8.189		2.85
2	18-02-2010	1	11169	3.774	8.751		6.81
		2		3.351	8.882		1.52
		3		3.554	7.762		0.69
3	09-03-2010	1	02099	3.373	9.509	*16.79*	3.00
		2		5.283	11.609	*14.93*	1.2
		3		4.367	12.282	*18.02*	5.63

ECLOX

Day No.	Date	Replicates	Batch no.	EC20 (mg/L)	EC50 (mg/L)	Validity check	
						Standard	Control
1	19-01-2010 (Test A)	1	10219	3.284	8.555	*19.5*	2.35
		2		3.832	8.537		2.05
		3		3.297	8.177		1.91
2	18-02-2010	1	11169	3.839	8.096	*15.12*	0.28
		2		Data not collected, error in PC			
		3		3.093	7.612		0.22
3	03-03-2010	1	11169	1.952	5.441		6.94
		2		2.862	6.80		0.11
		3		1.571	5.05		0.55
4		1	02099	3.191	10.234	*18.79*	6.02
		2		5.02	11.858	*14.95*	2.59
		3		2.324	14.344	*10.44*	4.15
		4		3.501	9.966		2.81

Replicate no. 4 at day 4, bacteria batch more than 4 hours old

Test D

Test time:	30 minutes
Test compound:	ZnSO4,7H2O as Zn

LUMIStox

Day No.	Date	Replicates	Batch no.	EC20 (mg/L)	EC50 (mg/L)	Validity check Standard	Validity check Control
1	19-01-2010 (Test A)	1	10129	2.093	4.283	27.12	1.18
		2		1.984	4.169	25.95	1.11
		3		1.698	3.928	20.17	2.34
2	18-02-2010	1	11169	2.135	4.473	21.69	3.76
		2		2.126	4.642	20.17	0.90
		3		1.864	3.924	31.90	0.21
3	09-03-2010	1	02099	2.369	5.378	16.79	0.07
		2		3.321	6.153	14.93	1.46
		3		2.635	5.417	18.02	4.13

ECLOX

Day No.	Date	Replicates	Batch no.	EC20 (mg/L)	EC50 (mg/L)	Validity check Standard	Validity check Control
1	19-01-2010 (Test A)	1	10219	1.668	4.169	19.5	2.41
		2		2.112	3.892	27.45	1.88
		3		1.89	4.234	25.21	0.55
2	18-02-2010	1	11169	2.43	4.367	15.12	1.22
		2		Data not collected, error in PC			
		3		1.915	4.201	31.08	1.94
3	03-03-2010	1	11169	1.445	3.244	30.73	6.37
		2		1.54	3.282	29.29	3.07
		3		1.206	2.785	33.95	2.31
4		1	02099	2.499	5.569	18.79	5.45
		2		3.017	5.932	14.95	3.75
		3		2.274	5.06	10.44	5.36
		4		2.358	5.073	21.21	1.14

Replicate no. 4 at day 4, bacteria batch more than 4 hours old

Test E

Test time 15 minutes
Temperature 23° C

ECLOX

Compound 1: ZnSO4, 7H2O (as Zn) Batch No.: 10129

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	83	3.4 mg/L	20%
3	0.2	0.3	0.5	96	8.5 mg/L	50%
4	0.2	no	0.8	99	13.6 mg/L	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	82	3.4 mg/L	20%
3	0.2	0.3	0.5	95	8.5 mg/L	50%
4	0.2	no	0.8	97	13.6 mg/L	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	85	3.4 mg/L	20%
3	0.2	0.3	0.5	96	8.5 mg/L	50%
4	0.2	no	0.8	99	13.6 mg/L	80%

Compound 2: SDS Batch No.: 10129

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	24	0.56 mg/L	20%
3	0.2	0.3	0.5	47	1.4 mg/L	50%
4	0.2	no	0.8	58	2.24 mg/L	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	24	0.56 mg/L	20%
3	0.2	0.3	0.5	44	1.4 mg/L	50%
4	0.2	no	0.8	54	2.24 mg/L	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	23	0.56 mg/L	20%
3	0.2	0.3	0.5	44	1.4 mg/L	50%
4	0.2	no	0.8	53	2.24 mg/L	80%

Test E

Test time 15 minutes
Temperature 16° C

ECLOX

Compound 1: ZnSO4, 7H2O (as Zn) Batch No.: 10129

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	49	3.4 mg/L	20%
3	0.2	0.3	0.5	82	8.5 mg/L	50%
4	0.2	no	0.8	90	13.6 mg/L	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	47	3.4 mg/L	20%
3	0.2	0.3	0.5	82	8.5 mg/L	50%
4	0.2	no	0.8	91	13.6 mg/L	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	50	3.4 mg/L	20%
3	0.2	0.3	0.5	84	8.5 mg/L	50%
4	0.2	no	0.8	92	13.6 mg/L	80%

Compound 2: SDS Batch No.: 10129

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	34	0.56 mg/L	20%
3	0.2	0.3	0.5	60	1.4 mg/L	50%
4	0.2	no	0.8	73	2.24 mg/L	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	31	0.56 mg/L	20%
3	0.2	0.3	0.5	62	1.4 mg/L	50%
4	0.2	no	0.8	75	2.24 mg/L	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	32	0.56 mg/L	20%
3	0.2	0.3	0.5	59	1.4 mg/L	50%
4	0.2	no	0.8	72	2.24 mg/L	80%

Test E

Test time 15 minutes
Temperature 5° C

ECLOX

Compound 1: ZnSO4, 7H2O (as Zn) Batch No.: 10129

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	11	3.4 mg/L	20%
3	0.2	0.3	0.5	26	8.5 mg/L	50%
4	0.2	no	0.8	34	13.6 mg/L	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	-14	3.4 mg/L	20%
3	0.2	0.3	0.5	8	8.5 mg/L	50%
4	0.2	no	0.8	15	13.6 mg/L	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	19	3.4 mg/L	20%
3	0.2	0.3	0.5	32	8.5 mg/L	50%
4	0.2	no	0.8	46	13.6 mg/L	80%

Compound 2: SDS Batch No.: 10129

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	44	0.56 mg/L	20%
3	0.2	0.3	0.5	70	1.4 mg/L	50%
4	0.2	no	0.8	77	2.24 mg/L	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	42	0.56 mg/L	20%
3	0.2	0.3	0.5	71	1.4 mg/L	50%
4	0.2	no	0.8	76	2.24 mg/L	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	16	0.56 mg/L	20%
3	0.2	0.3	0.5	40	1.4 mg/L	50%
4	0.2	no	0.8	52	2.24 mg/L	80%

Test F

	Test time:	15 minutes
	Test compound:	SDS
Conc ~EC 20	Actual start conc:	0,80 mg/L

Temperatures are measured in reference hole in termoblock

LUMIStox

Temp. (°C)	Replicate	Batch No.	% inhibition	Validity check Standard	Control
14.0	1	10129	24.20		2.02
Block A	2		21.82	*19.42*	2.52
	3		20.49		2.72
15.4	1		27.18		2.52
Block B	2		23.00		0.02
	3		17.15		1.98
16.1	1		13.19	*18.96*	1.33
Block C	2		17.60	*17.39*	0.13
	3		15.83		0.87

ECLOX

Temp. (°C)	Replicate	Batch No.	% inhibition	Standard	Control
14.0	1	10129	23.25		0.92
Block A	2		19.38		2.85
	3		9.82		0.90
15.4	1		24.67		2.06
Block B	2		16.73		3.01
	3		18.43		1.54
16.1	1		20.83		1.00
Block C	2		17.34		2.64
	3		15.99		1.07

Test F

	Test time:	30 minutes
	Test compound:	SDS
Conc ~EC 20	Actual start conc:	0,80 mg/L

Temperatures are measured in reference hole in termoblock

LUMIStox

Temp. (°C)	Replicate	Batch No.	% inhibition	Standard	Control
14.0	1	10129	30.44	22.69	2.47
Block A	2		29.09	19.42	0.05
	3		26.22	24.55	2.64
15.4	1		31.43	20.36	1.29
Block B	2		29.66	26.73	0.99
	3		20.48	31.2	0.20
16.1	1		19.74	18.96	0.58
Block C	2		19.97	17.39	1.29
	3		18.53	22.11	0.17

ECLOX

Temp. (°C)	Replicate	Batch No.	% inhibition	Standard	Control
14.0	1	10129	30.31	21.00	1.06
Block A	2		24.75	21.23	0.14
	3		18.17	21.61	1.27
15.4	1		27.64	21.96	2.15
Block B	2		20.84	26.04	4.08
	3		24.81	27.49	2.62
16.1	1		23.13	23.61	1.52
Block C	2		19.38	25.73	1.30
	3		20.06	23.04	0.45

Test G

	Test time: 15 minutes	
	Test compound:	SDS
Conc ~EC 20	Actual start conc:	0.80

LUMIStox

pH	Replicate	Batch No.	% inhibition
6.0	1	10129	22.92
	2		16.41
	3		14.67
7.0	1		24.41
	2		17.64
	3		14.09
8.5	1		23.95
	2		20.16
	3		12.38

Validity check

Standard	Control
	1.95
	1.27
17.55	2.29
	1.95
	1.27
17.55	2.29
	1.95
	1.27
17.55	2.29

ECLOX

pH	Replicate	Batch No.	% inhibition
6.0	1	10129	23.50
	2		18.38
	3		9.60
7.0	1		18.49
	2		18.19
	3		9.65
8.5	1		19.56
	2		16.76
	3		10.46

Validity check

Standard	Control
	0.54
	4.57
18.25	1.19
	0.54
	4.57
18.25	1.19
	0.54
	4.57
18.25	1.19

Test G

	Test time:	30 minutes
	Test compound:	SDS
Conc ~EC 20	Actual start conc:	0.80

LUMIStox

pH	Replicate	Batch No.	% inhibition	Validity check Standard	Control
6.0	1		31.91	26.62	1.56
	2		23.18	20.52	1.26
	3		18.69	17.55	4.36
7.0	1		28.31	26.62	1.56
	2	10129	24.45	20.52	1.26
	3		14.09	17.55	4.36
8.5	1		28.25	26.62	1.56
	2		26.49	20.52	1.26
	3		16.96	17.55	4.36

ECLOX

pH	Replicate	Batch No.	% inhibition	Validity check Standard	Control
6.0	1		29.20	28.91	0.25
	2		25.52	20.65	3.89
	3		12.83	18.25	2.30
7.0	1		25.31	28.91	0.25
	2	10129	23.84	20.65	3.89
	3		10.45	18.25	2.30
8.5	1		27.00	28.91	0.25
	2		23.30	20.65	3.89
	3		12.33	18.25	2.30

Test H

	Test time: 15 minutes
	Test compound: SDS
Conc ~EC 20	Actual start conc: 0,80 mg/L

LUMIStox

Color conc.	Replicate	Batch No.	Color correction Without % inhibition	Color correction With % inhibition	Validity check Standard	Validity check Control
0,2 % dye	1	10129	23.28	20.58		1.06
Sample	2		15.35	15.35		0.15
	3		18.92	18.92		3.84
6,25 % dye	1		36.95	24.16		1.06
Sample	2		28.83	16.88		0.15
	3		33.84	22.17		3.84
12,5 % dye	1		45.83	24.94		1.06
Sample	2		39.64	18.94		0.15
	3		43.89	24.50		3.84
0,2 % dye	1		0.45	0.45		1.06
No sample	2		-3.19	-6.32		0.15
	3		7.29	7.29		3.84
6,25 % dye	1		14.56	-2.52		1.06
No sample	2		13.13	-1.30		0.15
	3		19.23	4.12		3.84
12,5 % dye	1		30.61	5.31		1.06
No sample	2		29.1	2.28		0.15
	3		30.54	4.99		3.84
No color	1		22.29			1.06
Sample	2		17.09			0.15
	3		19.19			3.84

The control value for replicate 2 changes to 3.22 when using color correction

ECLOX

Color conc.	Replicate	Batch No.	Color correction Without % inhibition	Color correction With % inhibition	Standard	Control
0,2 % dye	1	10129	18.23	21.51		2.16
Sample	2		12.61	16.12		2.46
	3		23.27	26.35		1.72
6,25 % dye	1		25.49	17.03		2.16
Sample	2		24.48	15.91		2.46
	3		30.12	22.19		1.72
12,5 % dye	1		33.15	17.59		2.16
Sample	2		35.22	20.14		2.46
	3		41.99	28.49		1.72
0,2 % dye	1		-1.08	2.98		2.16
No sample	2		-1.39	2.68		2.46
	3		0.99	4.97		1.72
6,25 % dye	1		11.06	0.97		2.16
No sample	2		15.73	6.17		2.46
	3		12.13	2.16		1.72
12,5 % dye	1		20.55	2.05		2.16
No sample	2		22.30	4.21		2.46
	3		18.62	-0.32		1.72
No color	1		15.40			2.16
Sample	2		15.20			2.46
	3		20.96			1.72

Test H

	Test time: 30 minutes
	Test compound: SDS
Conc ~EC 20	Actual start conc: 0,80 mg/L

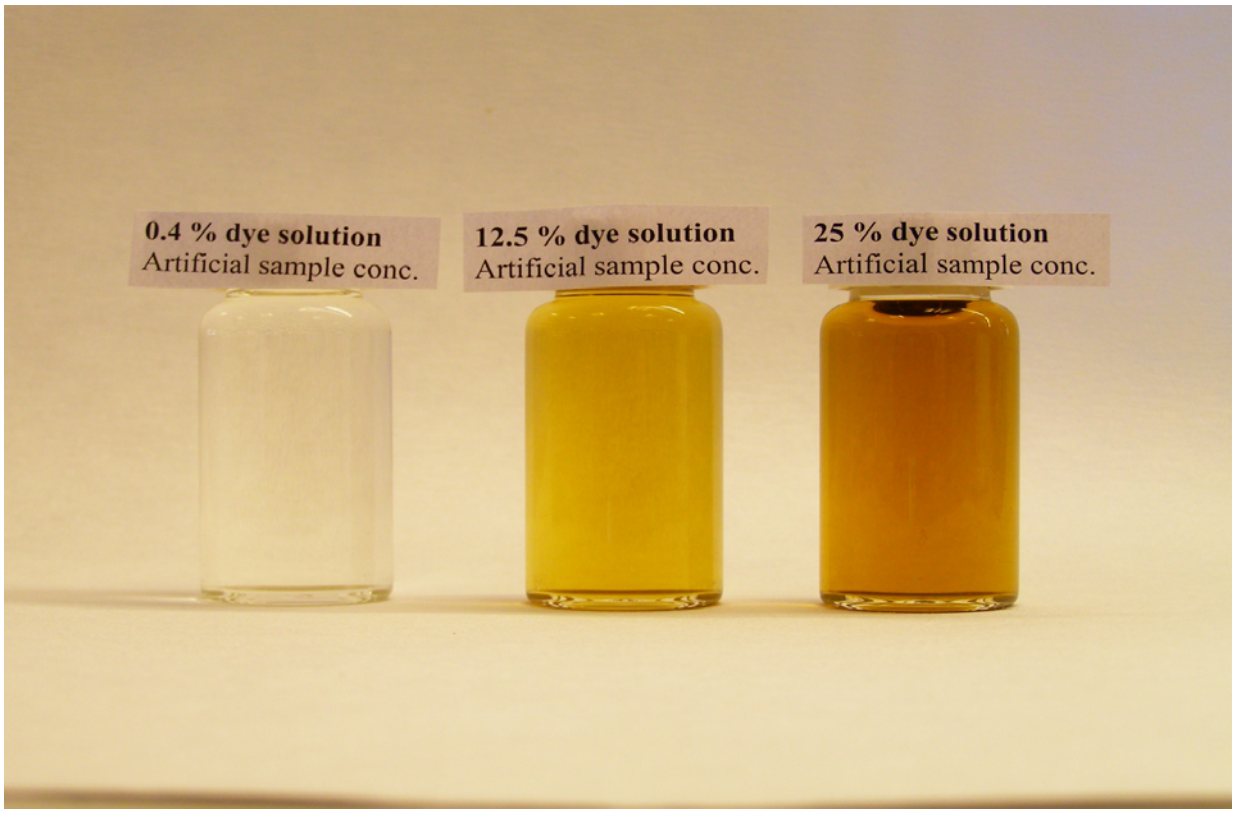
LUMIStox

Color conc.	Replicate	Batch No.	Color correction Without % inhibition	Color correction With % inhibition	Validity check Standard	Validity check Control
0,2 % dye	1	10129	29.93	27.92	27.62	0.16
Sample	2		20.95	20.95	25.17	5.05
	3		21.83	21.83	26.13	3.35
6,25 % dye	1		40.97	29.94	27.62	0.16
Sample	2		32.79	21.48	25.17	5.05
	3		34.19	22.74	26.13	3.35
12,5 % dye	1		49.41	31.87	27.62	0.16
Sample	2		41.5	21.35	25.17	5.05
	3		44.75	25.63	26.13	3.35
0,2 % dye	1		2.55	-0.39	27.62	0.16
No sample	2		-3.94	-3.94	25.17	5.05
	3		2.00	2.00	26.13	3.35
6,25 % dye	1		14.4	-2.03	27.62	0.16
No sample	2		10.63	-4.58	25.17	5.05
	3		14.77	-1.11	26.13	3.35
12,5 % dye	1		28.18	0.48	27.62	0.16
No sample	2		25.88	-0.14	25.17	5.05
	3		27.73	2.43	26.13	3.35
No color	1		27.26		27.62	0.16
Sample	2		19.62		25.17	5.05
	3		22.14		26.13	3.35

ECLOX

Color conc.	Replicate	Batch No.	Color correction Without % inhibition	Color correction With % inhibition	Standard	Control
0,2 % dye	1	10129	21.33	24.49	23.88	1.60
Sample	2		20.49	23.68	27.30	4.83
	3		30.44	33.23	27.15	2.23
6,25 % dye	1		29.23	21.20	23.88	1.60
Sample	2		30.31	22.40	27.30	4.83
	3		37.08	29.94	27.15	2.23
12,5 % dye	1		35.75	20.79	23.88	1.60
Sample	2		38.20	23.81	27.30	4.83
	3		44.00	30.96	27.15	2.23
0,2 % dye	1		-4.91	-0.70	23.88	1.60
No sample	2		1.44	5.40	27.30	4.83
	3		2.65	6.56	27.15	2.23
6,25 % dye	1		8.49	-1.90	23.88	1.60
No sample	2		13.66	3.86	27.30	4.83
	3		11.27	1.20	27.15	2.23
12,5 % dye	1		16.56	-2.86	23.88	1.60
No sample	2		24.08	6.41	27.30	4.83
	3		18.5	-0.47	27.15	2.23
No color	1		18.24		23.88	1.60
Sample	2		21.73		27.30	4.83
	3		25.46		27.15	2.23

Test H


0.4 % dye solution
Artificial sample conc.
12.5 % dye solution
Artificial sample conc.
25 % dye solution
Artificial sample conc.

Test I

	Test time:	15 minutes
	Test compound:	SDS
Conc ~EC 20	Actual start conc:	0.80 mg/L

LUMIStox

Turbide conc.	SDS	Replicate	Batch No.	Color correction Witout % inhibition	With % inhibition	Validity check Standard	Control
No	Yes	1	10129	6.71	6.71		2.16
		2		21.99	19.44		3.35
		3		10.79	5.31	*18.26*	2.40
		4		11.42	11.42		0.62
0,05 g BaSO4/L	Yes	1		13.23	3.12		2.16
		2		16.26	9.79		3.35
		3		12.98	6.55	*18.26*	2.40
		4		14.48	8.31		0.62
0,10 g BaSO4/L	Yes	1		12.56	-1.19		2.16
		2		18.8	9.49		3.35
		3		11.17	-2.05	*18.26*	2.40
		4		10.75	-2.06		0.62
0,20 g BaSO4/L	Yes	1		8.56	-16.54		2.16
		2		16.6	-4.23		3.35
		3		11.49	-13.51	*18.26*	2.40
		4		12.74	-12.07		0.62
Blind 0,05 g BaSO4/L	No	1		1.80	-10.09		2.16
		2		3.46	-6.89		3.35
		3		0.64	-10.27	*18.26*	2.40
		4		1.53	-6.85		0.62
Blind 0,10 g BaSO4/L	No	1		-2.77	-21.12		2.16
		2		1.22	-13.07		3.35
		3		0.61	-18.61	*18.26*	2.40
		4		-1.24	-18.26		0.62
Blind 0,20 g BaSO4/L	No	1		-5.96	-36.77		2.16
		2		-0.20	-27.2		3.35
		3		-0.82	-38.15	*18.26*	2.40
		4		-0.74	-35.7		0.62

Replicate no. 4, bacteria batch more than 4 hours old

ECLOX

Turbide conc.	SDS	Replicate	Batch No.	Color correction Witout % inhibition	With % inhibition	Validity check Standard	Control
No	Yes	1	10129	9.2			0.58
		2		13.11			1.42
		3		9.5		*19.76*	1.32
		4		13.3			3.3
0,05 g BaSO4/L	Yes	1		12.25	15.25		0.58
		2		12.05	15.05		1.42
		3		11.36	14.39	*19.76*	1.32
		4		13.46	16.41		3.3
0,10 g BaSO4/L	Yes	1		13.48	17.54		0.58
		2		16.77	20.67		1.42
		3		10.25	14.46	*19.76*	1.32
		4		12.8	16.89		3.3
0,20 g BaSO4/L	Yes	1		12.52	15.1		0.58
		2		8.72	11.41		1.42
		3		11.95	14.55	*19.76*	1.32
		4		8.18	10.89		3.3
Blind 0,05 g BaSO4/L	No	1		-3.79	-0.25		0.58
		2		-2.92	0.59		1.42
		3		-7.51	-3.84	*19.76*	1.32
		4		1.21	4.58		3.3
Blind 0,10 g BaSO4/L	No	1		0.44	5.11		0.58
		2		-7.95	-2.89		1.42
		3		-8.41	-3.32	*19.76*	1.32
		4		2.27	6.89		3.3
Blind 0,20 g BaSO4/L	No	1		-2.61	0.42		0.58
		2		-13.04	-9.71		1.42
		3		-10.46	-7.2	*19.76*	1.32
		4		3.6	6.44		3.3

Replicate no. 4, bacteria batch more than 4 hours old

Test I

	Test time:	30 minutes
	Test compound:	SDS
Conc ~EC 20	Actual start conc:	0.80 mg/L

LUMIStox

Turbide conc.	SDS	Replicate	Batch No.	Color correction Witout % inhibition	With % inhibition	Validity check Standard	Control
No	Yes	1	10129	10.47	10.47	25.37	3.27
		2		25.25	25.25	23.22	0.36
		3		11.20	11.20	18.26	0.37
		4		14.64	14.64	24.96	1.76
0,05 g BaSO4/L	Yes	1		15.23	8.88	25.37	3.27
		2		17.74	11.84	23.22	0.36
		3		14.46	11.77	18.26	0.37
		4		17.55	10.61	24.96	1.76
0,10 g BaSO4/L	Yes	1		17.29	7.49	25.37	3.27
		2		20.12	12.55	23.22	0.36
		3		12.01	2.67	18.26	0.37
		4		10.54	2.52	24.96	1.76
0,20 g BaSO4/L	Yes	1		10.07	-7.96	25.37	3.27
		2		19.64	-0.3	23.22	0.36
		3		12.22	-1.96	18.26	0.37
		4		12.37	-2.52	24.96	1.76
Blind 0,05 g BaSO4/L	No	1		0.61	-12.03	25.37	3.27
		2		-0.45	-9.93	23.22	0.36
		3		0.08	-9.95	18.26	0.37
		4		0.04	-7.9	24.96	1.76
Blind 0,10 g BaSO4/L	No	1		-4.17	-17.59	25.37	3.27
		2		-1.37	-16.39	23.22	0.36
		3		-4.43	-20.13	18.26	0.37
		4		1.19	-12.26	24.96	1.76
Blind 0,20 g BaSO4/L	No	1		-8.60	-31.21	25.37	3.27
		2		-5.92	-32.29	23.22	0.36
		3		-7.12	-34.22	18.26	0.37
		4		-0.35	-23.31	24.96	1.76

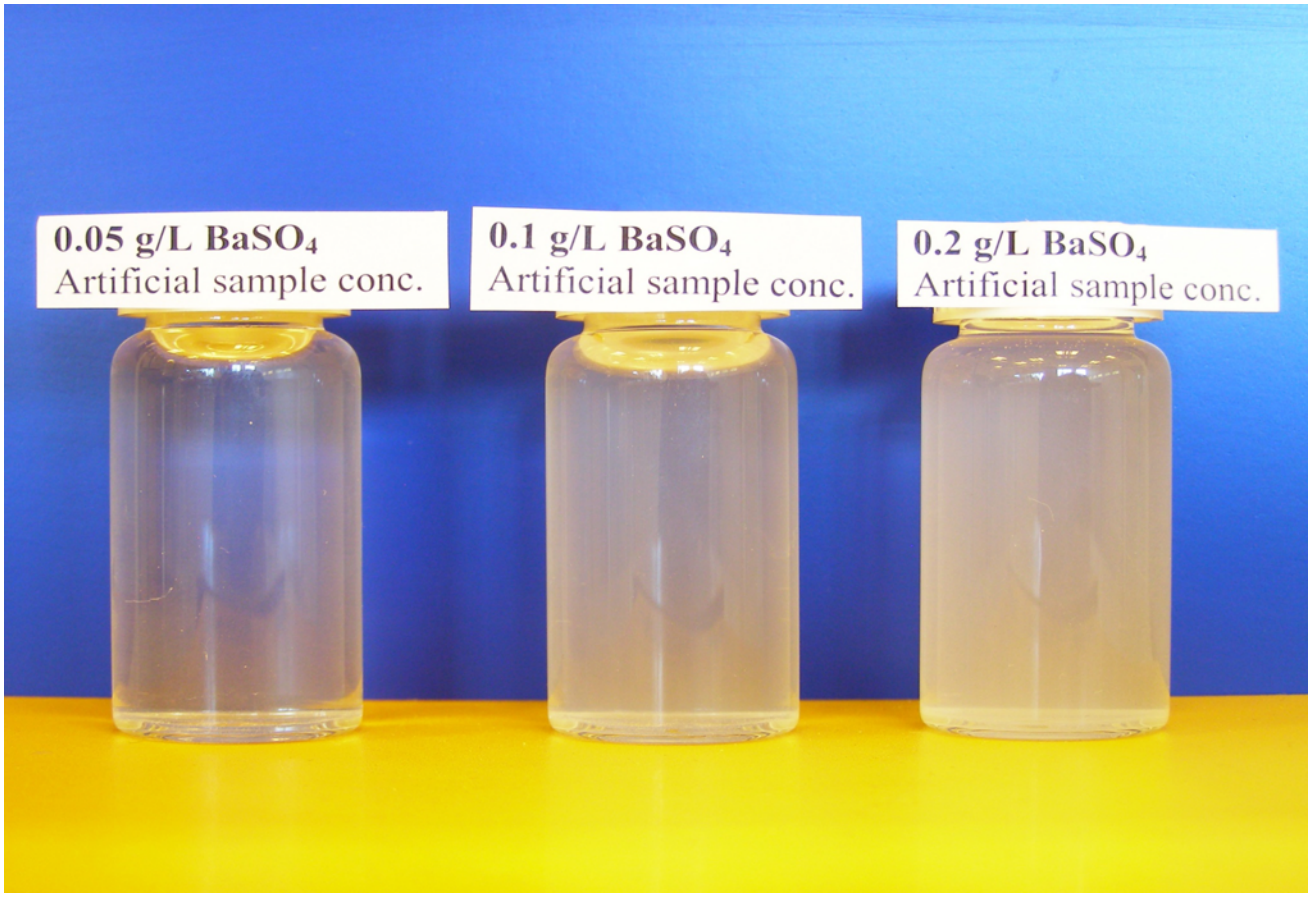
Replicate no. 4, bacteria batch more than 4 hours old

ECLOX

Turbide conc.	SDS	Replicate	Batch No.	Color correction Witout % inhibition	With % inhibition	Validity check Standard	Control
No	Yes	1	10129	15.89		25.01	1.34
		2		23.03		24.37	2.29
		3		12.96		19.76	0.28
		4		13.85		22.18	7.89
0,05 g BaSO4/L	Yes	1		12.76	15.74	25.01	1.34
		2		18.86	21.63	24.37	2.29
		3		15.02	17.92	19.76	0.28
		4		14.52	17.44	22.18	7.89
0,10 g BaSO4/L	Yes	1		18.52	22.34	25.01	1.34
		2		20.62	24.34	24.37	2.29
		3		16.43	20.35	19.76	0.28
		4		14.72	18.72	22.18	7.89
0,20 g BaSO4/L	Yes	1		15.51	18.00	25.01	1.34
		2		17.54	19.97	24.37	2.29
		3		15.25	17.75	19.76	0.28
		4		8.18	10.89	22.18	7.89
Blind 0,05 g BaSO4/L	No	1		-2.81	0.70	25.01	1.34
		2		-0.71	2.73	24.37	2.29
		3		-5.42	-1.82	19.76	0.28
		4		-1.42	2.04	22.18	7.89
Blind 0,10 g BaSO4/L	No	1		0.75	5.41	25.01	1.34
		2		-3.94	0.94	24.37	2.29
		3		-10.21	-5.04	19.76	0.28
		4		0.28	4.96	22.18	7.89
Blind 0,20 g BaSO4/L	No	1		-1.86	1.14	25.01	1.34
		2		-4.19	-1.12	24.37	2.29
		3		-11.95	-8.65	19.76	0.28
		4		0.71	3.64	22.18	7.89

Replicate no. 4, bacteria batch more than 4 hours old

Test I


0.05 g/L $BaSO_4$
Artificial sample conc.
0.1 g/L $BaSO_4$
Artificial sample conc.
0.2 g/L $BaSO_4$
Artificial sample conc.

Test J

Bacteria batches as in Test A

Conc ~EC 20

LUMIStox Test time: 15 minutes

Industrial wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition
No	Waste water		1	10129	5.11
			2		-5.60
			3		4.03
CTAB	2% NaCl MilliQ water	1.20	1		7.08
			2		10.54
			3		23.74
CTAB	Waste water	1.20	1		16.24
			2		9.99
			3		16.09
Cr	2% NaCl MilliQ water	2.80	1		18.36
			2		7.34
			3		11.80
Cr	Waste water	2.80	1		7.90
			2		-2.07
			3		5.79
SDS	2% NaCl MilliQ water	0.80	1		14.01
			2		17.28
			3		21.25
SDS	Waste water	0.80	1		11.82
			2		10.77
			3		13.19
Triclosan	2% NaCl MilliQ water	0.60	1		21.22
			2		31.29
			3		38.5
Triclosan	Waste water	0.60	1		27.63
			2		35.24
			3		41.29
Zn	2% NaCl MilliQ water	4.00	1		9.84
			2		7.55
			3		9.80
Zn	Waste water	4.00	1		11.62
			2		4.03
			3		5.16

Validity check

Standard	Control
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.20
	3.30
	1.08
	0.89
	1.04
	2.63
	0.89
	1.04
	2.63

Test J

Bacteria batches as in Test A

Conc ~EC 20

LUMIStox

Test time: 15 minutes

Domestic wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition
No	Waste water		1	10129	-6.99
			2		-15.41
			3		-1.95
CTAB	2% NaCl MilliQ water	1.20	1		19.00
			2		17.07
			3		20.20
CTAB	Waste water	1.20	1		13.69
			2		9.63
			3		18.67
Cr	2% NaCl MilliQ water	2.80	1		17.12
			2		7.38
			3		13.11
Cr	Waste water	2.80	1		-6.18
			2		-13.45
			3		0.93
SDS	2% NaCl MilliQ water	0.80	1		22.11
			2		15.82
			3		21.95
SDS	Waste water	0.80	1		13.75
			2		8.96
			3		16.85
Triclosan	2% NaCl MilliQ water	0.60	1		34.88
			2		36.36
			3		43.91
Triclosan	Waste water	0.60	1		22.66
			2		31.79
			3		41.74
Zn	2% NaCl MilliQ water	4.00	1		7.69
			2		7.84
			3		9.85
Zn	Waste water	4.00	1		4.98
			2		-0.78
			3		3.74

Validity check

Standard	Control
	1.78
	0.16
	2.48
	1.78
	0.16
	2.48
	1.78
	0.16
	2.48
	1.78
	0.16
	2.48
	1.78
	0.16
	2.48
	1.78
	0.16
	2.48
	1.78
	0.16
	2.48
	1.78
	0.16
	2.48
	3.03
	1.58
	0.38
	3.03
	1.58
	0.38

Test J

Bacteria batches as in Test A

Conc ~EC 20

ECLOX

Test time:	15 minutes

Industrial wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition
No	Waste water		1	10129	2.80
			2		-2.19
			3		3.93
CTAB	2% NaCl MilliQ water	1.20	1		12.15
			2		11.28
			3		22.33
CTAB	Waste water	1.20	1		15.49
			2		13.83
			3		16.11
Cr	2% NaCl MilliQ water	2.80	1		20.53
			2		10.17
			3		14.93
Cr	Waste water	2.80	1		2.86
			2		-1.04
			3		3.77
SDS	2% NaCl MilliQ water	0.80	1		13.06
			2		12.89
			3		22.81
SDS	Waste water	0.80	1		13.76
			2		7.74
			3		11.75
Triclosan	2% NaCl MilliQ water	0.60	1		23.27
			2		27.56
			3		36.25
Triclosan	Waste water	0.60	1		27.12
			2		31.82
			3		41.76
Zn	2% NaCl MilliQ water	4.00	1		7.56
			2		11.01
			3		8.76
Zn	Waste water	4.00	1		6.16
			2		5.27
			3		4.00

Validity check

Standard	Control
	2.39
	1.37
	0.25
	2.39
	1.37
	0.25
	2.39
	1.37
	0.25
	2.39
	1.37
	0.25
	2.39
	1.37
	0.25
	2.39
	1.37
	0.25
	2.39
	1.37
	0.25
	2.39
	1.37
	0.25
	3.45
	0.11
	2.18
	3.45
	0.11
	2.18

Test J

Bacteria batches as in Test A

Conc ~EC 20

ECLOX

Test time: 15 minutes

Domestic wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition	Validity check Standard	Validity check Control
No	Waste water		1	10129	-4.68	*19.54*	2.93
			2		-8.26		4.06
			3		-4.18		1.93
CTAB	2% NaCl MilliQ water	1.20	1		20.33	*19.54*	2.93
			2		23.59		4.06
			3		21.49		1.93
CTAB	Waste water	1.20	1		13.45	*19.54*	2.93
			2		14.12		4.06
			3		14.12		1.93
Cr	2% NaCl MilliQ water	2.80	1		13.36	*19.54*	2.93
			2		11.03		4.06
			3		8.04		1.93
Cr	Waste water	2.80	1		-6.98	*19.54*	2.93
			2		-9.15		4.06
			3		-3.52		1.93
SDS	2% NaCl MilliQ water	0.80	1		20.11	*19.54*	2.93
			2		19.36		4.06
			3		22.60		1.93
SDS	Waste water	0.80	1		15.27	*19.54*	2.93
			2		12.23		4.06
			3		16.82		1.93
Triclosan	2% NaCl MilliQ water	0.60	1		31.50	*19.54*	2.93
			2		39.09		4.06
			3		42.04		1.93
Triclosan	Waste water	0.60	1		23.94	*19.54*	2.93
			2		33.30		4.06
			3		42.68		1.93
Zn	2% NaCl MilliQ water	4.00	1		8.80	*19.54*	1.89
			2		11.22		1.44
			3		5.51		1.58
Zn	Waste water	4.00	1		1.50	*19.54*	1.89
			2		6.11		1.44
			3		1.80		1.58

Test J

Bacteria batches as in Test A

Conc ~EC 20

LUMIStox

Test time:	30 minutes

Industrial wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition
No	Waste water		1	10129	3.26
			2		-10.55
			3		-1.44
CTAB	2% NaCl MilliQ water	1.20	1		12.00
			2		14.36
			3		40.32
CTAB	Waste water	1.20	1		16.71
			2		10.46
			3		17.84
Cr	2% NaCl MilliQ water	2.80	1		30.84
			2		20.02
			3		21.73
Cr	Waste water	2.80	1		5.23
			2		-7.27
			3		-1.26
SDS	2% NaCl MilliQ water	0.80	1		21.01
			2		21.23
			3		26.19
SDS	Waste water	0.80	1		8.68
			2		2.43
			3		7.83
Triclosan	2% NaCl MilliQ water	0.60	1		8.58
			2		12.01
			3		21.98
Triclosan	Waste water	0.60	1		16.63
			2		16.80
			3		26.78
Zn	2% NaCl MilliQ water	4.00	1		21.37
			2		20.15
			3		20.39
Zn	Waste water	4.00	1		17.45
			2		4.54
			3		4.51

Validity check

Standard	Control
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	0.34
21.88	0.56
23.12	1.84
24.03	2.57
21.88	1.88
23.12	2.18
24.03	2.57
21.88	1.88
23.12	2.18

Test J

Bacteria batches as in Test A

Conc ~EC 20

LUMIStox

Test time: 30 minutes

Domestic wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition
No	Waste water		1	10129	-5.33
			2		-13.87
			3		-0.57
CTAB	2% NaCl MilliQ water	1.20	1		30.79
			2		33.05
			3		31.21
CTAB	Waste water	1.20	1		16.07
			2		11.34
			3		22.45
Cr	2% NaCl MilliQ water	2.80	1		27.22
			2		19.57
			3		20.81
Cr	Waste water	2.80	1		-2.95
			2		-10.91
			3		4.47
SDS	2% NaCl MilliQ water	0.80	1		28.21
			2		21.70
			3		26.86
SDS	Waste water	0.80	1		16.84
			2		13.24
			3		19.25
Triclosan	2% NaCl MilliQ water	0.60	1		17.26
			2		18.71
			3		25.61
Triclosan	Waste water	0.60	1		2.35
			2		9.61
			3		23.02
Zn	2% NaCl MilliQ water	4.00	1		19.64
			2		22.85
			3		19.64
Zn	Waste water	4.00	1		17.22
			2		19.12
			3		15.73

Validity check

Standard	Control
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	3.15
24.59	0.79
21.59	4.63
24.62	0.55
24.59	0.99
21.59	1.76
24.62	0.55
24.59	0.99
21.59	1.76

Test J

Bacteria batches as in Test A

Conc ~EC 20

ECLOX

Test time: 30 minutes

Industrial wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition	Validity check Standard	Validity check Control
No	Waste water		1	10129	1.28	24.20	1.50
			2		-8.03	21.63	2.86
			3		-3.02	25.82	1.48
CTAB	2% NaCl MilliQ water	1.20	1		14.38	24.20	1.50
			2		15.54	21.63	2.86
			3		38.51	25.82	1.48
CTAB	Waste water	1.20	1		15.14	24.20	1.50
			2		12.93	21.63	2.86
			3		13.44	25.82	1.48
Cr	2% NaCl MilliQ water	2.80	1		31.51	24.20	1.50
			2		21.50	21.63	2.86
			3		22.45	25.82	1.48
Cr	Waste water	2.80	1		-0.24	24.20	1.50
			2		-5.82	21.63	2.86
			3		-4.78	25.82	1.48
SDS	2% NaCl MilliQ water	0.80	1		20.09	24.20	1.50
			2		16.86	21.63	2.86
			3		25.34	25.82	1.48
SDS	Waste water	0.80	1		11.21	24.20	1.50
			2		4.83	21.63	2.86
			3		5.75	25.82	1.48
Triclosan	2% NaCl MilliQ water	0.60	1		9.51	24.20	1.50
			2		11.85	21.63	2.86
			3		18.87	25.82	1.48
Triclosan	Waste water	0.60	1		15.71	24.20	1.50
			2		14.30	21.63	2.86
			3		26.74	25.82	1.48
Zn	2% NaCl MilliQ water	4.00	1		21.45	24.20	2.46
			2		22.66	21.63	0.19
			3		21.27	25.82	1.48
Zn	Waste water	4.00	1		10.29	24.20	2.46
			2		2.68	21.63	0.19
			3		1.69	25.82	1.48

Test J

Bacteria batches as in Test A

Conc ~EC 20

ECLOX

Test time:	30 minutes

Domestic wastewater

Compound	Matrix	Conc (mg/L)	Replicate	Batch No.	% inhibition	Validity check Standard	Control
No	Waste water		1	10129	-2.82	19.54	0.89
			2		-9.02	24.40	4.5
			3		-4.11	24.53	1.86
CTAB	2% NaCl MilliQ water	1.20	1		33.91	19.54	0.89
			2		35.35	24.40	4.5
			3		32.36	24.53	1.86
CTAB	Waste water	1.20	1		15.93	19.54	0.89
			2		14.68	24.40	4.5
			3		18.99	24.53	1.86
Cr	2% NaCl MilliQ water	2.80	1		27.11	19.54	0.89
			2		21.85	24.40	4.5
			3		17.81	24.53	1.86
Cr	Waste water	2.80	1		-6.59	19.54	0.89
			2		-8.86	24.40	4.5
			3		-0.34	24.53	1.86
SDS	2% NaCl MilliQ water	0.80	1		22.18	19.54	0.89
			2		23.80	24.40	4.5
			3		26.25	24.53	1.86
SDS	Waste water	0.80	1		15.50	19.54	0.89
			2		14.00	24.40	4.5
			3		18.91	24.53	1.86
Triclosan	2% NaCl MilliQ water	0.60	1		14.08	19.54	0.89
			2		20.60	24.40	4.5
			3		27.26	24.53	1.86
Triclosan	Waste water	0.60	1		2.66	19.54	0.89
			2		12.40	24.40	4.5
			3		23.20	24.53	1.86
Zn	2% NaCl MilliQ water	4.00	1		18.18	19.54	2.66
			2		21.95	24.40	3.02
			3		19.68	24.53	1.05
Zn	Waste water	4.00	1		14.09	19.54	2.66
			2		18.80	24.40	3.02
			3		17.72	24.53	1.05

Test K

Bacteria batches as in Test J

Mark test time:	15 minutes

LUMIStox

Wastewater	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Validity check Standard	Control
Industrial	1	10129	n.c.	n.c.	*-2.71*	3.13
	2		n.c.	n.c.		2.83
	3		n.c.	n.c.		4.8
Domestic	1	10129	n.c.	n.c.		1.66
	2		n.c.	n.c.		4.04

ECLOX

Wastewater	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Standard	Control
Industrial	1	10129	n.c.	n.c.	*-3.77*	2.35
	2		n.c.	n.c.		5.39
Domestic	1	10129	n.c.	n.c.		3.67
	2		n.c.	n.c.		2.83

Test K

Bacteria batches as in Test J

Mark test time:	30 minutes

LUMIStox

Wastewater	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Validity check Standard	Control
Industrial	1	10129	n.c.	n.c.	-2.71	3.44
	2		n.c.	n.c.	25.27	2.70
	3		n.c.	n.c.	25.14	5.42
Domestic	1	10129	n.c.	n.c.	38.63	0.78
	2		n.c.	n.c.	33.46	0.67

ECLOX

Wastewater	Replicate	Batch No.	EC20 (mg/L)	EC50 (mg/L)	Standard	Control
Industrial	1	10129	n.c.	n.c.	-3.77	3.26
	2		n.c.	n.c.	28.71	4.10
Domestic	1	10129	n.c.	n.c.	33.63	2.47
	2		n.c.	n.c.	31.55	3.31

n.c. Not calculated. No toxic inhibition.

Test L

Compound 1	Mark test time:	15 minutes
	Test compound 1:	SDS
Conc ~EC 20	Actual start conc:	0.80 mg/L

LUMIStox			LL100205-69	LL100205-70	LL100205-71
Cuvette	Replicate	Batch No.	% inhibition	% inhibition	% inhibition
Glass	1		23.17	20.02	14.82
	2		22.23	20.55	16.86
	3	10129	23.37	20.43	15.53
Plastic	1		20.87	19.15	13.87
	2		25.43	22.56	17.38
	3		27.66	24.07	12.74

Validity check

LL100205-69		LL100205-70		LL100205-71	
Standard	Control	Standard	Control	Standard	Control
	0.78		0.12		0.26
	0.78		0.12		0.26
	0.78		0.12		0.26
	1.33		1.33		2.16
	1.33		1.33		2.16
	1.33		1.33		2.16

Compound 2	Mark test time:	15 minutes
	Test compound 2:	Zn
Conc ~EC 20	Actual start conc:	4.00 mg/L

LUMIStox			LL100205-69	LL100205-70	LL100205-71
Cuvette	Replicate	Batch No.	% inhibition	% inhibition	% inhibition
Glass	1		8.64	8.24	8.02
	2		10.06	6.44	5.31
	3	10129	9.19	10.14	5.84
Plastic	1		8.01	11.63	4.33
	2		10.61	14.15	7.03
	3		9.08	13.83	7.94

LL100205-69		LL100205-70		LL100205-71	
Standard	Control	Standard	Control	Standard	Control
	0.78		0.12		0.26
	0.78		0.12		0.26
	0.78		0.12		0.26
	1.33		1.33		2.16
	1.33		1.33		2.16
	1.33		1.33		2.16

Test L

Compound 1	Mark test time:	30 minutes
	Test compound 1:	SDS
Conc ~EC 20	Actual start conc:	0.80 mg/L

LUMIStox			LL100205-69	LL100205-70	LL100205-71
Cuvette	Replicate	Batch No.	% inhibition	% inhibition	% inhibition
Glass	1		29.10	25.22	19.77
	2		27.71	25.56	20.20
	3	10129	29.43	26.82	18.37
Plastic	1		26.09	23.59	16.88
	2		30.13	26.42	20.48
	3		30.52	27.09	15.37

LL100205-69		LL100205-70		LL100205-71	
Standard	Control	Standard	Control	Standard	Control
28.21	0.52	21.34	0.18	20.77	0.89
28.21	0.52	21.34	0.18	20.77	0.89
28.21	0.52	21.34	0.18	20.77	0.89
28.21	1.78	21.34	1.52	20.77	1.79
28.21	1.78	21.34	1.52	20.77	1.79
28.21	1.78	21.34	1.52	20.77	1.79

Compound 2	Mark test time:	30 minutes
	Test compound 2:	Zn
Conc ~EC 20	Actual start conc:	4.00 mg/L

LUMIStox			LL100205-69	LL100205-70	LL100205-71
Cuvette	Replicate	Batch No.	% inhibition	% inhibition	% inhibition
Glass	1		21.53	17.69	17.94
	2		22.3	17.74	15.60
	3	10129	22.13	18.79	14.90
Plastic	1		22.47	20.69	16.02
	2		25.15	20.5	19.15
	3		22.66	22.41	16.05

LL100205-69		LL100205-70		LL100205-71	
Standard	Control	Standard	Control	Standard	Control
28.21	0.52	21.34	0.18	20.77	0.89
28.21	0.52	21.34	0.18	20.77	0.89
28.21	0.52	21.34	0.18	20.77	0.89
28.21	1.78	21.34	1.52	20.77	1.79
28.21	1.78	21.34	1.52	20.77	1.79
28.21	1.78	21.34	1.52	20.77	1.79

A P P E N D I X 7

Amendment and deviation reports for test

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:

Dev. No.	Experiment label Test Plan	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature test responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada
1	Test A	CuSO4 testing was not completed.	Precipitation was observed. Not toxic at concentration with no precipitation. Frozen samples very different from fresh samples. Inaccurate pH adjustment gave different toxicity even when final pH was identical.	The Test A evaluation for the heavy metal category will be based on one fewer compound. However, results for two other heavy metals (Zn and Cr) will still be performed and should adequately represent performance for the heavy metal category.	None	6/5/10		6/5 2010				10/5/2010	
2	Test A	Flutriafol testing was not completed.	Flutriafol was not sufficiently toxic at concentrations with no precipitation.	The Test A evaluations for the organic pesticide category will not be completed. Performance will still be evaluated for organic compounds by testing with Triclosan and the detergents.	None	6/5/10		6/5 2010				10/5/2010	

3	Test D	Will be performed with 3 instead of 4 bacteria batches.	Only 3 different batches could be delivered from Hach Lange.	Test on reproducibility is reduced from 4 to 3 bacteria batches. By switching to three bacteria batch means for the calculation of relative standard deviation (RSD), the 95 percent confidence interval range of values for RSD is likely to be approximately 15% larger than it would be with 4 batches. This was determined by a simulation study assuming that the data are normally distributed.	None	6/5/10		6/5 2010				10/5/2010	

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:

Dev. No.	Experiment label Test Plan	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature test responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada
4	Test A	Repeatability for 4-NPE was not completed. The sample was not sent for chemical analyses or Microtox testing.	Found solubility in literature around 1-3 mg/l. Dissolved 3 mg in one liter. Pre-screening showed that this concentration was non-toxic.	The evaluation of repeatability will be based on fewer compounds. 4-NPE, 4 detergent 2 other detergents are present in the results for evaluation.	None	25/2 10	Claus Jorgensen	25/2 -2010	Anders Andersen	4-28-10	Gary Williams	29/4/2010	Mark Hall

① Spelling error BJW 4-28-10

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:

Dev. No.	Experiment label Test Plan	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature test responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada
5	K	The test on industrial waste water was only performed as replicate, not triplicate as specified	One tube was accidentally measured twice. When trying to correct the error the test was aborted.	The waste water is non toxic. Therefore duplicate measurements are considered sufficient.	None	29/4 10		29/4-10		4-29-10		30-4-10	
6	K	The test of the municipal waste water will be run in duplicate in case of non toxicity.	To be consistent with the test on industrial waste water.	If the waste water is non toxic, a duplicate measurement will be sufficient and consistent with the industrial water test. If toxic, there is no deviation and test will be run in triplicate per the original test plan.	None	29/4 10		29/4-10		4-29-10		30-4-10	

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:

Dev. No	Experiment label/ Test Plan	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature test responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada
7	Test D, Test Plan section 9.4.4, Appendix 4	The ISO requests test of standards and acceptance of control for bacteria batches. Each delivered batch shall be checked with three reference substances. That has been done; however, batch 02099 did not meet the criteria of being within 20-80% inhibition for all three reference substances, but this batch has been used in Test D to evaluate batch to batch variability	It is not possible to change the activity of the bacteria. We have asked Hach Lange for additional batches; however, Hach Lange could only provide three and 02099 did not meet all of the ISO bacteria quality control. Since the purpose of Test D is to evaluate reproducibility with different bacteria batches it is important to have at least three batches included, and the three batches represent real-world availability from the vendor; therefore, we have left batch 02099 in the evaluation.	When the bacteria batch does not pass the criteria for all three reference standards, it will cause slightly higher standard deviations on the calculated results and slightly higher relative standard deviation.	It will be noted in the report, that results for Test D have been calculated using one bacteria batch which did not meet the ISO reference standard criteria and the impact on the results will be noted.	2015-10		27/5 2010		5-27-10		4-6-10	

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:

Dev. No.	Experiment label Test Plan	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature test responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada
9 part B	4 4.3	Reference luminescent bacteria tests were not performed	The tests should be performed according to ISO 11348-3 and under ISO 17025 accreditation and with different equipment than tested in the verification. The selected laboratory ALcontrol (using Microtox equipment) was after test of 3 samples found not to fulfill the requirements.	The results were only intended to be used to give indication of toxicity level and e.g. as false positive or negative. The results were not intended to be used as true values. The value of the tests were from the beginning limited.	It was investigated if any other laboratory could fulfill the requirements, but none could be found (expect for laboratories using the HACH-LANGE LUMIStox). In the test report, text will be included that describes the planned reference tests and why they were not performed.	9/6 - 2010		9/6-2010		6-9-10		12-6-2010	

Dev. No.	Experiment label Test Plan	Deviation	Cause	Impact assessment	Corrective action, if any	Date	Signature test responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada
9 part C	6.3	Reference luminescent bacteria test of blanks was performed on one instead of two samples	The tests should be performed according to ISO 11348-3 and under ISO 17025 accreditation and with different equipment than tested in the verification. The selected laboratory ALcontrol (using Microtox equipment) was after test of 3 samples found not to fulfill the requirements.	2 blanks of DHI MilliQ water should be tested as part of test system control. One result will be listed.	One of the tested 3 sampies was a blank. The results will be listed with a comment related to the reference laboratory not fulfilling the ISO 11348-3 requirement and a reference to the associated deviation in the test method.	27-10		2010-12-15		5-7-10		4-6-10	

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:

Dev No.	Experiment label Test Plan	Deviation	Cause	impact assessment	Corrective action, if any	Date	Signature test responsible	Date	Signature verification responsible	Date	Signature Battelle AMS QM	Date	Signature ETV Canada
10	Section 4.3, Tabel 4.2	Method for SDS analyses changed from MK8230-LC-MS to DS 237	Eurofins had mixed up SDS with LAS. MK8230-LC-MS is for LAS while DS 237 is for anionic surfacts (as SDS)	Correct method used, therefore no impact	No	25/5-10		25/5 2010		5-26-10		4-6-2010	
11 part A	Section 2.4	Review of test report will only be performed by external expert Kresten Ole Kusk	US EPA and ETV Canada do not want their experts to review test report, they find review of verification report sufficient	Since one external expert is reviewing report requirements in DANETV QA manual are still fulfilled.	No	25/5-10		25/5 2010		5-26-10		4-6-2010	