BS ISO 21338:2010

BSI Standards Publication

# Water quality — Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of Vibrio fischeri (kinetic luminescent bacteria test)



*raising standards worldwide*™

Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

**National foreword**

This British Standard is the UK implementation of ISO 21338:2010.

The UK participation in its preparation was entrusted to Technical Committee EH/3/5, Biological Methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.



ISBN 978 0 580 56634 9

ICS 13.060.60

**Compliance with a British Standard cannot confer immunity from legal obligations.**

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 August 2010.

**Amendments issued since publication**

| Date | Text affected |
|---|---|

BS ISO 21338:2010

# INTERNATIONAL STANDARD

# ISO 21338

First edition
2010-07-15

# Water quality — Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* (kinetic luminescent bacteria test)

*Qualité de l'eau — Détermination cinétique des effets inhibiteurs des échantillons de sédiment, autres solides et des échantillons colorés sur la luminescence de* Vibrio fischeri *(essai cinétique de bactéries luminescentes)*

Reference number
ISO 21338:2010(E)



ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

# Contents

Page







Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

# Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 21338 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

# Introduction

The method specified in this International Standard is a kinetic modification of the luminescent bacteria test specified in ISO 11348. The kinetic method overcomes the problems arising from intense colour and turbidity in the samples. There is no need for sedimentation or centrifugation of turbid samples, or for the correction of colour as described in ISO 11348.

This kinetic method uses luminometers capable of dispensing luminescent bacteria to the samples and measuring the luminescent intensity over a period of time. In the method, the bacterial suspension is dispensed and mixed with the sample in the measurement chamber of the luminometer. Several suitable instruments are commercially available, but only a few of them are capable of cooling the measurement chamber to (15 ± 1) °C as specified in ISO 11348. However, if the bacterial suspension and test samples are kept at (15 ± 1) °C in a thermo-block before the measurement and during the whole incubation, the actual temperature during the contact time is (15 ± 1) °C.

The measurements specified in this International Standard can be carried out using freshly prepared bacteria, as well as freeze- or liquid-dried bacterial preparations. The various bacterial preparations can deliver different results, especially in the presence of heavy metals (see ISO 11348). The laboratories responsible for the results have the opportunity to select the most suitable bacterial preparation based on expert judgement and information about the samples to be tested.

Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

**INTERNATIONAL STANDARD** **ISO 21338:2010(E)**

# Water quality — Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* (kinetic luminescent bacteria test)

**WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.**

**IMPORTANT — It is absolutely essential that tests conducted in accordance with this International Standard be carried out by suitably trained staff.**

## 1 Scope

This International Standard specifies the kinetic direct-contact method for determining the inhibitory effect of suspensions of sediment and other solid samples, and also for problematic turbid or coloured aqueous samples on the light emission of the marine bacterium *Vibrio fischeri* (NRRL B-11177).

This method is applicable to:

a) sediment samples and water suspensions of sediments (fresh water, brackish, and seawater sediments);

b) effluents (especially turbid and coloured);

c) aqueous extracts (e.g. leachates, eluates, elutriates) of soil, solid waste, and other solid material (especially turbid and coloured).

## 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 5667-16:1998, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*

ISO 11348-1, *Water quality — Determination of the inhibitory effect of water samples on the light emission of* Vibrio fischeri *(Luminescent bacteria test) — Part 1: Method using freshly prepared bacteria*

ISO 11348-2, *Water quality — Determination of the inhibitory effect of water samples on the light emission of* Vibrio fischeri *(Luminescent bacteria test) — Part 2: Method using liquid-dried bacteria*

ISO 11348-3:2007, *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*





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

## 3 Terms and definitions

For the purpose of this document, the following terms and definitions apply.

**3.1**
**contact time**
duration of contact between one object or substance and another

NOTE In the test, the contact time is the time available to control or sample for contact with the test bacteria.

**3.2**
**control sample**
sample used in a laboratory in order to check or monitor the instrument or measurement performance or to monitor changes in a sample under investigation

**3.3**
**correction factor**
dimensionless multiplier to correct data for known influences affecting their values as measured

NOTE In the test, the correction factor, $f_{kt}$, serves to correct the initial luminescence intensity of the sample.

**3.4**
**peak value**
maximum signal recorded in response to a stimulus

NOTE In the test, the peak value is the maximum signal which is recorded immediately after all the bacteria are in contact with the sample.

**3.5**
**reference sample**
when the effect or behaviour of a substance is known from previous tests (reference substance) and when this substance is examined within the framework of a test series as test sample, this is called the reference sample

NOTE Adapted from ISO 5667-16:1998.

**3.6**
**test sample**
test sample is made from the sample by means of various preparatory steps specific to the sample and the test, e.g. by dissolving, homogenizing, sedimenting, filtering, neutralizing or aeration

[ISO 5667-16:1998]

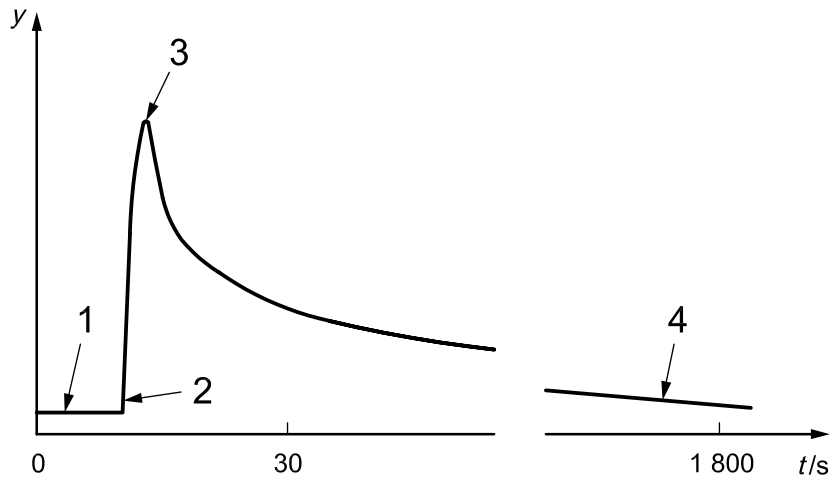
## 4 Principle

The inhibition of light emission by cultures of *Vibrio fischeri* is measured kinetically by following the light emission of cultures from the very beginning of the assay. This is accomplished by dispensing the luminescent bacteria suspension into the sample in a cuvette or other suitable vessel (e.g. microtiter plate) already in the measuring position in the luminometer. The light emission is measured and recorded from the moment of dispensing of the bacterial suspension to the sample until the maximum value has been reached and not only at the maximum value of intensity (peak value) which usually occurs within 5 s of mixing, and after a contact time of 15 min and 30 min or optionally 5 min (Figure 1).





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013




**Key**

$t$ time
$y$ relative light units
1 start measurement
2 inject bacteria
3 record peak value from 0 s to 5 s
4 mix the sample before recording signal at 30 min

**Figure 1 — Principal schematic protocol for the kinetic luminescent bacteria test**

*Vibrio fischeri* suspension is dispensed and mixed into the sample in the measurement chamber of the luminometer.

The test criterion is the decrease of the luminescence at each endpoint compared to the peak value, taking into account a correction factor, $f_{kt}$, which is measured from intensity changes of control samples during the exposure time. The inhibitory effect of the sample can be determined as the lowest ineffective dilution (LID) value, or as effective concentration ($EC_{20}$ or $EC_{50}$) values by means of dilution series (e.g. as described in Annex C). The LID value is the most concentrated test batch tested at which the inhibition of light emission is <20 %. For higher levels of inhibition, the dilution effect relationship can be determined graphically or by statistical analysis. The inhibition by a sample is expressed as the concentrations which results in 20 % and 50 % light reduction compared to the blank ($EC_{20}$ and $EC_{50}$). This value is interpolated within the dilution series.

No extra correction procedures for colour and turbidity are needed because these factors remain the same during the whole measurement. Inhibition at different contact times and different sample concentrations yields complete kinetic toxicological data about the sample (inhibition, expressed as a percentage vs. concentration vs. time) and enables assumptions to be made about the nature of the contaminants if compared with existing data (see Annex B).

## 5 Interferences

Volatile substances or substances which react with the dilution water or the test suspension, or alter their state during the test period, may affect the result or impair the reproducibility of the test results.

Since oxygen is required for the bioluminescence (Reference [18]), samples with high oxygen demand (or low oxygen concentration) may cause deficiency of oxygen and be inhibitory.

Readily biodegradable nutrients in the sample may cause a pollutant-independent reduction in bioluminescence (Reference [19]).





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

Samples with a pH outside the range 6,0 to 8,5 affect the luminescence of bacteria (References [18][20]). Adjust the sample when the toxic effect of pH is not of interest.

As the test organism *Vibrio fischeri* is a marine bacterium, testing salt water samples with the standard procedure often leads to stimulation effects of bioluminescence, which may mask inhibition effects (see ISO 11348-3:2007, Annex D).

Salt concentrations in the initial sample exceeding 30 g/l NaCl or contents of other compounds giving equal osmolarity may lead, together with the salt spiking required by the test, to hyperosmotic effects. The resulting salt concentration in the test samples should not exceed the osmolarity of a 35 g/l NaCl solution in order to avoid these effects.

## 6 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled or demineralized water or water of equivalent purity.

**6.1 Test bacteria**. Use a strain of luminescent bacteria belonging to the species *Vibrio fischeri* NRRL B-11177.

The bacterial suspensions used for toxicity measurements are prepared according to the instructions in ISO 11348. Dilute the bacterial suspension before the assay from the stock suspension to the measuring concentration (example: ISO 11348-3:2007, variant B).

**6.2 Sodium chloride solution**, as diluent.

Dissolve 20 g of sodium chloride (NaCl) in water and make up to 1 l with water.

**6.3 Sodium hydroxide solution**, e.g. $c$(NaOH) = 1 mol/l.

**6.4 Hydrochloric acid**, e.g. $c$(HCl) = 1 mol/l.

For the adjustment of the pH it may be necessary to use acids or bases of lower or higher concentration.

**6.5 Solution for freeze-dried bacteria**.

— 20,0 g sodium chloride (NaCl);

— 2,035 g magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$);

— 0,30 g potassium chloride (KCl).

Dissolve in water and make up to 1 l with water. Store the solution in portions in a freezer at −18 °C to −20 °C.

**6.6 Reference substances**.

Prepare the following three separate reference substance solutions with sodium chloride solution (6.2) as diluent without adjustment of the pH for the use of freeze-dried bacteria:

a) 6,8 mg/l 3,5-dichlorophenol (DCP, $C_6H_4OCl_2$);

b) 19,34 mg/l zinc sulfate heptahydrate ($ZnSO_4 \cdot 7\ H_2O$);

c) 105,8 mg/l potassium dichromate ($K_2Cr_2O_7$).





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

The concentrations in a) to c) are approximately twice the expected $EC_{50}$ values for the respective reference substances in ISO 11348-3. For concentrations of reference substance solutions for freshly cultured or liquid-dried bacteria, see ISO 11348-1 or ISO 11348-2. The volumes required depend on the test set-up.

NOTE 1 It is possible to use commercially available chemical preparations with defined concentrations of $ZnSO_4$ and $K_2Cr_2O_7$ for the preparation of the solutions of the reference substances.

NOTE 2 For more information about reference substances, see Reference [22].

## 7 Apparatus

Usual laboratory equipment, and in particular the following.

**7.1 Freezer**, for the storage of preserved bacteria.

**7.2 Incubator** or **refrigerator**, to maintain the solution for freeze-dried bacteria (6.5) at a temperature of (4 ± 3) °C.

**7.3 Thermostatically controlled thermo-block**, to maintain the test samples and the *Vibrio fischeri* suspension at a temperature of (15 ± 1) °C. Within one test, the temperature deviation should be at most ±0,3 °C.

**7.4 Luminometer**, equipped with at least one dispenser (minimum injection volume 0,2 ml).

The instrument shall be capable of measuring and recording the luminescence continuously at least for 5 s in 0,2 s intervals or shorter. The injection and recording shall be performed simultaneously.

**7.5 Test tubes, cuvettes, test plates** or any other suitable **test vessels**, made of chemically inert material, appropriate for the selected luminometer.

**7.6 pH-meter**.

**7.7 Chronometer**.

**7.8 Piston pipettes**, nominal capacity 100 µl to 1 000 µl, ISO 8655-2[3].

**7.9 Mixer**, e.g. vortex mixer, for mixing the samples before the measurements.

**7.10 Conductometer**.

**7.11 Oxygen probe**, as specified in ISO 5814.

**7.12 Sieve**, nominal size of openings 2 mm, ISO 565[1].

## 8 Sampling and sample pre-treatment

### 8.1 Sampling

Collect the samples in chemically inert, clean containers as specified in ISO 5667-16. Fill the containers completely and seal them. Cool the samples on ice, or in a refrigerator or a cooling box at 2 °C to 5 °C and test them as soon as possible after collection. Where necessary, store samples at a temperature of 2 °C to 5 °C in the dark for no longer than 48 h. If the samples have to be frozen store them at a temperature of −18 °C or below in the dark for no longer than 2 months. Prepare and measure frozen samples immediately after thawing in a water bath. For long-term storage, the samples may be freeze-dried and stored in the dark at room temperature. Do not use chemicals to preserve the samples. Perform the necessary pH adjustment and salt addition just before testing.

### 8.2 Sample preparation

Sieve the samples to remove any coarse material (e.g. plant roots and particles larger than 2 mm).

Suspend sediment samples in water (e.g. 20 % mass fraction) by vigorous shaking (vortexing) in order to obtain homogeneous suspensions (Reference [22]). Also, mass fractions higher than 20 % can be measured if the suspensions stay homogeneous during the whole measurement.

Measure the oxygen concentration in all samples and sample water suspensions. An oxygen concentration >3 mg/l is required for the test. If the oxygen concentration of the undiluted sample is less than 3 mg/l, use appropriate methods to oxygenate the sample, e.g. aeration or stirring.

Measure the pH of all samples and sample water suspensions. If the pH is between 6,0 and 8,5, no adjustment is necessary. The adjustment of the pH value, however, may alter the nature of the sample. On the other hand, the pH of the sample and the pH of the test batch can differ because of the buffer capacity of the test medium. It may be necessary to carry out tests on both the pH-adjusted and the non-pH-adjusted samples.

If necessary, adjust the pH of the samples by adding either hydrochloric acid (6.4) or sodium hydroxide solution (6.3). Depending on the purpose of the test, the pH may be adjusted to $7{,}0 \pm 0{,}2$ or to the upper ($8{,}5 \pm 0{,}2$) or lower ($6{,}0 \pm 0{,}2$) limits. Choose the concentration of the hydrochloric acid or the sodium hydroxide solution to restrict the volume added to not more than 5 % of total volume.

Add 20 g of sodium chloride per litre to the sample or to the neutralized sample.

For salt water samples, ISO 11348-3:2007, Annex D gives further information.

## 9 Procedure

### 9.1 Initial preparations

#### 9.1.1 Preparation of test solutions

Prepare the reference samples according to 6.6. Test each batch of bacteria after delivery with all three reference substances. Test at least one of the three reference substances with each vial of stock suspension reconstituted.

Prepare the samples according to 8.2.

Prepare, in the first set of test tubes (7.5), the sample dilution series, the reference sample (6.6) and the control (6.2) required.

Serially dilute the sample. Make the dilutions in separate tubes and transfer them to the measurement cuvettes or plates. Normally, in this test, equal volumes of test suspension and sample (or diluted sample) are mixed, giving tested dilution levels within a series of $D \geqslant 2$.

The minimum dilution which can be tested is 1,00 in 1,25, prepared by mixing 4 volumes of sample with 1 volume of test suspension (e.g. 800 µl sample plus 200 µl test suspension). The corresponding $D$ value is 1. For this $D$ value, an extra control batch is needed which is made up by adding 200 µl of the test suspension to 800 µl sodium chloride solution (see Annex C).

Maintain the test tubes containing the sodium chloride solution (6.2) for controls, the reference samples (6.6), the samples (8.2) and the samples of the dilution series (Annex C) at $(15 \pm 1)$ °C.

Choose test conditions which ensure that the maximum temperature deviation in the thermo-block within one test is no more than ±0,3 °C.

#### 9.1.2 Preparation of test suspensions

Prepare the luminescent bacterial stock suspension for the test according to the instructions for freshly cultured (ISO 11348-1), liquid-dried (ISO 11348-2) or freeze-dried (ISO 11348-3) bacteria test.

Prepare the test suspension used in the kinetic test as specified, for example, in ISO 11348-3:2007, 8.3.3, Variant B, where the test suspension is prepared outside the test tubes. Dilute reconstituted bacterial suspension with solution (6.5) to form the test suspension before starting the test.

Store the luminescent bacteria test suspension for the measurement (cell concentration about $2 \times 10^6$ cells/ml) at (4 ± 3) °C. The suspension is ready to use after a minimum of 30 min in a refrigerator and can be used for testing purposes as long as the validity criteria stated in Clause 12 are met.

Before initiating the measurement, store the test suspension at (15 ± 1) °C for at least 30 min.

### 9.2 Test procedure

Carry out duplicate or triplicate determinations per dilution level at a test temperature of (15 ± 1) °C.

Set the dispensing volume from 200 µl to 500 µl (equal to the sample volume) and prime the dispenser with the *Vibrio fischeri* bacterial test suspension.

Mix (7.9) the samples well and pipette 200 µl to 500 µl of each well-homogenized sample into the luminometer cuvettes or microtiter plate wells depending on the instrument used. If the sample contains large particles, cutting the sharp point of the pipette tip can facilitate the pipetting. Wider pipette tips are also commercially available.

Load the sample cuvette (or microtiter plate) into the luminometer and start the run by dispensing *Vibrio fischeri* bacterial suspension into the cuvette. Determine and record the maximum luminescence value of the test suspension during the first 5 s of contact (at least 5 measurements/s). The maximum luminescence intensity is the peak-value, $I_p$. Immediately after the measurement, put the cuvette back into the incubator and measure the next cuvette. Repeat this process for each sample and control. When a microtiter plate reader is used, run the controls and test sample dilution series immediately before transferring the plate to the incubator. Store the cuvettes at (15 ± 1) °C during the incubation.

Determine and record the luminescence intensity after, optionally 5 min, $I_5$, and again after 15 min, $I_{15}$, and 30 min, $I_{30}$, as required. Mix the samples by hand before each measurement. Repeat this procedure for every control and sample.

Intervals of 30 s between the samples have been found adequate to allow sufficient time for the readings to be made and the change of the cuvettes.

## 10 Evaluation

### 10.1 Inhibitory effect on luminescent bacteria

Calculate the correction factor, $f_{kt}$, for the contact time (5 min, 15 min or 30 min) from the measured luminescence intensity using Equation (1). This factor serves to correct the initial values, $I_p$, of all test samples before they can be used as reference values for the determination of the water-dependent decrease of luminescence.

$$f_{kt} = \frac{I_{kt}}{I_p} \tag{1}$$

where

$I_{kt}$ is the luminescence intensity in the control sample after the contact time of 5 min, 15 min or 30 min, in relative luminescence units;





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

$I_p$ is the maximum luminescence intensity (the peak value), in relative luminescence units, of the control test suspension immediately after dispensing the bacteria into the sample.

Calculate the mean correction factor $\overline{f}_{kt}$. Find the deviation of the individual replicates from the mean, expressed as a percentage to one significant figure, using Formula (2):

$$\left[\frac{\left(\overline{f}_{kt} \pm f_{kt,\,i}\right)}{\overline{f}_{kt}}\right] \times 100 \tag{2}$$

where $f_{kt,i}$ is either of the two individual values of the correction factor.

Calculate the corrected value of $I_p$ for test sample cuvettes (corrected peak intensity value), $I_{ct}$, using Equation (3):

$$I_{ct} = I_p \cdot \overline{f}_{kt} \tag{3}$$

where

$\overline{f}_{kt}$ is the mean of $f_{kt,i}$;

$I_p$ is the maximum luminescence intensity (the peak value), in relative luminescence units, of the test suspension immediately after dispensing the bacteria into the sample.

Calculate the inhibitory effect of a test sample after the contact time (5 min, 15 min or 30 min), $H_t$, expressed as a percentage, using Equation (4):

$$H_t = \frac{(I_{ct} - I_t)}{I_{ct}} \times 100 \tag{4}$$

where

$I_{ct}$ is the corrected peak intensity value given by Equation (3);

$I_t$ is the luminescence intensity of the test sample after the contact time (5 min, 15 min or 30 min), in relative luminescence units.

Calculate the mean of the inhibitory effect $\overline{H}_t$ for each dilution level, expressed as a percentage.

Calculate the arithmetic difference, expressed in per cent points to one significant figure:

$$\overline{H}_t(\%) - H_{t,i}(\%)$$

where

$H_{t,i}$ is either of the two individual values of the inhibitory effects of at least one test sample;

$\overline{H}_t$ is the respective mean value.

## 10.2 Determination of EC values

Calculate the concentration-effect relationship for each exposure time using suitable standard linear or non-linear regression analysis (see Reference [19]).





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

For evaluation of the relationship between concentration and effect using a linear regression technique, calculate the gamma value (ratio of light lost to the amount of light remaining at time $t$), $\Gamma_t$, for each dilution level of the test sample after the contact time (5 min, 15 min or 30 min) using Equation (5):

$$\Gamma_t = \frac{\overline{H}_t}{(100 - \overline{H}_t)} \tag{5}$$

where $\overline{H}_t$ is the mean of the values of $H_t$ obtained by Equation (4).

NOTE When a certain test concentration gives 0 % or 100 % inhibition of bioluminescence, the gamma value cannot be calculated. Therefore, usually only $H_t$ values between 10 % and 90 % are used in the calculation of the concentration-effect relationship.

The concentration-effect relationship at a given exposure time can often be described by the linear equation:

$$\lg c_t = b \lg \Gamma_t + \lg a \tag{6}$$

where

$c_t$ is the portion of the sample (e.g. water or sediment) within the test sample, expressed as a percentage;

$\Gamma_t$ is given by Equation (5);

$b$ is the value of the slope of the described line;

$\lg a$ is the value of the intercept of the described line.

By means of standard least-squares regression statistics, calculate the $EC_{20}$ and $EC_{50}$ values with corresponding confidence limits, in which:

$c_t = EC_{20t}$ at $\Gamma_t = 0{,}25$

$c_t = EC_{50t}$ at $\Gamma_t = 1{,}00$

For non-linear regression analysis, various models are available within standard graphic or statistical software packages. They are typically based on functions of the normal distribution (i.e. Probit analysis), the logistic distribution (i.e. Logit analysis), or the Weibull distribution (i.e. Weibull analysis). Calculated inhibitory effects, $H_t$, can be used directly to estimate parameters of the non-linear concentration-effect relationship, from which EC values for any level might subsequently be derived (see Reference [19]).

If a curve cannot be fitted to the range of value pairs, the EC values can be estimated graphically using a double logarithmic coordinate system.

## 11 Expression of results

Report the results in accordance with the example in Table 1.





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

**Table 1 — Example of test evaluation — Sample: River sediment suspension 50 g/l**

| Control experiments | | | | | | |
|---|---|---|---|---|---|---|
| Control batch number | Dilution level $D$ | Measured values $I_p$ | Measured values $I_{k30}$ | $I_{k30}/I_p = f_{k30}$ | $\overline{f}_{k30}$ | Validity test: Deviation from the mean $\overline{f}_{k30}$ % [b] |
| 1 | 2[a] | 1 393 | 1 025 | 0,7358 | 0,7381 | 0,31 |
| 2 | | 1 390 | 1 029 | 0,7403 | | |

| Test experiments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Test batch number | Dilution level $D$ | Measured values $I_p$ | Measured values $I_{30}$ | $I_{c30}$ | $H_{30}$ % | $\overline{H}_{30}$ % | Validity test: Deviation from the mean [c] % points | $\Gamma_{30}$ |
| 1 | 2 | 246 | 7,1 | 181,6 | 96,1 | 95,55 | 0,5 | 21,460 |
| 2 | | 228 | 8,4 | 168,3 | 95,0 | | | |
| 3 | 4 | 403 | 13,2 | 297,4 | 95,6 | 95,44 | 0,1 | 20,920 |
| 4 | | 369 | 13,7 | 292,3 | 95,3 | | | |
| 5 | 8 | 640 | 66 | 472,4 | 86,0 | 85,15 | 0,9 | 6,492 |
| 6 | | 646 | 75 | 476,8 | 84,3 | | | |
| 7 | 16 | 897 | 280 | 662,0 | 57,7 | 54,80 | 2,9 | 1,305 |
| 8 | | 893 | 317 | 659,1 | 51,9 | | | |
| 9 | 32 | 1 089 | 562 | 803,7 | 30,1 | 29,64 | 0,4 | 0,494 |
| 10 | | 1 064 | 556 | 785,3 | 29,2 | | | |
| 11 | 64 | 1 225 | 740 | 904,1 | 18,2 | 16,08 | 2,1 | 0,207 |
| 12 | | 1 188 | 754 | 876,8 | 14,0 | | | |
| 13 | 128 | 1 311 | 863 | 967,6 | 10,8 | 8,61 | 2,2 | 0,094 |
| 14 | | 1 261 | 871 | 930,7 | 6,4 | | | |
| | Reference substance | | | | | | | |
| 15 | 3,4 mg/l DCP or 2,2 mg/l Zn or 18,7 mg/l Cr | 966 | 296 | 713,0 | 58,5 | 57,42 | 1,1 | 1,348 |
| 16 | | 925 | 298 | 682,7 | 56,3 | | | |

NOTE 1 In this example, the lowest value of $D$ tested, at which the mean inhibitory effect, $H_{30} < 20$ %, $LID_{lb} = 64$.

NOTE 2 In this example, $EC_{20} = 905$ mg/l; $EC_{50} = 2{,}34$ g/l (standard least-square statistics).

NOTE 3 Dilution levels $D = 2$ and $D = 4$ were rejected from the calculation.

[a] See Annex C.

[b] For the control batch, the deviation from the mean $\overline{f}_{k30}$ is determined by the arithmetic difference of the parallel determinations from the mean, divided by the mean expressed in % [Formula (2)].

[c] For the test batch, the deviation of the $H_{30}$-values (in %) of the parallel measurements from the mean is calculated as the arithmetic difference of each $H_{30}$-value (in %) from the mean $\overline{H}_{30}$ (in %) (called % points).





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

Report the test duration.

Report the $EC_{20}$ and $EC_{50}$ values and the method for the derivation of these values.

If determined, report the lowest value of $D$ tested, at which the mean inhibitory effect, $H_{30} < 20$ %, $LID_{lb}$ (see ISO 11348-3:2007, Annex B).

Report the type of bacterial preparation used.

## 12 Criteria of validity

The test is valid if:

a) the $\overline{f}_{kt}$ value for 30 min incubation ranges between 0,6 and 1,8;

b) the $I_p$ (peak value) of the sample is >5 % of $I_p$ of the control sample;

c) the parallel determinations do not deviate from their mean by more than 3 % for the control samples;

d) for the test samples which determine the $LID_{lb}$, $EC_{20}$ or $EC_{50}$ values, respectively, the deviation of the parallel determinations from their mean, in % points, does not exceed 3 % points (see Table 1, Footnote c).

Because different bacterial preparations (freeze-dried, liquid-dried and freshly prepared) have slightly different $EC_{50}$ values with the reference chemicals, the operators are recommended to follow the validity criteria set for their bacterial preparation. For the batch of freeze-dried bacteria delivered, the three reference substances (6.6) (solutions not neutralized, check separately) cause 20 % to 80 % inhibition after 30 min of contact time at the following concentrations in the final test suspension:

1) 3,4 mg/l 3,5-dichlorophenol;

2) 2,2 mg/l Zn(II) (equivalent to 9,67 mg/l zinc sulfate heptahydrate);

3) 18,7 mg/l Cr(IV) (equivalent to 52,9 mg/l potassium dichromate).

One of these three reference substances (6.6) (solution not neutralized) tested in parallel to each stock suspension vial reconstituted for the actual test (9.2) causes 20 % to 80 % inhibition after 30 min contact time.

## 13 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this International Standard (ISO 21338:2010);

b) identity of the sample, including sampling, storage time and conditions;

c) pH and oxygen concentration, in milligrams per litre or as a percentage saturation, of the original water sample or the water suspension of the solid sample before testing;

d) date of carrying out the test;

e) sample pre-treatment, if any, e.g. pH before and after adjustment;

f) bacterial preparation;





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

g) origin of the bacteria, batch number, date of delivery, and expiration date;

h) storage temperature of the bacteria;

i) expression of the results, as specified in Clause 11, Table 1;

j) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

k) test results with reference substances for the batch of bacteria and the actual test.

# Annex A
(informative)

# Precision data

To evaluate the applicability of the method for testing toxicity of coloured and turbid samples, an interlaboratory trial was organized in April 2007. Altogether, eight laboratories participated in the comparison. Two participants reported results from measurements using two different instruments.

## A.1 Samples

The following four samples were sent to the participants:

a) V1: synthetic water sample, spiked with 20 mg/l 3,5-DCP (toxic reference substance);

b) V2: coloured river water sample (non-toxic);

c) S1: sediment sample (freeze-dried), river sediment from the recipient of an old sawmill (toxic);

d) S2: sediment sample (freeze-dried), clean river sediment (non-toxic).

The samples were prepared and their homogeneity as well as preservation were checked in Western Finland Environment Centre, Kokkola laboratory.

Before testing, samples S1 and S2 were prepared by mixing 0,5 g freeze-dried sediment into 10 ml NaCl solution (6.2).

## A.2 Analytical methods

| **Laboratory** | **Instrument**[c] |
|---|---|
| 1[a] | LKB-Wallac 1251 Luminometer, Tube Luminometer, Carousel |
| 2[b] | Triathler, Tube Luminometer |
| 3 | Sirius-Berthold Detection Systems, Tube Luminometer |
| 4 | Luminoscan 1251 Carousel, Tube Luminometer |
| 5[b] | Plate Chameleon V, Microtiter Plate Luminometer |
| 6 | Luminometer Sirius 142, Tube Luminometer |
| 7 | 1251 Luminometer (Bio-Orbit), Tube Luminometer |
| 8 | Sirius 1-Berthold, Tube Luminometer |
| 9 | 1251 Luminometer (Bio-Orbit), Tube Luminometer |
| 10[a] | Labsystems Fluorosan Ascent FL, Microtiter Plate Luminometer |

[a, b] Participants reported results from measurements using two different instruments (tube luminometer and plate luminometer); they were numbered separately. Number of participants = 8, number of data sets = 10.

[c] The trade names listed in this column are given for the information of users of this document. Such listing does not constitute an endorsement by ISO of the products named.

## A.3 Results

Two participants reported results from measurements using two different instruments. The results of laboratories 2 and 5 were rejected from the performance evaluation because of errors in the measurements and calculations.

A summary of the results is presented in Table A.1 and the more detailed results of the comparison are presented in Tables A.2, A.3 and A.4.

The $EC_{20}$ and $EC_{50}$ values were calculated according to Equations (5) and (6).

The mean value, the standard deviation, and the coefficient of variation were calculated after rejection of the outliers by the Hampel test. The robust mean value was chosen as the assigned value. Performance of the participants was evaluated by using $z$-scores.

The repeatability, $s_w$%, and reproducibility, $s_b$%, of the method were tested according to ANOVA statistics from two parallel measurements (Table A.3).

All participants found samples V1 and S1 toxic and samples V2 and S2 non-toxic.

The performance evaluation was performed only for the toxic samples (V1 and S1). In samples V2 and S2, no inhibition of luminescence intensity was detected, indicating that the samples were not toxic.

In this comparison, 84 % of the results were acceptable, when the deviation of 30 % for the water sample V1 and 50 % deviation for the sediment sample S1 was permitted (equivalent to 95 % significance).

Water sample V1 was spiked with 3,5-dichlorophenol in order to calculate the $EC_{50}$ value for this reference substance. The reference substance caused the inhibition between 20 % to 80 % after 30 min contact time.

**Table A.1 — Summary of results received: $EC_{20}$ and $EC_{50}$ values**

| Laboratory | Sample V1 | | Sample V2 | | Sample S1 | | Sample S2 | |
|---|---|---|---|---|---|---|---|---|
| | $EC_{20}$ | $EC_{50}$ | $EC_{20}$ | $EC_{50}$ | $EC_{20}$ | $EC_{50}$ | $EC_{20}$ | $EC_{50}$ |
| | % | % | % | % | mg/l | mg/l | mg/l | mg/l |
| 1 | 9,7 | 14,1 | >50[a] | >50 | 540 | 1 516 | >25 000[a] | >25 000 |
| 2 | 11,8 | 17,3 | >50 | >50 | 2 268 | 5 895 | >25 000 | >25 000 |
| 3 | 9,1 | 12,2 | >50 | >50 | 1 660 | 3 298 | >25 000 | >25 000 |
| 4 | 16,0 | 20,8 | >50 | >50 | 813 | 2 123 | >25 000 | >25 000 |
| 5 | 17,1 | 22,6 | >50 | >50 | 2 593 | 7 961 | >25 000 | >25 000 |
| 6 | 6,1 | 12,6 | >50 | >50 | 833 | 2 237 | >25 000 | >25 000 |
| 7 | 11,5 | 15,6 | >50 | >50 | 548 | 1 490 | >25 000 | >25 000 |
| 8 | 8,1 | 11,4 | >50 | >50 | 833 | 1 166 | >12 572 | >25 000 |
| 9 | 11,5 | 13,9 | >50 | >50 | 449 | 914 | >25 000 | >25 000 |
| 10 | 9,9 | 13,9 | >50 | >50 | 613 | 2 216 | >25 000 | >25 000 |

NOTE 1 The values are means of two measurements of each laboratory.

NOTE 2 The results of laboratories 2 and 5 have been rejected from the performance evaluation.

[a] Highest tested concentration.

**Table A.2 — Summary of the proficiency test: robust values**

| Data | Effective concentration causing | | | |
|---|---|---|---|---|
| | 20 % luminescence inhibition $EC_{20}$ | 50 % luminescence inhibition $EC_{50}$ | 20 % luminescence inhibition $EC_{20}$ | 50 % luminescence inhibition $EC_{50}$ |
| Sample | V1 | V1 | S1 | S1 |
| Unit | % | % | mg/l | mg/l |
| Assigned value | 9,9 | 13,4 | 707 | 1 798 |
| Mean value | 10,4 | 13,9 | 786 | 1 867 |
| Robust mean value | 9,9 | 13,4 | 707 | 1 798 |
| Median value | 11,1 | 14,2 | 821 | 2 203 |
| Standard deviation, $s_{rob}$, absolute value | 2,6 | 2,1 | 225 | 704 |
| Standard deviation, $s_{rob}$, % | 26,2 | 16,5 | 31,9 | 39,2 |
| No. of labs (participants) | 10 | 10 | 10 | 10 |
| Twice target standard deviation[a], $2s_{targ}$,% | 30 | 30 | 50 | 50 |
| Accepted $z$-value[b],% | 60 | 70 | 70 | 70 |

[a] 95 % Confidence level.

[b] Satisfied $z$-values: the results where $|z| \leqslant 2$.

**Table A.3 — Results of the duplicate determinations: ANOVA-statistics**

| Data | Effective concentration causing | | | |
|---|---|---|---|---|
| | 20 % luminescence inhibition $EC_{20}$ | 50 % luminescence inhibition $EC_{50}$ | 20 % luminescence inhibition $EC_{20}$ | 50 % luminescence inhibition $EC_{50}$ |
| Sample | V1 | V1 | S1 | S1 |
| Unit | % | % | mg/l | mg/l |
| Assigned value | 9,9 | 13,4 | 707 | 1 798 |
| Mean | 10,4 | 14,3 | 795 | 1 893 |
| Median value | 9,9 | 13,6 | 654 | 1 713 |
| Repeatability standard error, $s_w$, absolute value | 0,68 | 0,66 | 161 | 234 |
| Repeatability standard error, $s_w$, % | 6,6 | 4,6 | 20 | 12 |
| Standard error between laboratories, $s_b$, absolute value | 2,9 | 3,0 | 390 | 762 |
| Standard error between laboratories, $s_b$, % | 28 | 21 | 49 | 40 |
| Reproducibility error, $s_t$, absolute value | 3,0 | 3,1 | 422 | 797 |
| Reproducibility error, $s_t$, % | 29 | 22 | 53 | 42 |
| No. labs | 9 | 9 | 8 | 8 |





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

**Table A.4 — Precision data for freeze-dried bacteria**

| **3,5-Dichlorophenol, sample V1** | **Effective concentration causing** | |
|---|---|---|
| | 20 % luminescence inhibition $EC_{20}$ | 50 % luminescence inhibition $EC_{50}$ |
| No. labs, $p$ | 8 | 8 |
| No. data sets, $n$ | 10 | 10 |
| No. outliers, $n_{AP}$ | 2 | 2 |
| Mean concentration, $\overline{\overline{\rho}}$, mg/l | 1,98 | 2,78 |
| Reproducibility standard deviation, $s_R$, mg/l | 0,57 | 0,61 |
| Coefficient of variation of reproducibility, $C_{V,R}$, % | 28,8 | 21,9 |



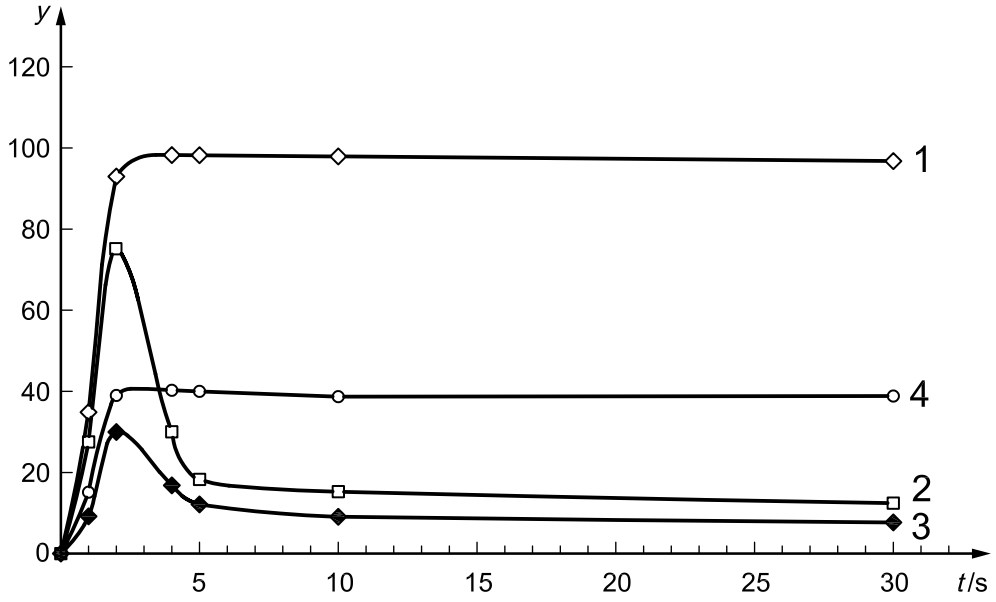


Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

# Annex B
(informative)

## Typical kinetic curves from different samples

See Figure B.1.




**Key**

$t$ time
$y$ relative light units
1 response from control (2 % mass fraction NaCl solution)
2 response from colourless toxicant DCP
3 response from a soil sample contaminated with oil
4 response from similar non-toxic soil

**Figure B.1 — Typical kinetic behaviour of toxic and non-toxic solid samples in the kinetic luminescent bacteria test during 30 s of exposure**

# Annex C
(informative)

# Dilution series

See Table C.1 and Figure C.1.

**Table C.1 — Preparation of dilution series**

| Dilution | Dilution level | Tube luminometer | | Plate luminometer | |
|---|---|---|---|---|---|
| | | Sample or diluted sample | Test suspension | Sample or diluted sample | Test suspension |
| | $D$ | µl | µl | µl | µl |
| 1→1,25 | 1 | 800 | 200 | 320 | 80 |
| 1→2 | 2 | 500 | 500 | 200 | 200 |
| 1→3 | 3 | 500 | 500 | 200 | 200 |
| 1→4 | 4 | 500 | 500 | 200 | 200 |
| 1→6 | 6 | 500 | 500 | 200 | 200 |
| 1→8 | 8 | 500 | 500 | 200 | 200 |
| 1→12 | 12 | 500 | 500 | 200 | 200 |
| 1→16 | 16 | 500 | 500 | 200 | 200 |
| 1→24 | 24 | 500 | 500 | 200 | 200 |
| 1→32 | 32 | 500 | 500 | 200 | 200 |
| Control batches | | | | | |
| for $D = 1$ | | 800 | 200 | 320 | 80 |
| for $D \geqslant 2$ | | 500 | 500 | 200 | 200 |

NOTE The easiest way to prepare this dilution series is to first make two stock dilutions:

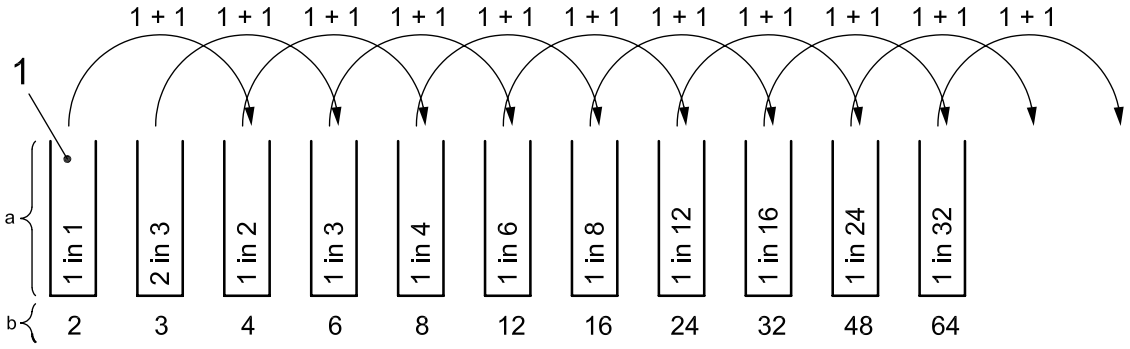
a) dilution 1→1, e.g. undiluted sample 3 000 µl (final dilution in test after mixing with equal volumes of test suspension is 1→2);

b) dilution 2→3, e.g. 2 000 µl of sample + 1 000 µl of solution 6.2 (final dilution in test after mixing with equal volumes of test suspension is 1→3).





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013




**Key**

1 sample

a Dilution of sample to perform in the first set of test tubes.

b Final dilution level $D$ after addition to test suspension.

**Figure C.1 — Dilution series**

# Bibliography

[1] ISO 565, *Test sieves — Metal wire cloth, perforated metal plate and electroformed sheet — Nominal sizes of openings*

[2] ISO 6107-6:2004, *Water quality — Vocabulary — Part 6*

[3] ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Piston pipettes*

[4] ISO/TS 20281:2006, *Water quality — Guidance on statistical interpretation of ecotoxicity data*

[5] LAPPALAINEN, J., JUVONEN, R., VAAJASAARI, K., KARP, M. A new flash method for measuring the toxicity of solid and colored samples. *Chemosphere* 1999, **38**, pp. 1069-1083

[6] RANTALA, P.-R., VAAJASAARI, K., JUVONEN, R., SCHULTZ, E., JOUTTI, A., MÄKELÄ-KURTTO, R. Composting of forest industry wastewater sludges for agricultural use. *Water Sci. Technol*. 1999, **40**, pp. 11-12, pp. 187-194

[7] PÖLLUMAA, L., KAHRU, A., EISENTRÄGER, A., REIMAN, R., MALOVERYAN, A., RÄTSEP, A. Toxicological investigation of soils with the solid-phase flash assay: Comparison with other ecotoxicological tests. *Altern. Lab. Anim.* 2000, **28**, pp. 461-472

[8] JUVONEN, R., MARTIKAINEN, E., SCHULTZ, E., JOUTTI, A., AHTIAINEN, J. LEHTOKARI, M. A battery of toxicity tests as indicators of decontamination in composting oily wastes. *Ecotoxicol. Environ. Saf*, 2000, **47**, pp. 156-166

[9] LAPPALAINEN, J., JUVONEN, R., NURMI, J., KARP, M. Automated color correction method for *Vibrio fischeri* toxicity test: Comparison of standard and kinetic assays. *Chemosphere* 2001, **45**, 2001, pp. 635-641

[10] DEGLI-INNOCENTI, F., BELLIA, G., TOSIN, M., KAPANEN, A., ITÄVAARA, M. Detection of toxicity released by biodegradable plastics after composting in activated vermiculite. *Polym. Degradation Stability* 2001, **73**, pp. 101-106

[11] LYYTIKÄINEN, M. SORMUNEN, A., RISTOLA, T., JUVONEN, R., KUKKONEN, J.V.K. Toxicity of freshwater sediments in the vicinity of an old sawmill: Application of three bioassays. *Arch. Environ. Contam. Toxicol*. 2001, **40**, pp. 318-326

[12] BLAISE, C., GAGNÉ, F., CHÈVRE, N., HARWOOD, M., LEE, K., LAPPALAINEN, J., CHIAL, B., PERSOONE, G., DOE, K. Toxicity assessment of oil-contaminated freshwater sediments. *Environ. Toxicol.* 2004, **19**, pp. 267-273

[13] PÖLLUMAA, L., KAHRU, A., MANUSADZIANAS, L. Biotests- and chemistry-based hazard assessment of soils, sediments and solid wastes. *J. Soils Sediments* 2004, **4**, pp. 267-275

[14] KAHRU, A., PÖLLUMAA, L., REIMAN, R., RÄTSEP, A. Predicting the toxicity of oil-shale industry wastewater by its phenolic composition. *Altern. Lab. Anim.* 1999, **27**, pp. 359-366

[15] KAHRU, A., PÖLLUMAA, L., REIMAN, R., RÄTSEP, A., LIIDERS, M., MALOVERYAN, A. The toxicity and biodegradability of eight main phenolic compounds characteristic to the oil-shale industry wastewaters: A test battery approach. *Environ. Toxicol.* 2000, **15**, pp. 431-442

[16] PÖLLUMAA, L., MALOVERYAN, A., TRAPIDO, M., SILLAK, H., KAHRU, A. Study of the environmental hazard caused by the oil-shale industry solid waste. *Altern. Lab. Anim.* 2001, **29**, pp. 259-267





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

[17] KAHRU, A., MALOVERYAN, A., SILLAK, H., PÖLLUMAA, L. The toxicity and fate of phenolic pollutants in the contaminated soils associated with the oil-shale industry. *Environ. Sci. Pollut. Res.* 2002, (Spec. 1), pp. 27-33

[18] POSTMA, J.F., DE VALK, S., DUBBELDAM, M., MAAS, J.L., TONKES, M., SCHIPPER, C.A., KATER, B.J. Confounding factors in bioassays with freshwater and marine organisms. *Ecotoxicol. Environ. Saf.*, 2002, **53**, pp. 226-237

[19] GRABERT, E., KÖSSLER, F. About the effect of nutrients on the luminescent bacteria test. In: HASTINGS, J.W., KRICKA, L.J., STANLEY, P.E., editors. *Bioluminescence and chemiluminescence: Molecular reporting with photons*, pp. 291-294. Chichester: Wiley, 1997

[20] KREBS, F. Toxizitätstest mit gefriergetrockneten Leuchtbakterien [Toxicity test with freeze-dried bacteria]. *Gewässerschutz, Wasser, Abwasser* 1983, **63**, pp. 173-230

[21] ENVIRONMENT CANADA (2002). *Biological test method: Reference method for determining the toxicity of sediment using luminescent bacteria in a solid-phase test*. Ottawa, ON: Environment Canada, 2002. 60 p. (EPS 1/RM/42.)

[22] MORTIMER, M., KASEMETS, K., HEINLAAN, M., KURVET, I., KAHRU, A. High throughput kinetic *Vibrio fischeri* bioluminescence inhibition assay for study of toxic effects of nanoparticles. *Toxicol.* in vitro 2008, **22**, pp. 1412-1417





Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

**ICS 13.060.60**
Price based on 21 pages




Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013

*This page deliberately left blank*

# British Standards Institution (BSI)

BSI is the independent national body responsible for preparing British Standards and other standards-related publications, information and services.
It presents the UK view on standards in Europe and at the international level.

It is incorporated by Royal Charter.

## Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover.

**Tel: +44 (0)20 8996 9001 Fax: +44 (0)20 8996 7001**

BSI offers Members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

**Tel: +44 (0)20 8996 7669 Fax: +44 (0)20 8996 7001**
**Email: plus@bsigroup.com**

## Buying standards

You may buy PDF and hard copy versions of standards directly using a credit card from the BSI Shop on the website **www.bsigroup.com/shop.** In addition all orders for BSI, international and foreign standards publications can be addressed to BSI Customer Services.

**Tel: +44 (0)20 8996 9001 Fax: +44 (0)20 8996 7001**
**Email: orders@bsigroup.com**

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

## Information on standards

BSI provides a wide range of information on national, European and international standards through its Knowledge Centre.

**Tel: +44 (0)20 8996 7004 Fax: +44 (0)20 8996 7005**
**Email: knowledgecentre@bsigroup.com**

Various BSI electronic information services are also available which give details on all its products and services.

**Tel: +44 (0)20 8996 7111 Fax: +44 (0)20 8996 7048**
**Email: info@bsigroup.com**

BSI Subscribing Members are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration.

**Tel: +44 (0)20 8996 7002 Fax: +44 (0)20 8996 7001**
**Email: membership@bsigroup.com**

Information regarding online access to British Standards via British Standards Online can be found at **www.bsigroup.com/BSOL**

Further information about BSI is available on the BSI website at **www.bsigroup.com/standards**

## Copyright



**BSI Group Headquarters**

389 Chiswick High Road London W4 4AL UK

Tel +44 (0)20 8996 9001
Fax +44 (0)20 8996 7001
www.bsigroup.com/standards

*raising standards worldwide™*

Licensed copy: Lee Shau Kee Library, HKUST, Version correct as of 03/01/2015, (c) The British Standards Institution 2013