Journal of Environmental Chemistry and Ecotoxicology Vol. 3(5), pp. 139-141, May 2011
Available online http://www.academicjournals.org/JECE
ISSN-2141-226X ©2011 Academic Journals

Short Communication

Measurement uncertainty for the determination of the inhibitory effect on *Vibrio fischeri*: A practical approach

Caterina Ledda*, Gea Oliveri Conti, Maria Fiore, Roberto Fallico, Salvatore Sciacca and Margherita Ferrante

Department G.F. Ingrassia- Hygiene and Public Health, University of Catania. Via Santa Sofia, 87- 95123 Catania-Italy.

Accepted 24 January, 2011

The 11348-3:2007 ISO method allows the evaluation of the inhibitory effect of water samples on the light emission of *Vibrio fischeri*; it is essential to quantify the measurement uncertainty, but there is no available guideline. The aim is to come up with a practical approach to calculate the measurement uncertainty. We propose a test with different 3,5-dichlorophenol dilutions to evaluate if the luminometer is functioning properly and, after, we suggest to consider the uncertainty contributions sum (x_i), to calculate the standard deviation of reproducibility (S_R) and, finally, to express the expanded uncertainty with the formula $U = 2S_R + u(x_i)$.

Key words: *Vibrio fischeri*, ISO 11348-3:2007, measurement uncertainty, microtox, uncertainty contributions.

INTRODUCTION

The 11348-3:2007 ISO method is used to assess the inhibitory effect on the light emission of *Vibrio fischeri* (NRRL-B-11177) of:Waste water, surface water, ground water, aqueous extracts and leachates, marine and brackish waters, sediments and eluates (ISO 11348-3:2007). Its advantages are primarily the ease of use, speed and relatively low cost. The laboratory that performs this test in accordance with 17025:2005 ISO/IEC (International Electrotechnical Commission) is suppose to estimate the measurement uncertainty. The calculation is not easy for ecotoxicological methods because they are considered empirical methods.

The measurement uncertainty of empirical methods is based on the use of reference materials and on the ring test (EURACHEM /CITAC Guide CG 4:2000). In the case of evaluating the inhibitory effect on *Vibrio fischeri,* there are no reference materials to be used; at the same time it is not possible to rely on the ring test, as this is not carried out regularly. As a consequence, though it is recommended the identification and quantification of sources of uncertainty (EURACHEM /CITAC Guide CG 4:2000), the sample analysis results are usually expressed with reference to the method but not corrected for bias (EURACHEM /CITAC Guide CG 4:2000; ISO/IEC Guide 98-1:2009; ISO Guide 98-3:2008).

The aim of this study is, therefore, to come up with a practical approach to calculate the measurement uncertainty.

MATERIALS AND METHODS

Apparatus, test reagents and reference material

The tests were performed using the Microtox ® Model 500 Toxicity Analyzer from Microbics Corporation (AZUR Environmental) programmed to obtain an integrated response over the wavelength range 400 to 700 nm with excitation source switched off. The analyzer was equipped with a 30-well temperature-controlled, incubator chamber regulated at 15°C. A small compartment held at 5°C was used to store the bacteria before dilution. The equipment is controlled by software MicrotoxOmni (SDI) that allows users of the Microtox ® Model 500 Analyzer to run tests, visualize data, calculate statistics and generate reports. The reagents used are:Reconstitution solution, osmotic adjusting solution and diluent (Strategic diagnostics Inc. SDI).

The reference material used for the implementation of the test is

*Corresponding author. E-mail: cledda@unict.it. Tel: +390953782133. Fax: +390953782177.

Table 1. The results obtained from inhibition of 3,5 Dichlorophenol at various concentrations.

Reference substance	mL/L	% Mean inhibition± SD*
3,5 Dichlorophenol	10	90.26 ± 0.81
3,5 Dichlorophenol	5	60.53 ± 7.31
3,5 Dichlorophenol	2.5	23.23 ± 2.08
3,5 Dichlorophenol	1.5	5.52 ± 4.03

*These data were calculated over 10 tests performed at different times of the year of different batches of reagents and strains of freeze-dried *Vibrio*.

3 to 5 dichlorophenol concentrated to 25 mg/L (Sclavo diagnostics). EC50 (half maximal effective concentration) values, defined as the concentration which provokes a 50% light reduction on *Vibrio fischeri*, were obtained by following the Microtox ® screening test protocol test (AZUR Environmental). Practically, the EC50 values were calculated by regression analysis of the linear relationship between the logarithm of the sample or control concentration against the logarithm of the lost/remaining light intensity ratio.

Test of the equipment with reference substance

Before starting any type of activity it is appropriate to test the efficiency of the luminometer. Screening test (AZUR environmental) was carried out with 3,5 Dichlorophenol substituting the sample as effector and adapted the standard test procedure for toxicity in a way that lets us evaluate every minute effects; concentrations of 10, 5, 2.5 and 1.5 mL/L were used. Results were considered acceptable if they fell within the SD (standard deviation) (Table 1).

Evaluation of the contribution of the uncertainty

To accredit the 11348-3:2007 ISO method according to ISO/IEC 17025:2005 one must calculate the measurement uncertainty. For this method the sources of uncertainty are: Various sampling, sample preservation, sample pre-treatment, equipment uncertainty, reagents purity, biological variability of *Vibrio fischeri*, measuring conditions, the sample itself, effects due to the operator and casual effects. We suggest to assess the uncertainty type *u(xi),* is obtained from a series of *n* independent measurements (x_1, x_2, x_3, x_4,…….x_n) under the same experimental conditions, calculating standard deviation, that allows us to consider the multiple variables related to the analysis. (ISO/TS 21748:2004):

$$u(x_i) = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

It requires at least 12 independent sample measurements carried out under the same experimental conditions. The greater the number of independent measurements n, the more reliable this type of evaluation is. It s appropriate to distinguish between the samples with toxicity <50% and those with toxicity >50%, as the uncertainty in the samples with toxicity <50 will be increased; because the different composition and concentration of toxic substances influence the bioavailability of toxicants and the light emission of luminescent bacteria. In this way we get a measurement uncertainty that covers all the variables previously mentioned.

Calculating the standard deviation of reproducibility

The standard deviation of reproducibility intralaboratory (S_R) must be calculated using the logarithms of the results; these are further processed according to the formula of ISO 19036 (ISO/TS 19036:2006):

$$S_R = \sqrt{\frac{\sum_{i=1}^{i=n} (\lg a_i - \lg b_i)^2}{2n}}$$

a_i e b_i = first and second test result on the same sample.
$(\lg a_i - \lg b_i)$ = difference between the two results, expressed in decimal logarithm.
n = number of tests performed in duplicate.
S_R provided gives us information on the degree of correlation between the results of successive measurements.

RESULTS

Calculation of measurement uncertainty

The measurement uncertainty of an analysis (U) is usually expressed as Y = X ± U.
Where,
Y= Measurement result
X= Quantity value
U= Expanded uncertainty

In the absence of guidelines, in the case of the 11348-3:2007 ISO method, that formula is adapted as follows: $U=2S_R+ u (x_{i})$. Coverage factor k =2 (p = 95%) (ISO/TS 19036:2006)

DISCUSSION AND CONCLUSIONS

The suggested approach is easy to apply in laboratories where the 11348-3:2007 ISO method is followed. It is a good laboratory practice to recalculate uncertainty once a year, of course, if the intermediate verify are out of control time is shortened, intermediate verify is done by following the instructions described in "test of the equipment with reference substance" every time you change a reagent, a lot of bacteria and generally at least every 20 samples analysis.

REFERENCES

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. Geneva, Switzerland
EURACHEM / CITAC (2000). Guide CG 4 Quantifying Uncertainty in

Analytical Measurement, Second Edition. ISBN 0 948926 15 5
ISO/IEC Guide 98-1 (2009). Uncertainty of measurement -- Part 1: Introduction to the expression of uncertainty in measurement. Geneva, Switzerland.
ISO Guide 98-3 (2008). Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM: 1995). Geneva,Switzerland.
ISO/TS 21748 (2004). Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation. Geneva, Switzerland.
ISO/TS 19036 (2006). Guidelines for the estimation of measurement uncertainty for quantitative determinations. Geneva, Switzerland.