



Evaluation of an automated luminescent bacteria assay for in situ aquatic toxicity determination

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ABSTRACT

A new system for monitoring toxicity TOXcontrol® (MicroLAN BV, The Netherlands) has been used to assess the toxicity of a selection of priority or emergent compounds in the laboratory. In this study, inhibition curves and EC50 – Effective Concentration causing 50% inhibition – of selected compounds (including pesticides, pharmaceuticals, surfactants and metals commonly detected in surface or drinking waters) were determined. This new technology is based on the measurement of *Vibrio fischeri* bioluminescence inhibition (ISO 11348). The main advantage of this equipment, compared to other laboratory assays, is the fully automation of the procedure. The instrument can be operated online in a simple, rapid and reproducible way. The variability of the results obtained with the TOXcontrol® biomonitoring system has been studied. A comparison with standardised technology based in *V. fischeri* (Microtox®) and additional test with *Daphnia magna* for selected organic compounds is presented. The results show that the methodology based on the TOXcontrol® system being validated is accurate and reproducible enough enabling this system to be used as an on-line automatic alert system to detect abnormal concentrations of toxic compounds.

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1. Introduction

Legislation related to the preservation of quality of water bodies is becoming more stringent both at national and international levels. In the European context, the Water Framework Directive (WFD) (2000/60/EC) is an example of the new risk-based attitude adopted in terms of environmental impacts. The WFD requires both a good chemical and biological status of water bodies; therefore new monitoring assays to detect changes in water quality on short notice are required. Although a list of priority substances to be determined in surface water bodies was established by Daughter Directive (2008/105/EC) according to their harmful potential, other substances exist that are not regulated nowadays, called emerging contaminants, suspicious to cause effects on living organisms but their concentrations are not routinely measured. A big number of studies have been performed with the aim of identifying those contaminants present in water flows (von der Ohe et al., 2011).

The objective of the Drinking Water Directive (DWD) (98/83/EC) is to protect human health in the European Union and to make sure that the water is healthy and clean. For this purpose, DWD sets

standards for the most common substances that can be found in drinking water. In the DWD a total of 48 microbiological and chemical parameters must be monitored and tested regularly. Thresholds of these substances (including pesticides, metals, bacteria, etc.) are based on the potential detrimental effects to organisms.

Chemical status of water bodies is determined in most cases by spot sampling campaigns and laboratory determinations. This off-line methodology is slow and in some cases ineffective to respond to sudden quality changes as a result of possible contamination. There is the need to use some alternative monitoring tools to complement traditional ones to provide a comprehensive overview of water quality. Biomonitoring protocols use sentinel species, defined as any living organism to be used as an indicator of the presence of a pollutant or the toxicity of a contaminant (Amiard and Amiard-Triquet, 2008). Biological analysis with the help of different biosensors is considered a highly informative testing system since the knowledge of the chemical characteristics of pollutants does not always provide sufficient information about their toxicity and danger for living organisms (Tsybulskii and Sazykina, 2010).

Toxicity tests are one example of a commonly used biomonitoring tool. In this case, a biological response of a test organism is measured as the result of the combined effect, including antagonism and synergism, of the mixture of all potential contaminants contained in water. One of the most common biosensor used for the risk assessment in

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aquatic environment is based on the inhibition of luminescence produced by marine bacteria *Vibrio fischeri*. The use of this bioluminescence based assay has been standardised (ISO, 11348-3) for the regulatory purposes because of its sensitivity and short time required to perform the test (Coz et al., 2007). Toxicity is usually represented as EC50, i.e. effective concentration of the tested chemical at which 50% of luminescence inhibition is observed.

Since the early 80's, many studies have been performed to determine toxicity of different families of chemicals at laboratory. In one of these studies, the drugs investigated (ibuprofen, ketoprofen, naproxen, diclofenac, salicylic acid and gemfibrozil) showed very similar EC50 values when comparing two techniques using the luminescent bacteria assay (14–36 mg L⁻¹ for Microtox® and 12–43 mg L⁻¹ for ToxAlert®) (Farré et al., 2001a). Other comparison performed with surfactants showed larger variability (0.36–127 mg L⁻¹ for ToxAlert® and 0.40–379 mg L⁻¹ for Microtox®) (Farré et al., 2001b). Antibiotics showed a moderate toxicity on *V. fischeri* and no significant effects at the maximum concentration tested corresponding to the water solubility were observed, but the compounds atrazine, simazine, glyphosate, deltamethrin and leucomalachite green showed EC50 values greater than 10 mg L⁻¹, and therefore they were classified as harmful, according to the Global Harmonized System of classification (UNECE, 2011; Hernando et al., 2007). EC50 values have also been determined for triclosan (0.28 mg L⁻¹) and methyl triclosan (0.21 mg L⁻¹) (Farré et al., 2008).

In other studies, EC50 values for individual metal added in the ionic form were obtained for cadmium, chromium, copper, lead and zinc using *V. fischeri* at exposure time of 30 min. Values ranged from 0.12 to 13.8 mg L⁻¹ (Guéguen et al., 2004). In another study, the toxicity of the 13 priority pollutant metals and non-metals was evaluated using the Microtox® chronic toxicity test. Among the metals, beryllium was found to be the most toxic in the test while thallium was the least toxic (Hsieh et al., 2004). The toxicity of arsenic, cadmium, lead, and mercury has been tested individually and as a composite mixture using the Microtox® bioassay. Among the individual metals and non-metals tested, the ranking of toxicity was mercury in first place, lead, cadmium and arsenic (Ishaque et al., 2006). More tests have been performed in assessing toxicity of metals based on this bacterium (Codina et al., 1993; Cho et al., 2004; Rosen et al., 2008; Tsybulskii and Sazykina, 2010). The response of luminescent bacteria to mercury compounds has also been investigated (JM Ribo et al., 1989).

Further studies have been performed for assessing the performance of different sensors. Ten toxicity sensors utilising enzymes, bacteria, or vertebrate cells were compared to rapidly identify toxicity in water samples containing one of 12 industrial chemicals. Microtox® was the highest scored at the ranking responding to 6 out of 12 compounds (van der Schalie et al., 2006). Another study performed a comparison between *V. fischeri*, *Selenastrum capricornutum* and *Daphnia magna* tests. A selection of pesticides, antifouling agents and pharmaceuticals were tested. *D. magna* resulted as the most sensitive test. *D. magna* and *V. fischeri* showed both to have discriminatory ability to separate compounds in different toxicity categories (Hernando et al., 2005). At a different study, the inhibitory effects of 81 chemicals, after 5 min contact time, were calculated at eight concentrations using three commercial assay systems based on the luminescent bacteria toxicity assay (ToxAlert®, Microtox® and LUMISTox®). Only five compounds gave EC50s that varied more than three-fold between assays (Jennings et al., 2001). Bioluminescent bacteria have been applied frequently to monitor toxicity in several environmental applications like wastewater, seawater, surface and ground water, tap water, soil and sediments, and air (Girotti et al., 2008; M.C. Riva et al., 2007). However those tests are based on discontinuous samples and provide only a partial response in terms of compliance with WFD and related legislations.

The objective of the study was the test of the automated equipment TOXcontrol® for measuring toxicity of contaminants that can

be found at surface and drinking waters. The response of the equipment at laboratory against a selection of contaminants has been assessed. Response for the organic compounds has been compared against response of toxicity tests performed with Microtox®. As it is a non-specific technique for measuring global toxicity, it's important to perform tests at laboratory with water spiked with single analytes so specific response to one compound can be obtained. Only one reference has been found concerning validation of on-line toxicology sensors, in which TOXcontrol®, was tested in combination with a spectrophotometer for the monitoring of sodium cyanide, azinphos-methyl, sodium fluoroacetate and difenacoum in surface waters (Appels et al., 2007). Additionally, sensitivity of *V. fischeri* for selected organic compounds has been compared against sensitivity of *D. magna*.

On one side, contaminants were selected taking into account their occurrence in semi-arid basins, like the Llobregat river (SE Spain) where a high contribution of treated wastewaters discharges in the total flow of the river is expected as the low flow makes dilution factor almost negligible. The pollutants can become a potential risk to the receiving bodies and, in addition, to the production of drinking water (Gasperi et al., 2008; Muñoz et al., 2009). On the other side, information on the possible presence of metals in drinking water according to their potential of being transferred from the corrosion of internally unprotected metallic water pipelines were taking into account for metals selection (Imran et al., 2009).

Experiments have been performed to assess the aquatic toxicity of a selection of target compounds. Those substances have been selected in view of their occurrence in surface water, especially in semi-arid regions where the water stress leads to low flow rates and higher concentration of dissolved pollutants, and their potential to show toxicity (González et al., 2012) and the possible presence in drinking water mainly due to migration of pipe material (Veschetti et al., 2010).

2. Materials and methods

2.1. Reagents and standard solution preparation

Chemical standards for terbutylazine, triclosan, dimethoate, sodium dodecylbenzene sulfonate (SDBS), diazinon, sodium diclofenac, nonylphenol, propanil, 2-methyl-4-chlorophenoxyacetic acid (MCPA) and iron (III) sulphate hydrate were purchased to Sigma Aldrich (St. Louis, MO, USA). Standards for chromium (III) nitrate nonahydrate, copper (II) sulphate pentahydrate, lead (II) nitrate and nickel (II) sulphate hexahydrate were purchased from Merck (Darmstadt, Germany). HPLC water and dimethyl sulfoxide (DMSO) were also purchased from Sigma-Aldrich. The freeze dry luminescent bacteria *V. fischeri*, cultivation media, zinc sulphate (2500 mg/l) and 20% sodium chloride were supplied by MicroLAN (Waalwijk, the Netherlands). Stock solutions were obtained by dissolving metal salts in HPLC water and organic compounds in DMSO (0.2% v/v). The pH of solutions was not adjusted but it was monitored and no sudden changes of pH were reported.

Luminescent marine bacteria of the species *V. fischeri* (NRRL B-11177) for the Microtox® determinations were obtained from SDI (Strategic Diagnostics Inc. Newark, DE USA). Luminescent bacteria for TOXcontrol® determinations were obtained from Microlan B.V. Waaljik. NL. *D. magna* used as test organisms were obtained from a CRIT-UPC maintained culture.

2.2. On-line toxicity monitoring

The instrument for online monitoring of toxicity of water samples is the TOXcontrol® Toxicity Monitoring System manufactured by Microlan, B.V.

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