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High-throughput screening assay for the environmental water samples using cellular response profiles

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ABSTRACT

Chemical and physical analyses are commonly used as screening methods for the environmental water. However, these methods can only look for the targeted substance but may miss unexpected toxicants. Furthermore, the synergistic effects of mixture cannot be detected. In order to set up the assay criteria for determining various biological activities at a cellular level that could potentially lead to toxicity of environmental water samples, a novel test based on cellular response by using Real-Time Cellular Analyzer (RTCA) is proposed in this study. First, the water sample is diluted to a series of strengths (80%, 60%, 40%, 30%, 20% and 10%) to get the multi-concentration cellular response profile. Then, the area under the cellular response profile (AUCRP) is calculated. Comparing to the normal cell growth of negative control, a new biological activity index named Percentage of Effect (PoE) has been presented which reflects the cumulative inhibitory activity of cell growth over the log-phase. Finally, a synthetical index PoE_{50} is proposed to evaluate the intensity of biological activities in water samples. The biological experiment demonstrates the effectiveness of the proposed method.

1. Introduction

Around the world, exposure to chemicals, whether acute poisoning through ingestion of large doses or chronic contact with low levels of contaminants, is a public health concern. Poisoning placed second behind automobile accidents as the leading cause of injury-related death in many developed countries. Certain poisonings (e.g., pesticides) occur most frequently in children, 40–60% of those affected being are under the age of six (WHO, 2010). Chronic adverse effects due to exposure to hazardous chemicals are more, subtle but they have been long recognized by occupational health studies conducted on workers. Important occupational toxicities such as neuro-, reproductive-, and immunotoxicity as well as mutagenicity and carcinogenicity are now well documented (Soldán and Badurová, 2013; Radić et al., 2013). Evidence is also accumulating that a range of adverse effects and even chronic diseases can occur in the general population at very low chemical concentrations after prolonged periods of time. Most of the population exposures are primarily through food, air, consumer products and water.

Conventional toxicity testing for environmental water monitoring has been performed by characterizing the specific substance, quantifying its level and then comparing it to known regulatory guidelines. A wide range of analytical chemistry methods are being used to achieve this goal (Kaza et al., 2007; IAEA, 2009; Comerton et al., 2009). However, there are many substances in the environmental water samples and only well-known toxic substances can be determined using these assay techniques. Thus it is difficult to perform toxicity analysis for substances which are new or not available in the library of the well-known toxic substances. Additionally, the use of only chemical analyses cannot predict the actual human health hazard, because they are limited by the sensitivity and determination of single compound (Lah et al., 2005; Elad et al., 2011). The environmental contaminants are usually present as mixtures and their synergistic effects cannot be obtained by simply summing up the toxicity from individual substances. Currently, little is known about the mixed

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cytotoxic effects caused by multiple environmental chemicals and their related human health risks. There is a great need for novel tests that can be used for the screening and monitoring of a wide range of toxic contaminants in the environment; then if desired, more detailed and expensive laboratory analysis can be performed.

To achieve a realistic estimation of human risks caused by the environmental water contaminations, the first thing to know is their toxic effects. The biotest is commonly used for the toxicity assessment in the literature (Faria et al., 2007; Mankiewicz-Boczek et al., 2008; Dragun et al., 2009). Lah et al. (2005) implemented the Comet assay and Ames test to monitor the genotoxicity in drinking water. Two human cell lines and protozoa cells were treated with non-concentrated and 50 × concentrated drinking water respectively, and the degree of nuclear DNA damage was proposed to assess the genotoxicity. Kaza et al. (2007) performed a battery of microbiotests to assay the toxicity of water samples from the rivers in Central Poland. As a result, the percentage effect depending on the effect criterion of the respective assay is proposed to rank the water samples into five hazard classes. Soldán and Badurová (2013) used exotoxicological approach to screen the risk of chronic effect of surface water pollution. The risk of toxicity and genotoxicity was investigated to detect the biological impact. Sansom et al. (2013) employed six fish cell lines in 24*h*/96*h* viability assays (EC_{50} and LC_{50}) for rapid fluorometric assessment of cellular integrity and functionality. The physicochemical composition of the tested waters confirmed that the proposed approach can be a biologically relevant tool in the initial assessment of the water toxicity.

Although the mentioned biological methods can be used for the evaluation of environmental water contamination, they either focused on the detection of special substances or used the end-point estimation only. Further, the dynamic information during organism (such as Daphnia magna, fish cells) growth was ignored when they were exposed to the environmental water samples. To this end, a Real-Time Cell Analyzer (RTCA) developed by the ACEA Biosciences Inc. (San Diego, USA) presents a real time monitoring platform to record the dynamic process of cell proliferation and changes induced by cell-toxicant interaction (Xing et al., 2012). Furthermore, the RTCA MP Station configured with six E-Plates (96 wells on one E-Plate) can improve the assay throughput (Roche, 2011). The basic principle of the RTCA is to monitor the changes in electrode impedance induced by the interactions between testing cells and electrodes. The presence of the cells leads to an increase in the electrode impedance: the more the cells attached to the sensor, the higher the impedance that could be picked up by RTCA. The dynamic data generated by the RTCA reflects cell proliferation. However, measuring the biological activity from this rich dynamic data is a challenge. The traditional indices (such as EC_{50}, IC_{50}, and LC_{50}) can also be used to achieve the target, but these indices are largely dependent on the incubation time. Different assayed time points may lead to different values of traditional indices (Pan et al., 2013a). The influence of incubation time can lead to questions about which time point provides the most scientifically valid results.

In this study, we develop a new high throughput screening method based on the RTCA that can be used to monitor environmental water for biological activities at a cellular level that could potentially lead to toxicity. Human cell lines are used to generate data that may be useful for human health risk assessment. The collected water samples are diluted to several concentrations and the human cells are exposed to each concentration of each individual water sample. As a result, the multi-concentration and time-dependent cellular response profile is recorded for each water sample. In order to quantify the cell growth effects, the area under the cellular response profile (AUCRP) is developed to evaluate the extent of exposure to each concentration of each individual sample. By integrating over time rather than looking at multiple endpoint measurements, a more accurate and robust estimate of the overall exposure to the chemical of various concentrations is obtained, which can describe the intensity of cellular level biological activities due to the environmental water. Compared to the negative control (without exposure to the collected water sample), a percentage of effect (PoE) index is proposed for identifying and analyzing the level of water contamination.

2. Materials and methods

2.1. Materials

2.1.1. Cell lines

Four cell lines (i.e. A549, ACHN, HepG2, and SK-N-SH) were purchased from the American Type Culture Collection (ATCC) and maintained in 37 °C incubators containing 5% CO_2. The cells were amplified and frozen in aliquots to ensure that the same source of cells was used for the investigation.

2.1.2. Controls

Controls are subjects closely resembling the experimental subjects but not receiving the treatment, thereby serving as a comparison group when treatment results are evaluated.

Positive control: Arsenic in Alberta's ground waters has been a concern since the early 1990s (Surveillance, 2000). The ground waters were monitored and arsenic was found to be at relatively high levels in some of the ground water samples. Therefore, *arsenic III* and a mixture of the trace elements were chosen as positive controls for the cytotoxicity assay, in which the affected result can be predicted.

Negative control: A negative control is a group that has not been administered the drug of interest. In this experiment, negative control contains the target cells, the culture medium, and the maximum concentration of the solvent used in dissolving chemicals, where no phenomena are expected. Here, H_2O was included as the negative control for environmental water analysis.

2.1.3. Water samples

As examples for testing the proposed method, three types of samples were collected from a specific well, lake or storm pond, which does not imply any similarity of these samples as other wells, lakes or storm ponds of the region (Pan et al., 2013a).

Well samples: Private domestic wells are the drinking and household water sources for rural families. The samples were analyzed for routine chemistry, trace elements, as well as cytotoxicity.

Storm pond samples: Storm water ponds are frequently built into urban areas in North America to provide storm water flow control and improve water quality. The suspended sediments are also collected in storm water, which are often found in high concentrations in storm water due to upstream construction and sand applications to roadways. Storm water ponds could be chemical soups of pesticides, fertilizers, pet wastes, oil, grease and other contaminants. The samples were analyzed for routine chemistry, trace elements, pesticides, VOCs, as well as cytotoxicity.

Lake samples: Water samples were collected from lakes across Alberta. The samples were analyzed for routine water chemistry, trace elements, total microcystins, as well as cytotoxicity.

Dilution rule: Each water sample was diluted to a series of strength (80%, 60%, 40%, 30% 20% and 10%) to get the multi-concentration cellular response profile. The advantage of this method is that it can achieve the concentration–response curve which is similar to the chemical compound exposure.

2.2. Methods

2.2.1. RTCA HT system and ecotoxicological testing

The xCELLigence RTCA HT system was used as the platform to facilitate this study. The system has been developed by the ACEA Biosciences Inc. (San Diego, USA) in the 96 × well plate format. It