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Simultaneous detection of multiple bioactive pollutants using a multiparametric biochip for water quality monitoring

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ABSTRACT

Water is a renewable resource but yet finite. Its sustainable usage and the maintenance of a good quality are essential for an intact environment, human life and a stable economy. Emerging technologies aim for a continuous monitoring of water quality, overcoming periodic analytical sampling, and providing information on the current state of inshore waters in real time. So does the here presented cell-based sensor system which uses RLC-18 cells (rat liver cells) as the detection layer for the detection of water pollutants. The electrical read-out of the system, cellular metabolism, oxygen consumption and morphological integrity detects small changes in the water quality and indicates a possible physiological damage caused. A generalized functional linear model was implemented in order to regress the chemicals present in the sample on the electrical read-out. The chosen environmental pollutants to test the system were chlorpyrifos, an organophosphate pesticide, and tetrabromobisphenol A, a flame retardant. Each chemical gives a very characteristic response, but the toxicity is mitigated if both chemicals are present at once. This will focus our attention on the statistical approach which is able to discriminate between these pollutants.

1. Introduction

The production of industrial chemicals, pharmaceutical drugs, personal care products, pesticides, herbicides and chemical additives is increasing continuously. These anthropogenic substances challenge the environment and some of them may represent an ecological and/or human-health risk. One of the main concerns is that these compounds end up in soil, ground and inshore waters. Humans may come in contact with these chemicals directly or via the food chain (Ritter et al., 2002; Eltzov and Marks, 2011).

The United Nations Environmental Program (UNEP) on a global scale, the Water Framework Directive (WFD) in the European Union and the U.S. Environmental Protection Agency (EPA) put special effort on reducing circulating organic pollutants.

Even if certain water quality parameters, such as oxygen deficiency or phosphate concentration, have ameliorated over the last decades in rivers and groundwater within the European Union (Fuerhacker, 2009), there are other contaminants, mainly organic pollutants, whose short and/or long-term consequences are yet not completely known and still remain to be elucidated (Eltzov and Marks, 2011). Moreover, the existence and presence of new chemicals or still unknown degradation products is conceivable. These substances could remain yet undetectable for conventional analytical methods. Currently, analytical techniques cover a large range of possibilities to evaluate the environmental quality. The most widely used techniques are chromatography, mass spectrometry, ion mobility spectrometry, chemical sensors, immunochemical techniques, optical spectrometry, radionuclear methods and remote sensing (Lopez-Avila and Hill, 1997; Jimenez-Jorquera et al., 2010). They are time-consuming and require a pretreatment of the acquired samples, enrichment and cleaning.

Novel and skillful chemical, physical and biological detection systems for environmental monitoring (Ho et al., 2005; Lieberzeit and Dickert, 2007, 2009; Jimenez-Jorquera et al., 2010; Chen et al., 2014) try to fill this gap. Such broadband or at least multiselective approaches detect a variety of chemicals, whether in gases or in an aqueous phase (Rogers and Gerlach, 1996). For instance, genetically engineered bacteria have been successfully used for the targeted detection of hazardous chemicals in water (Brandt et al., 2008; Elad et al., 2011), and Kubisch et al. (2012) monitor the physiological damage caused to living cells by heavy metals in aqueous phases.

The present study was carried out using a technology switching from selective and single substance detection towards overall

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toxicity detection systems. This could enable rapid quality assessment of inshore waters as complex matrices and specify the toxic effects for living organisms which get in contact with these media. The combination of three sensor types combined on the chip surface ensures a broad band monitoring of the cells where different physiological parameters, like respiration, metabolism, small changes in morphology, actin assembly, cell-to-cell-contact, and ion channel activity, are taken into consideration in order to determine changes or impairment of the cellular physiological activity.

In the case that a pollutant is present in a water sample, it is of importance to determine which substance or at least which class of compound is contaminating the water. A first step in this direction is made in this study by applying a statistical signal evaluation method on the signals resulting from the electrical readout mentioned above. The chosen method belongs to the field of functional data analysis (FDA). FDA has become increasingly important in the fields of health and biomedicine (Sorensen et al., 2013; Ullah and Finch, 2013; Goldsmith and Scheipl, 2014) since it allows us to analyze, model and predict most of the quasi-continuous data measured in these fields of research. Metabolic processes are inherently continuous. Therefore, physiological data can be taken as punctual realizations of a continuous process (Ramsay and Silverman, 2005).

In this study chlorpyrifos and tetrabromobisphenol (TBBPA) were chosen as model substances to analyze the suitability of a broadband cell-based sensor system for the detection of pollutants in an aqueous phase. Both chemicals are a matter of environmental and human risk concern (Duirk and Collette, 2006; Zhang et al., 2014). Chlorpyrifos is one of the 33 priority chemicals defined by the Water Framework Directive of the European Union. It is an organophosphate insecticide used in the European Union mainly in vineyards (European Food Safety Authority, 2011), with an annual average worldwide usage of 24.5 thousand tons (Eaton et al., 2008). The primary mechanism of action of chlorpyrifos is the inhibition of the acetylcholinesterase (AChE) in target tissues by its oxon metabolite (Eaton et al., 2008). Besides this acute toxicity, the exposure to chlorpyrifos is associated with neurodevelopmental effects (Engel et al., 2011; Bouchard et al., 2011).

TBBPA represents the worldwide most heavily produced flame retardant (Betts, 2013; Sun et al., 2014) with an annual worldwide market demand of more than 120,000 tons (de Wit et al., 2010). It was first not believed to reach the environment, but actually TBBPA as well as its metabolites can be found in the air, soil and in sediments (Birnbaum and Staskal, 2004). As well as chlorpyrifos, TBBPA is categorized as a persistent organic pollutant that does not degrade for several years. Toxicological studies reveal adverse effects consisting in the impairment of the central and peripheral nervous system and disruption of thyroid homeostasis (Hendriks et al., 2012; Meijer et al., 2014).

Both compounds, chlorpyrifos and TBBPA, were applied to the system as single compounds and then the cellular layer was co-exposed to both chemicals in different concentrations. This should elucidate how the interaction of two compounds alter their potential toxicity, and this in turn the impact on cellular healthiness.

2. Experimental

2.1. Cell culture

RLC-18 cells (rat liver cells) were purchased from DSMZ (German Collection of Microorganisms and Cell Lines, Braunschweig, Germany) and grown at 37 °C in 5% CO_2 in a humidified atmosphere. RLC-18 cells were cultivated in Dulbecco's modified Eagle medium (DMEM; Gibco, Darmstadt, Germany), supplemented with 10% heat inactivated fetal bovine serum (FBS; Biochrom, Berlin, Germany), 100 units/mL penicillin (BioWhittaker, Heidelberg, Germany) and 100 μg/mL streptomycin (BioWhittaker).

2.2. Chemicals

Chlorpyrifos (catalog #45395), and 3, 3′, 5, 5′–tetrabromobisphenol A were purchased from Sigma Aldrich (Steinheim, Germany). Dimethylsulfoxid (DMSO, catalog #A36720250) was bought from AppliChem (Darmstadt, Germany) and Triton-X (TX, catalog #93418) from Fluka (Steinheim, Germany).

2.3. Cultivation on the metabolic chip

The same growth conditions were used for the cultivation of RLC-18 rat liver cells on the sensor chip (SC 1000 Metabolic Chip) as for cell culturing. Cells were kept in Dulbecco's modified Eagle medium supplemented with penicillin/streptomycin and 10% FBS. Approximately $1.75 \cdot 10^5$ cells were seeded on the chip in 400 μL nutrient medium, and incubated over night at 37 °C in 5% CO_2 and 95% humidity until confluency was reached. The sensor chips with cells were then transferred to the Bionas 2500 Analyzing System.

2.4. Bionas 2500 analyzing system

To detect the presence of organic pollutants, the cell-loaded chips were placed into the Bionas 2500 Analyzing System (Fig. 1A). This system allows the recording and processing of the acquired data from the sensor chip. Three types of electrodes cover the surface of the chip beneath the cells. Interdigitated electrode structures (IDES) measure the cellular impedance (Fig. 1C). The cell membrane is a strong insulator. If a current is applied it will disable the current flow in a passive way and the impedance will decrease (Ehret et al., 1998). In this way the steadiness of the cell membrane can be analyzed. But the signals recorded by the IDES electrode do not completely correlate with the mere morphological integrity of the cell membrane, also other factors like ion channel activity, cell-to-cell contacts or changes in actin assembly contribute to the impedance signal (Giaever and Keese, 1993; Xiao and Luong, 2003; Hong et al., 2011).

For the detection of the oxygen consumption two Clark-type electrodes are integrated on surface of the chip (Ceriotti et al., 2007; Thévenot et al., 2001; Ramamoorthy et al., 2003). Five ion-sensitive field-effect transistors (ISFET) measure the pH value of the extracellular medium and thus the metabolic activity of the cells (Thévenot et al., 2001; Covington, 1994).

The measurement was performed according to the following protocol:

(a) 5 h equilibration time in order to let the cells adapt to the system and get stable physiological signals.
(b) 10 h exposure to polluted samples in different concentrations. Cells notice the presence of a contamination or a toxic substance as soon as they come in contact with it, but the effect becomes more pronounced after a longer exposure time.
(c) 7 h recovering time with running medium to determine if effects are lasting or transient.
(d) Removing of cells by the addition of 0.2% Triton X-100 to obtain a basic signal without living cells on the sensor surface as a negative control.

2.5. Functional data analysis

A statistical signal evaluation method was applied to evaluate the presence of a certain pollutant in an unknown sample. This