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Research paper

A portable chemical toxicity biochip based on electronic enzymatic monitoring of cytotoxic effects on fish cells

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ABSTRACT

Herein we describe a miniaturised chemical toxicity analyser with capacity to assess water quality without intervention of laboratory equipment. The device, as presented, integrates living cells and electrode-based sensors to monitor cell viability upon exposure to toxic chemicals. The methodology involved cultured fish cells *Poeciliopsis lucidia* hepatocellular carcinoma (PLHC-1) and *Oncorhynchus mykiss* rainbow trout gonad (RTG-2) cells, exposed to toxic chemicals ($CdCl_2$ and pentachlorophenol (PCP)) after which the activity of a cellular enzyme, acid phosphatase (AP) was measured. Reduced AP activity was quantified electrochemically, allowing IC_{50} values (50% reduction in AP activity) to be determined. Fish cells are a relevant model of the toxicity risks posed by contaminated water to aquatic health. Such cells are relatively slow growing and do not require a source of CO_2 for incubation, thus ensuring portability for single-use point of site applications. The 24 hour IC_{50} values determined for $CdCl_2$ in the fish cell lines (16.2 ± 0.7 and 91 ± 11 μM for PLHC and RTG-2, respectively) were in agreement with those found using the MTT assay (16.3 ± 1.6 and 164 ± 96 μM). In the case of PCP, the IC_{50} value in PHLC cells (127 ± 22 μM) suggested enhanced sensitivity towards PCP compared with the MTT assay ($IC_{50} > 160$ μM). The optimised electronic assay was transferred to a prototype integrated assay cartridge with associated fluidic and transducer elements. This device (TOXOR) monitored changes in the metabolic state of fish cells, realising high-value toxicity information as a potential early warning on-site screening system.

1. Introduction

Access to clean unpolluted water is essential for human health and the ecosystem, yet European water quality frequently fails to meet basic biological and chemical standards with point and diffuse sources of pollution originating from private homes, industry and agriculture [1]. Directive 2008/105/EC has identified 33 priority surface water chemical pollutants in which action by member states was required [2]. As a result of this and other directives, there is a need for regular monitoring of water for the presence of potentially toxic chemicals. On-site water testing still relies on strategic sampling with considerable sample preparation and analytical laboratory work. Conventional chemical analysis such as high performance liquid chromatography (HPLC), gas chromatography (GC) and spectroscopic methods are both accurate and sensitive but can be time consuming, requiring sample transport to the laboratory for analysis by trained personnel [3].

In addition to chemical analysis, biological monitoring for early detection and ecotoxicological evaluation is also a requirement [4]; however identifying the extent of toxicity followed by elucidation of the source of the contamination in near real-time is beyond current technology. Existing continuous analytical monitoring instruments provide only limited information regarding exposure and are reliant on bulk parameters e.g. dissolved gases, turbidity/conductivity measurements, BOD estimates, nitrates, phosphates, metal contamination [5]. The Toxi-Chromo™ bioassay which measures inhibition of β-galactosidase activity in *Escherichia coli* following toxicant exposure, and the Microtox® bioassay, based on bioluminescence fading of bacterial species *Vibrio fischeri* in the presence of toxic chemicals represent the most widely used methods for water toxicity assessment [6]. The Microtox® assay is sensitive, cost effective and requires only 5 to 30 minutes to make toxicity measurements [7].

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In addition to optical based assays, electrochemical biosensors employing bacteria [8–12] and microalgae [13,14] as biological recognition elements have been successfully used to screen heavy metals, pesticides and phenols. Although microbial cells offer advantages such as robustness and good viability in harsh environments, the use of eukaryotic cells for biosensing is of greater physiological relevance to human health [15]. A number of researchers have employed mammalian cells for cytotoxicity testing of key environmental toxicants including disinfection by-products, pesticides and heavy metals [16–19]. However, one of the major challenges in the development of portable mammalian cell biosensors for on-site analysis is the requirement for growth at 37 °C in a 5% CO_2 atmosphere and the need for frequent medium changes and subculture[20]. As an alternative, a number of groups have employed fish cells as a biological indicator [21–24] with electric cell substrate impedance sensing (ECIS) or amperometric measurement of redox mediators used to assess changes in cell viability upon toxicant exposure. Brennan *et al. (*2012) developed an ECIS biochip that employed rainbow trout gill cells (RTgill-W1) as the biological recognition element [25]. Some advantages of this biochip included no requirement for CO_2 during culture, sensitivity comparable to that of mammalian cells and cell viability for up to 78 weeks in the absence of medium changes (stored at 6 °C).

In our previous work we reported development of an electrochemical cytotoxicity assay that measured changes in cellular enzyme AP in A549 human lung epithelial cells upon exposure to toxic chemicals [26]. Intracellular AP enzymes catalyses the conversion of assay substrate 2-naphthyl phosphate to 2-naphthol which upon release from cells can be measured electrochemically (See Supplementary information Scheme S-1). The development and optimisation of the AP based electrochemical assay in PLHC-1 and RTG-2 cell lines is presented here and to the best of our knowledge represents the first such report of electrochemical cytotoxicity measurements being made in these fish cell lines. Alterations in cellular AP activity following exposure to toxic $CdCl_2$ and PCP was assessed and validated against the MTT assay. The electrochemical cytotoxicity assay methodology was subsequently transferred onto a disposable electronic biochip for proof of principle testing. This next generation integrated device (TOXOR) was designed and fabricated *in house* via combined screen printing/layer by layer polyester lamination. Cytotoxicity testing towards RTG-2 cells cultured on-chip was demonstrated, proving the capability of the device for provision of high-value toxicity information as part of a future suite of environmental remote sensing deployment units.

2. Materials and methods

2.1. Apparatus

All electrochemical measurements employed a CH Instruments Inc CH1630C potentiostat with single use in-house fabricated carbon-Ag/AgCl screen printed electrodes (working electrode 4 mm^2) or the on-board two electrode detector of the TOXOR prototype device (electrode area 3.8 mm^2). MTT assay absorbance measurements were made using a Biotek synergy H1 Hybrid Reader.

2.2. Chemicals

$CdCl_2$ (99.99%), PCP (98%), dimethyl sulphoxide (DMSO) (≥99.6%), phosphate buffered saline (PBS) tablets, sodium acetate (>99%) and magnesium chloride hexahydrate (≥98%) were purchased from Sigma Aldrich. Dulbecco's Modified Eagles Medium (DMEM)/Hams F-12 (1:1), fetal bovine serum (FBS) and poly-L-lysine (0.1 mg/ml MW >300,000) were Biochrom brand. Trypsin-EDTA 1X in PBS and HEPES buffer were Biowest brand. Ag/AgCl and carbon ink were supplied by Gwent Ltd, polyester O 100 μm was Melinex brand while polyester A grade 500 μm was Mylar brand. Other chemicals and suppliers included penicillin/streptomycin 100X (Applichem GmBH), acetic acid (99% Riedel de Haen), non-essential amino acids 100X (Lonza Group Ltd), tetrahydrofuran (99.9% Lab Scan Analytical Sciences), 0.22 μm hydrophilic filters (Whatman) and 3 M adhesive A4 sheets.

2.3. Cell culture

RTG-2 and PLHC-1 cells were a gift from Dr Paul Corcoran at the Radiation and Environmental Science Centre (RESC), FOCAS Research Institute, Dublin Institute of Technology, Kevin Street, Dublin 8 and were cultured following the procedures reported by Naha et al. [27,28]. Briefly RTG-2 and PLHC-1 cells, two adherent cell lines, were cultured in T 75 cm^2 tissue culture flasks in DMEM/Hams F-12 (1:1) supplemented with 5% v/v (PLHC-1) or 10% v/v (RTG-2) FBS, 45 IU ml^{-1} penicillin/45 μg ml^{-1} streptomycin, 25 mM HEPES buffer and 1% v/v non-essential amino acids. All reagents used were cell culture grade. Both cell lines were grown under normoxic conditions at 30 °C (PLHC-1) or 20 °C (RTG-2).

2.4. Electrochemical cytotoxicity assay development

Electrochemical measurement of AP activity in PLHC-1 and RTG-2 cells was achieved by seeding 4×10^4 cells on the microwells of flat bottom transparent 96 well plates, followed by incubation under previously described conditions for 24 hours (Section 2.3). After this time, the culture medium was removed and the microwells washed three times with sterile PBS. The substrate 2-naphthyl phosphate (10 mM) dissolved in pH 4.5 acetate buffer (pre-sterilised using a 0.22 μm filter) was added in triplicate to microwells seeded with cells. Non-faradaic background (BG) measurements were made by triplicate addition of pre-sterilised pH 4.5 acetate buffer to other microwell seeded cells. The plates were incubated for 3 hours under culture conditions after which time the supernatant was removed and aliquoted into fresh microwells. Cellular generation of 2-naphthol was measured via cyclic voltammetry (CV) over the potential range 0-0.9 V vs. Ag/AgCl at 0.1 V/s. Assay parameters including cell number, substrate concentration and substrate incubation time were investigated and optimised in both cell lines (See Supplementary information S-1to S-3).

2.5. Electrochemical cytotoxicity assay

$CdCl_2$ and PCP are widely known to be cytotoxic [29,30], and were selected as model inorganic and organic chemicals as they are among a list of 33 priority pollutants in surface water that have been identified by the European Union in the Directive on Environmental Quality Standards (Directive 2008/105/EC) [2]. Briefly, 96 well plates were seeded with either 2×10^4 (RTG-2) or 4×10^4 (PLHC-1) cells per microwell and incubated for 24 hours under previously described conditions (Section 2.3). Following incubation, the medium was removed and each microwell washed three times with PBS. Fresh medium (100 μl) was added to each microwell plus 100 μl of $CdCl_2$ dissolved in deionised water (0–60 μM in the case of PLHC-1 cells and 0–1000 μM for RTG-2 cells), or PCP (0–320 μM) prepared in PBS. All toxicant reagents were pre-sterilised using a 0.22 μm hydrophilic filter. PCP standards were prepared from a stock solution of 150 mM PCP dissolved in DMSO with no greater than 0.1% v/v DMSO present in each sample. Cells were exposed to toxicants for 24 hours prior to washing with PBS, followed by the addition of 10 mM 2-naphthyl phosphate to the microwells for the appropriate time period (one hour for PLHC-1 cells and four hours for RTG-2 cells). The supernatants were then aliquoted into

fresh microwells with 2-naphthol generation measured by chronocoulometry (potential step 0.2 to 0.8 V, pulse width 10 s, sample interval 0.01 s, quiet time 0 s and sensitivity 1×10^6 A/V). All cytotoxicity measurements were made in triplicate (including controls) and three independent times.

2.6. MTT cell viability assay

The cytotoxic effects of $CdCl_2$ and PCP were also assessed using an MTT cytotoxicity assay kit (Cayman Chemical). PLHC-1 (4×10^4 cells per microwell) or RTG-2 (3×10^4 cells per microwell) were cultured for 24 hours on a 96 well plate and exposed for a further 24 hours to the same concentrations of $CdCl_2$ or PCP as previously described. Following 24 hour incubation, 10 μl of 5 mg/ml MTT reagent were added to each microwell. The plate was placed on an orbital shaker for one minute and then incubated for four hours under culture conditions, after which the toxicant and medium was removed from the microwells and 100 μl of crystal dissolving solution added to each well. The plate was then returned to an orbital shaker for five minutes and then into the incubator for an additional 20 minutes to ensure dissolution of formazan crystals. Formazan generation by cells was measured at 570 nm. All cytotoxicity measurements were made in triplicate (including controls) and three independent times.

2.7. Design and fabrication of TOXOR prototype device

Our previous report [26] described the initial design and fabrication process for the TOXOR prototype device which was capable of electrochemical measurements of AP activity in A549 human lung epithelial cells cultured on-chip for 24 hours. The next iteration overcomes fluidic and engineering challenges encountered previously (See Fig. 1A and B). The device was designed using CorelDraw X5 software and assembled using a thin film lamination approach of polyester layers bonded through chemical adhesion. Each layer (11 in total) was machined individually using an Epilog Zing CO_2 laser cutter and included the following fluidic and sensing subcomponents.

"Membrane touch" activated reagent cavity – (volume 220 μl) filled with assay substrate 2-naphthyl phosphate. This patented "membrane touch" component [31] functioned by manual application (finger pressure) of a force (6 N) to the 100 μm polyester membrane that sealed the cavity, expelling the substrate through a 0.2×8.0 mm channel into the microwell.

Microwell – (capacity 45 μl, surface area 55.7 mm^2) fish cells were cultured here and exposed to toxicants for the relevant duration.

Sink – (volume 122 μl) waste collection chamber connected to the microwell via channels (10×1.9 mm^3) – after flooding the microwell with assay substrate, chemical toxicants and medium were washed through the channels into waste.

Suction pump mechanism – consisted of a sealed air filled cavity (volume 25 μl) activated by finger pressure application of a force (6 N) to a membrane directly above the cavity which expelled air into the microwell. Upon removal of applied force, liquid was then drawn into the electrochemical cell from the microwell cavity for analysis.

Electrochemical cell – (volume 2.4 μl) consisted of stencil printed carbon working (3.8 mm^2) and Ag/AgCl reference electrodes sealed by a Melinex O PET layer (100 μm). The cell was connected to the microwell through a 0.2×5.9 mm channel.

2.8. TOXOR prototype device on-chip cytotoxicity measurement

Fifteen devices were assembled and the microwells of each device washed with deionised water and dried under a stream of N_2 gas. They were then placed in a Telstar class II biosafety cabinet and sterilised under UV (254 nm) for 20 minutes. Sterility was confirmed by standard microbiological testing. In order to improve cell attachment and growth, the microwells of each device were precoated with poly-L-lysine (0.1 mg/ml) for 30 minutes, followed by washing with PBS and drying in a laminar flow hood for one hour prior to cell seeding. Aliquots, 20 μl of RTG-2 cells suspended in culture medium (2×10^4 cells) were added to the microwells followed by the addition of 20 μl of either of pre-sterilised deionised water or 600 μM $CdCl_2$. After 24 hours incubation, cell culture medium and

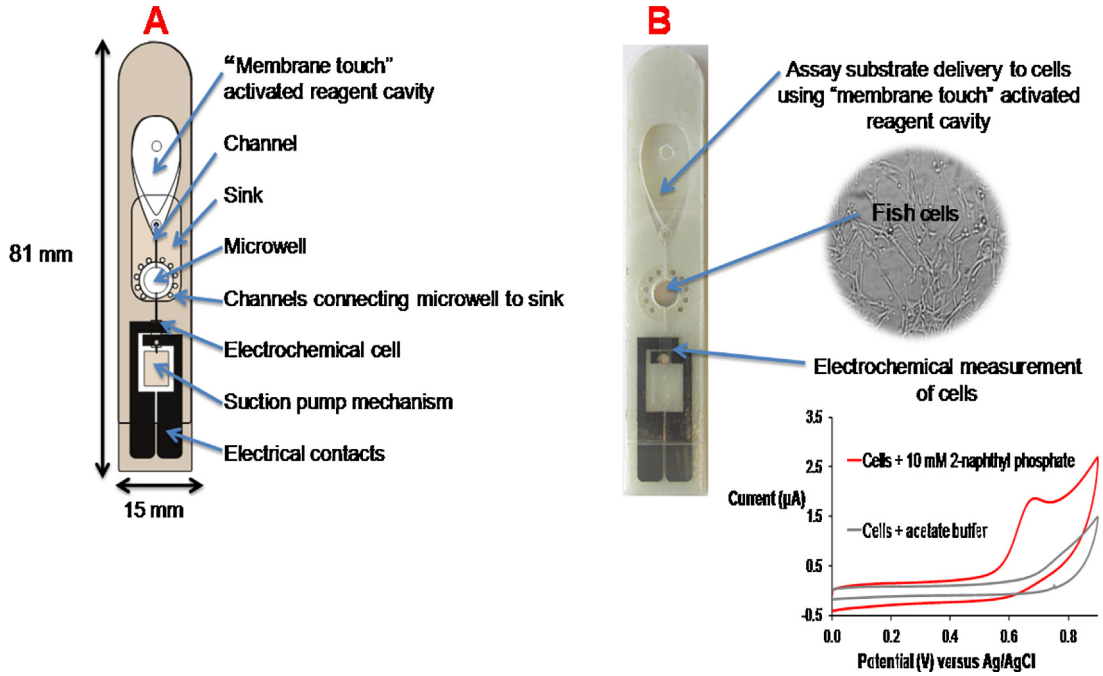

Fig. 1. CorelDraw schematic of TOXOR prototype device (A) showing integrated microwell, fluidic and electrode components and image (B) of the device illustrating the role the reagent cavity, microwell and electrochemical cell play in on-chip culture and measurement of enzyme activity in fish cells.

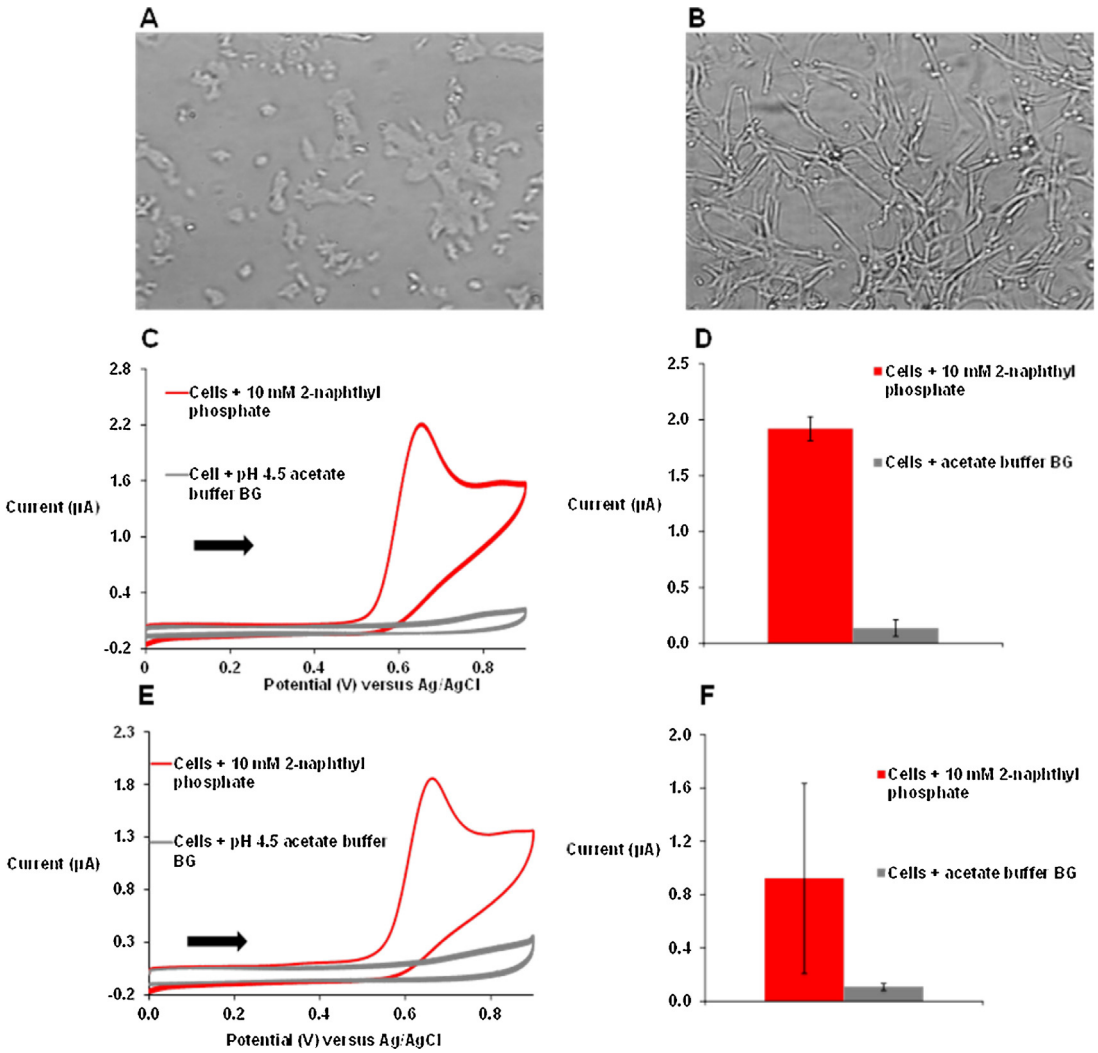

Fig. 2. Light microscopy (200 X) images of (A) PLHC-1 and (B) RTG-2 cells after 24 hours in culture. CV data showing electrochemical detection of AP activity in PLHC-1 (C and D) and RTG-2 (E and F) cells following 24 hours culture. Arrows indicate direction of scan. Results are from a single experiment carried out in triplicate. Error bars are standard deviation n = 3.

toxicant were removed from the microwells which were washed with PBS. Aliquots (40 μl) of either pre-sterilised 10 mM 2-naphthyl phosphate or pH 4.5 acetate buffer were then added to the microwells of each device and incubated for an additional four hours. A sample was then drawn from the microwell of each device via the suction pump mechanism into the on-chip electrochemical detector for analysis.

2.9. Statistical analysis

Experiments to investigate the effect of substrate concentration on measured charge were analysed using a one way ANOVA while experiments to examine the influence of cell number and substrate incubation time on 2-naphthol generation by cells were analysed using a one way ANOVA plus Dunnett's comparison. Additionally, all electrochemical and MTT cytotoxicity assay experiments were analysed using a one way ANOVA plus Dunnett's comparison. Finally, the results of the on-chip cytotoxicity experiments were analysed using a two-sample *t*-test. For all experiments $P < 0.05$ was considered statistically significant. All statistical analysis was undertaken using Minitab 17 statistical software.

3. Results and discussion

3.1. Electrochemical cytotoxicity assay development

Prior to electrochemical detection of AP activity, PLHC-1 and RTG-2 cells were viewed under a light microscope, 24 hours after plating (Fig. 2A and B). Both PLHC-1 and RTG-2 cells formed an even layer over the surface of the microwell but were not fully confluent, with RTG-2 cells having a distinctive elongated shape which has been previously reported [32]. AP activity was detected by CV in both PLHC-1 and RTG-2 cells with an average current of 1.9 and 0.9 μA measured respectively for 4×10^4 cells (Fig. 2C–F). The current measured in PLHC-1 cells was larger than that measured for the equivalent number of RTG-2 cells suggesting greater AP activity in this cell line. In addition there was greater variation in current measured in RTG-2 cells which was likely to be related to cell density and was subsequently optimised.

The optimum assay conditions selected for PLHC-1 cells were 4×10^4 cells per microwell, 10 mM 2-naphthyl phosphate and a substrate incubation time of one hour. In the case of RTG-2 cells, 2×10^4 cells per microwell, 10 mM 2-naphtyl phosphate and a substrate incubation time of four hours were deemed appropriate (See Supplementary information S-1 to S-3).

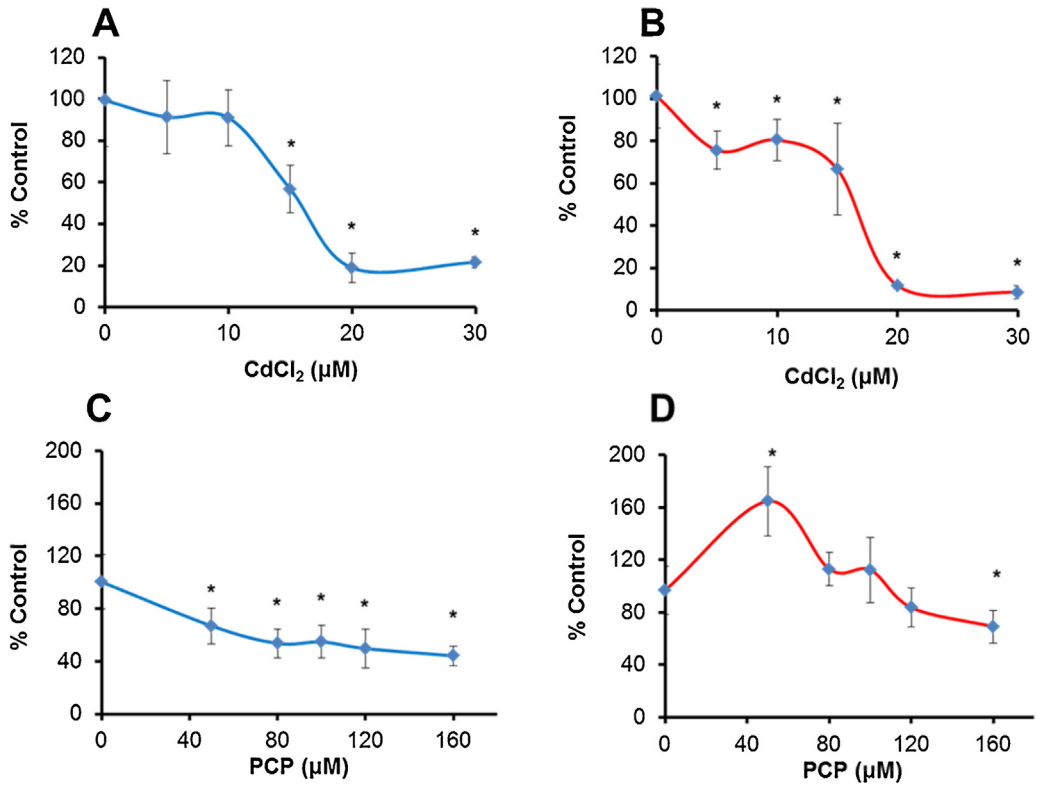

Fig. 3. Electrochemical and MTT cytotoxicity assay results following 24 hours exposure of PLHC-1 cells to $CdCl_2$ (0 to 30 μM) (A and B) and PCP (0 to 160 μM) (C and D). Electrochemical assay data is presented in blue while MTT assay data is presented in red. Results are the mean of three independent experiments. Error bars represent SD (n = 9). Points marked with an asterisk are statistically significant from controls $P < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. *Electrochemical cytotoxicty assessement of $CdCl_2$ and PCP*

A concentration dependent reduction in AP activity was detected in both PLHC-1 and RTG-2 cells following 24 hours exposure to $CdCl_2$ and PCP (Figs. 3A and C (PLHC-1 cells)) and (Fig. 4A (RTG-2 cells $CdCl_2$ only)). The cytotoxicity of these chemicals was also examined using the MTT assay (Fig. 3B and D (PLHC-1 cells)) and Fig. 4B (RTG-2 cells $CdCl_2$ only). From this data, the IC_{50} values at 24 hours, i.e. the concentration of $CdCl_2$ or PCP resulting in 50% inhibition in AP or mitochondrial dehydrogenase activity in cells were determined. The electrochemically determined IC_{50} values for $CdCl_2$ in both cell lines were found to be in agreement with that of the MTT cytotoxicity assay (Table 1). The IC_{50} values were also comparable with those reported in the literature [33]. Della Torre *et al.* (2012) used the MTT assay to investigate the cytotoxicity of $CdCl_2$ towards PLHC-1 cells and reported an IC_{50} value of 39.66 μM following 24 hours exposure [34]. Morcillo *et al.* (2015) examined the toxicity of $CdCl_2$ towards fibroblast SAF-1 cells from the gilthead seabream, using the MTT, LDH and neutral red uptake assays and determined IC_{50} values at 24 hours of 100, 703 and 100 μM respectively [35]. The reduced sensitivity of the LDH assay compared to the neutral red and MTT assays was suggested to be due to lysosomal and mitochondrial damage occurring at lower $CdCl_2$ concentrations rather than cell membrane damage. Recently the cytotoxicity of $CdCl_2$ towards sea bass brain cells (DLB-1) was investigated using both MTT and neutral red uptake assays with IC_{50} values at 24 hours of 15 and 4 μM reported respectively [29].

Table 1
Comparison of IC_{50} values following Electrochemical and MTT assays.

Cell line	Chemical	Electrochemical IC_{50} (μM)[a]	MTT IC_{50} (μM)[a]
PLHC-1	$CdCl_2$	16.2 ± 0.7	16.3 ± 1.6
PLHC-1	PCP	127 ± 22	>160
RTG-2	$CdCl_2$	91 ± 11	164 ± 96

[a] IC_{50} values are the average of those obtained from three independent experiments.

PLHC-1 cells were found to be more sensitive towards PCP than RTG-2 cells and as was the case in our previous study with mammalian cells, the electrochemical cytotoxicity assay showed enhanced sensitivity towards PCP compared with the MTT assay. The MTT assay data showed increased formazan generation by PLHC-1 cells when exposed to 50 μM PCP (Fig. 3D). At higher concentrations, formazan generation decreased with a statistically significant reduction detected at 160 μM PCP compared with controls. In RTG-2 cells, the electrochemical assay detected cytotoxicity when cells were exposed to ≥120 μM concentrations of PCP compared with the MTT assay which did not detect any cytotoxicity over the range of PCP concentrations investigated (Data not shown).

The MTT assay has been used by a number of groups to investigate the cytotoxicity of PCP towards different cell lines with a range of sensitivities reported [36–38]. Repetto et al. (2001) determined an IC_{50} value at 24 hours of > 200 μM when investigating the cytotoxicity of PCP towards RTG-2 cells [39] which was comparable with what was found in this study. A more recent study has suggested that rainbow trout liver (RTL-W1) cells are more sensitive to PCP than RTG-2 cells with IC_{50} values of 1.55 and 8.91 μM determined after 24 hours using the neutral red uptake and MTT assays respectively [40]. Significant cytotoxicity towards primary hepatocytes of *Carassius carassius* (crucian carp) has also been shown following exposure to as little as 1 μM of PCP for 8 hours [30].

The neutral red uptake assay, an indicator of lysosomal damage, has been found to be more sensitive to PCP than the MTT assay

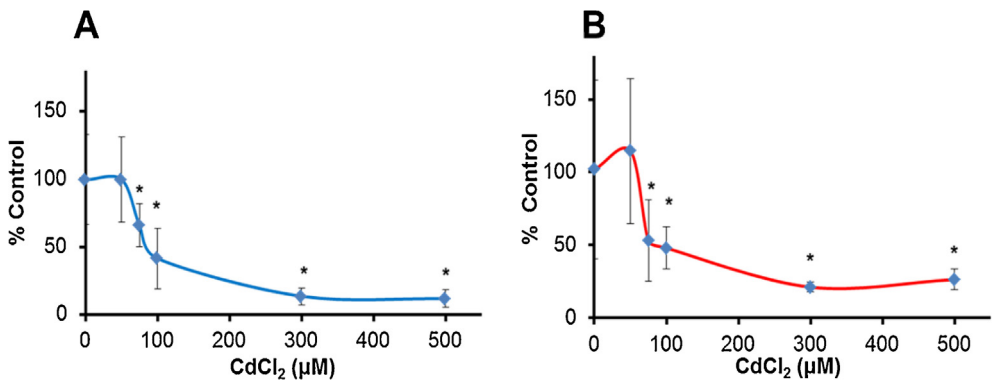

Fig. 4. Electrochemical and MTT cytotoxicity assay results (B) following 24 hours exposure of RTG-2 cells to $CdCl_2$ (0 to 500 µM). Electrochemical assay data is presented in blue while MTT assay data is presented in red. Results are the mean of three independent experiments. Error bars represent SD (n = 9). Points marked with an asterisk are statistically significant from controls. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

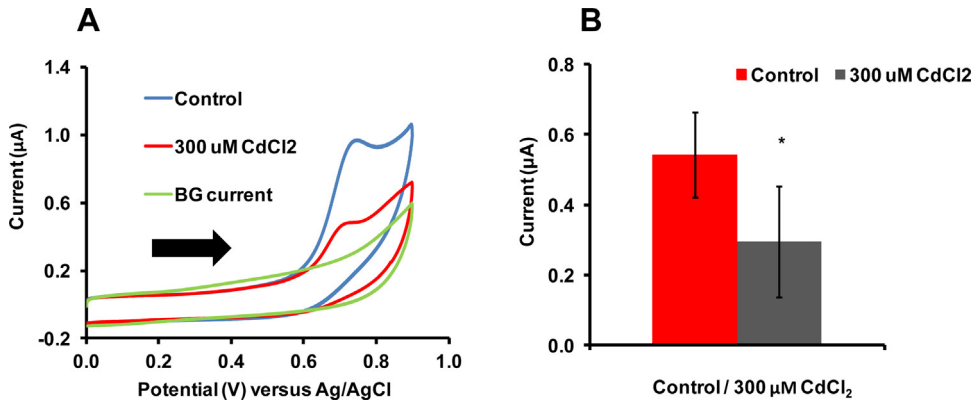

Fig. 5. (A) CV measurements made using TOXOR biochip showing a clear reduction in AP in RTG-2 cells following 24 hours exposure to 300 µM $CdCl_2$. Arrow indicates direction of scan. (B) Average current (µA) measured following 24 hours exposure of cells to 300 µM $CdCl_2$ or 50/50 medium sterile water (control). Results are the mean measurements from five devices with standard deviation (n = 5). *Statistically signficant from controls $P < 0.05$.

[41]. Since AP enzymes are located in the lysosomes and cytosol of cells [42], it is possible that AP is also more sensitive to PCP than mitochondrial dehydrogenase enzymes, which might explain the enhanced sensitivity of the electrochemical assay towards PCP.

3.3. TOXOR on-chip cytotoxicity measurement

In order to demonstrate that the biochip could make simple cytotoxicity measurements, AP activity in RTG-2 cells was analysed after exposure to 300 µM $CdCl_2$ using the TOXOR portable cytotoxicity device with data revealing a 46% reduction in AP activity compared to controls ($P < 0.05$, Fig. 5A and B), demonstrating for the first time that this novel device was capable of performing on-chip cytotoxicity measurements. Traditional cytotoxicity assays such as the MTT assay are limited to a laboratory setting, requiring addition of a colorimetric dye and solvent solution to microwells prior to making measurements using a microplate reader. In contrast, the TOXOR biochip proposed here simplifies cytotoxicity measurements, requiring simple addition of the "on device" assay substrate to cultured cells by application of finger pressure to the pressure sensitive membrane that seals the "membrane touch" activated substrate reagent cavity (Fig. 1A). Electrochemical measurements of cellular AP activity may be easily achieved using the screen printed transducer. The device can be combined with a micropotentiostat controller which can be phone or tablet operated facilitating use by personal with minimum training in electrochemistry.

4. Conclusion

High-value information on the toxic state of watercourses, on-site and immediately after a pollution incident is an emerging technology. The fish cell based biosensor device proposed here relies on quantifiable cellular enzyme AP under optimised assay conditions in response to toxicant exposure. The IC_{50} values for $CdCl_2$ determined using the AP based electrochemical cytotoxicity assay were in agreement with those found using the MTT assay in both cell lines while in the case of PCP, the electrochemical assay demonstrated enhanced sensitivity. A hand held cytotoxicity test device suitable for on-site aquatic screening successfuly demonstrated capability for simple cytotoxicity measurements in RTG-2 cells cultured on-chip using the AP based electrochemical approach. Utilising fish cells as the biological reognition element, the TOXOR prototype had proven advantages including no requirement for a source of CO_2 during incubation, ease of use and low cost fabrication from simple polyester based and conductive ink materials. Additionally, measurement of cytotoxic effects towards biologically relevant fish cells gives a more accurate indication of the toxicity risk that such water poses to aquatic health. However, additional benchmarking is required to ascertain how cytotoxicity data generated by the TOXOR prototype relates to water quality. At present, it takes 24 hours for the biochip to generate cytotoxicity data and further work will investigate time dependent cytotoxicity with the view to reducing total time to result. It is envisioned that this low cost device can contribute to on-site water toxicity

screening by generating cytotoxicity data which will inform remedial action via specialist analytical testing techniques.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2016.11.097.

References

[1] European-Environment-Agency, Water Pollution-Overview, 2008, http://www.eea.europa.eu/themes/water/water-pollution (Accessed 8 October 2015).
[2] European-Commission, Priority Substances-Water-Environment, European Commission, 2015, http://ec.europa.eu/environment/water/water-dangersub/pri_substances.htm (Accessed 25 April 2016).
[3] H.J. Shin, Genetically engineered microbial biosensors for in situ monitoring of environmental pollution, Appl. Microbiol. Biotechnol. 89 (2011) 867–877, http://dx.doi.org/10.1007/s00253-010-2990-8.
[4] X. Zhu, H. Qin, J. Liu, Z. Zhang, Y. Lu, X. Yuan, D. Wu, A novel electrochemical method to evaluate the cytotoxicity of heavy metals, J. Hazard. Mater. 271 (2014) 210–219, http://dx.doi.org/10.1016/j.jhazmat.2014.02.030.
[5] S.H.A. Hassan, S.W. Van Ginkel, M.A.M. Hussein, R. Abskharon, S.-E. Oh, Toxicity assessment using different bioassays and microbial biosensors, Environ. Int. 92 (2016) 106–118, http://dx.doi.org/10.1016/j.envint.2016.03.003.
[6] C. Liu, D. Yong, D. Yu, S. Dong, Cell-based biosensor for measurement of phenol and nitrophenols toxicity, Talanta 84 (2011) 766–770, S0039914011001378.
[7] S. Parvez, C. Venkataraman, S. Mukherji, A review on advantages of implementing luminescence inhibition test (Vibrio fischeri) for acute toxicity prediction of chemicals, Environ. Int. 32 (2006) 265–268, http://dx.doi.org/10.1016/j.envint.2005.08.022.
[8] S.H.A. Hassan, S.W. Van Ginkel, S.-E. Oh, Detection of Cr 6+ by the sulfur oxidizing bacteria biosensor: effect of different physical factors, Environ. Sci. Technol. 46 (2012) 7844–7848, http://dx.doi.org/10.1021/es301360a http://0-pubs.acs.
[9] J. Li, Y. Yu, Y. Wang, J. Qian, J. Zhi, The benzoquinone-mediated electrochemical microbial biosensor for water biotoxicity assay, Electrochim. Acta 97 (2013) 52–57, S0013468613003058.
[10] Q. Zhang, J. Ding, L. Kou, W. Qin, A potentiometric flow biosensor based on ammonia-oxidizing bacteria for the detection of toxicity in water, Sensors 13 (2013) 6936–6945 http://www.mdpi.com/1424-8220/13/6/6936/htm.
[11] M.U. Anu Prathap, A.K. Chaurasia, S.N. Sawant, S.K. Apte, Polyaniline-based highly sensitive microbial biosensor for selective detection of lindane, Anal. Chem. 84 (2012) 6672–6678, http://dx.doi.org/10.1021/ac301077d.
[12] F. Cortés-Salazar, S. Beggah, J.R. van der Meer, H.H. Girault, Electrochemical As(III) whole-cell based biochip sensor, Biosens. Bioelectron. 47 (2013) 237–242, S095656631300170X.
[13] C. Chouteau, S. Dzyadevych, C. Durrieau, J. Chovelon, A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples, Biosens. Bioelectron. 21 (2005) 273–281, S0956566304004907.
[14] A. Tsopela, A. Lale, E. Vanhove, O. Reynes, I. Séguy, P. Temple-Boyer, P. Juneau, R. Izquierdo, J. Launay, Integrated electrochemical biosensor based on algal metabolism for water toxicity analysis, Biosens. Bioelectron. 61 (2014) 290–297, S0956566314003364.
[15] P. Banerjee, S. Kintzios, B. Prabhakarpandian, Biotoxin detection using cell-based sensors, Toxins (Basel) 5 (2013) 2366–2384 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3873691&tool=pmcentrez&rendertype=abstract.
[16] T.M. Curtis, M.W. Widder, L.M. Brennan, S.J. Schwager, W.H. van der Schalie, J. Fey, N. Salazar, A portable cell-based impedance sensor for toxicity testing of drinking water, Lab Chip 9 (2009) 2176–2183, http://dx.doi.org/10.1039/b901314h.
[17] H. Du, J. Li, B. Moe, C.F. McGuigan, X.-F. Li, A real-time cell-electronic sensing method for comparative analysis of toxicity of water contaminants, Anal. Methods 6 (2014) 2053–2058 http://0-pubs.rsc.org.millennium.it-tallaght.ie/en/content/articlehtml/2014/ay/c3ay41686k.
[18] R. Kubisch, U. Bohrn, M. Fleischer, E. Stütz, Cell-based sensor system using L6 cells for broad band continuous pollutant monitoring in aquatic environments, Sensors (Basel) 12 (2012) 3370–3393 http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3376625&tool=pmcentrez&rendertype=abstract.
[19] F. Liu, A.N. Nordin, F. Li, I. Voiculescu, A lab-on-chip cell-based biosensor for label-free sensing of water toxicants, Lab Chip 14 (2014) 1270–1280 http://0-pubs.rsc.org.millennium.it-tallaght.ie/en/content/articlehtml/2014/lc/c3lc51085a.
[20] T.M. Curtis, A.M. Collins, B.D. Gerlach, L.M. Brennan, M.W. Widder, W.H. van der Schalie, N.T.K. Vo, N.C. Bols, Suitability of invertebrate and vertebrate cells in a portable impedance-based toxicity sensor: temperature mediated impacts on long-term survival, Toxicol. In Vitro 27 (2013) 2061–2066, S0887233313001859.
[21] L.M. Brennan, M.W. Widder, M.K. McAleer, M.W. Mayo, A.P. Greis, W.H. van der Schalie, Preparation and testing of impedance-based fluidic biochips with RTgill-W1Cells for rapid evaluation of drinking water samples for toxicity, J. Vis. Exp. 109 (2016) 53555, http://dx.doi.org/10.3791/53555.
[22] E. Hondroulis, C. Liu, C.-Z. Li, Whole cell based electrical impedance sensing approach for a rapid nanotoxicity assay, Nanotechnology 21 (2010) 315103 http://iopscience.iop.org/article/10.1088/0957-4484/21/31/315103.
[23] M.E. Polak, D.M. Rawson, B.G.D. Haggett, Redox mediated biosensors incorporating cultured fish cells for toxicity assessment, Biosens. Bioelectron. 11 (1996) 1253–1257, 0956566396880900.
[24] M.W. Widder, L.M. Brennan, E.A. Hanft, M.E. Schrock, R.R. James, W.H. van der Schalie, Evaluation and refinement of a field-portable drinking water toxicity sensor utilizing electric cell-substrate impedance sensing and a fluidic biochip, J. Appl. Toxicol. 35 (2015) 701–708, http://dx.doi.org/10.1002/jat.3017.
[25] L.M. Brennan, M.W. Widder, L.E.J. Lee, W.H. van der Schalie, Long-term storage and impedance-based water toxicity testing capabilities of fluidic biochips seeded with RTgill-W1 cells, Toxicol. In Vitro. 26 (2012) 736–745, S0887233312000719.
[26] T. O'Hara, B. Seddon, S. McClean, E. Dempsey, TOXOR: design and application of an electrochemical toxicity biosensor for environmental monitoring, Electroanalysis 27 (2015) 58–66, http://dx.doi.org/10.1002/elan.201400433.
[27] P.C. Naha, H.J. Byrne, Generation of intracellular reactive oxygen species and genotoxicity effect to exposure of nanosized polyamidoamine (PAMAM) dendrimers in PLHC-1 cells in vitro, Aquat. Toxicol. 132–133 (2013) 61–72, S0166445X13000301.
[28] P.C. Naha, A. Casey, T. Tenuta, I. Lynch, K.A. Dawson, H.J. Byrne, M. Davoren, Preparation, characterization of NIPAM and NIPAM/BAM copolymer nanoparticles and their acute toxicity testing using an aquatic test battery, Aquat. Toxicol. 92 (2009) 146–154, S0166445X09000472.
[29] P. Morcillo, E. Chaves-Pozo, J. Meseguer, M.Á. Esteban, A. Cuesta, Establishment of a new teleost brain cell line (DLB-1) from the European sea bass and its use to study metal toxicology, Toxicol. In Vitro 38 (2016) 91–100, http://dx.doi.org/10.1016/j.tiv.2016.10.005.
[30] Y.-L. Dong, P.-J. Zhou, S.-Y. Jiang, X.-W. Pan, X.-H. Zhao, Induction of oxidative stress and apoptosis by pentachlorophenol in primary cultures of Carassius carassius hepatocytes, Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 150 (2009) 179–185, S153204560900091X.
[31] Micro-reagent handler and cartridge assembly P10977EP00, 12192551.5, (2013).
[32] D.D. Mosser, J.J. Heikkila, N.C. Bols, Temperature ranges over which rainbow trout fibroblasts survive and synthesize heat-shock proteins, J. Cell. Physiol. 128 (1986) 432–440, http://dx.doi.org/10.1002/jcp.1041280312.
[33] W.W.L. Chan, K.M. Chan, Cloning and characterization of a tilapia (Oreochromis aureus) metallothionein gene promoter in Hepa-T1 cells following the administration of various heavy metal ions, Aquat. Toxicol. 86 (2008) 59–75, S0166445X07003761.
[34] C. Della Torre, R. Zaja, J. Loncar, T. Smital, S. Focardi, I. Corsi, Interaction of ABC transport proteins with toxic metals at the level of gene and transport activity in the PLHC-1 fish cell line, Chem. Biol. Interact. 198 (2012) 9–17, http://dx.doi.org/10.1016/j.cbi.2012.04.008.
[35] P. Morcillo, M.Á. Esteban, A. Cuesta, Heavy metals produce toxicity, oxidative stress and apoptosis in the marine teleost fish SAF-1 cell line, Chemosphere 144 (2015) 225–233, S0045653515300217.
[36] Y. Guo, B. Zhou, Thyroid endocrine system disruption by pentachlorophenol: an in vitro and in vivo assay, Aquat. Toxicol. 142–143 (2013) 138–145, S0166445X13002129.
[37] H. Sun, L.-C. Xu, J.-F. Chen, L. Song, X.-R. Wang, Effect of bisphenol A, tetrachlorobisphenol A and pentachlorophenol on the transcriptional activities of androgen receptor-mediated reporter gene, Food Chem. Toxicol. 44 (2006) 1916–1921, S0278691506001712.
[38] S. Yang, X. Han, C. Wei, J. Chen, D. Yin, The toxic effects of pentachlorophenol on rat Sertoli cells in vitro, Environ. Toxicol. Pharmacol. 20 (2005) 182–187, S1382668905000128.
[39] G. Repetto, A. Jos, M.J. Hazen, M.L. Molero, A. del Peso, M. Salguero, P. de. Castillo, M.C. Rodríguez-Vicente, M. Repetto, A test battery for the ecotoxicological evaluation of pentachlorophenol, Toxicol. In Vitro 15 (2001) 503–509, S0887233301000558.
[40] C. Pietsch, J. Hollender, F. Dorusch, P. Burkhardt-Holm, Cytotoxic effects of pentachlorophenol (PCP) and its metabolite tetrachlorohydroquinone (TCHQ) on liver cells are modulated by antioxidants, Cell Biol. Toxicol. 30 (2014) 233–252, http://dx.doi.org/10.1007/s10565-014-9283-4.
[41] P. Fernández Freire, V. Labrador, J.M. Pérez Martín, M.J. Hazen, Cytotoxic effects in mammalian Vero cells exposed to pentachlorophenol, Cell Biol. Toxicol. 210 (2005) 37–44, S0300483X05000624.

[42] A. Siddiqua, A. Saeed, R. Naz, M. Sherazi, S. Abbas, A. Saeed, Purification and biochemical properties of acid phosphatase from Rohu fish liver, Int. J. Agric. Biol. 14 (2012) 223–228, http://www.cabdirect.org/abstracts/20123099873.html;jsessionid=0B878ED6BBAE9B94EADFFD345C6BE788.

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