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Electrical Impedance for Non-Destructive, Real Time Measurement of Neural Cell Viability on Microelectrode Arrays

Seline Choo^{1,2}, Kathleen Wallace², Theresa Freudenrich², Timothy Shafer²

¹ORISE Research Participant ²Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency

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choo.seline@epa.gov

Introduction

Background :

- Microelectrode arrays (MEAs) capture compound effects on neural network activity in vitro and are used to characterize potential chemical neurotoxicity hazard
- Cell viability is typically determined with CellTiter-Blue® (CTB) and lactate dehydrogenase (LDH) assays
- Previously, tracking network activity and cell viability concomitantly was not possible
- Recently, it has become possible to measure electrical impedance of cells without interrupting cell neural activity
- Impedance is the amount of opposition that a circuit presents to current or voltage change
- In MEA assays, impedance can serve as a surrogate measure of cell number

Aim :

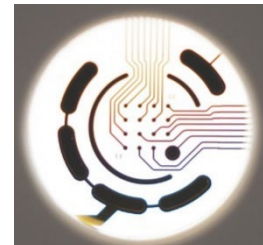
- To compare impedance to other cell viability assays to determine the utility of impedance as a metric of cell viability

Approach :

- Comparing impedance measures with CTB and LDH in its response to compounds with known cytotoxicity



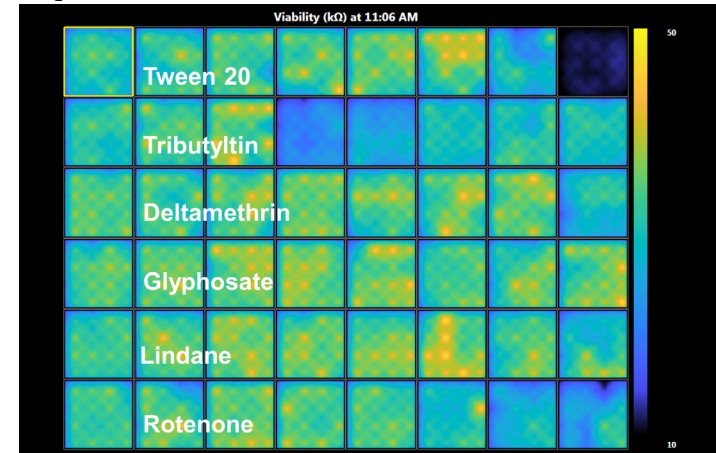
48well CytoView MEA plate



Electrodes in a well

Compounds Tested	Expected Outcome
Tween 20	Cytotoxic (Positive control)
Thiamethoxam	Non-Cytotoxic (Negative control)
Tributyltin	Cytotoxic (Positive control)
Deltamethrin	Cytotoxic at higher concentration
Glyphosate	Non-Cytotoxic (Negative control)
Lindane	Non-Cytotoxic (Negative control)
Rotenone	Cytotoxic (Positive control)

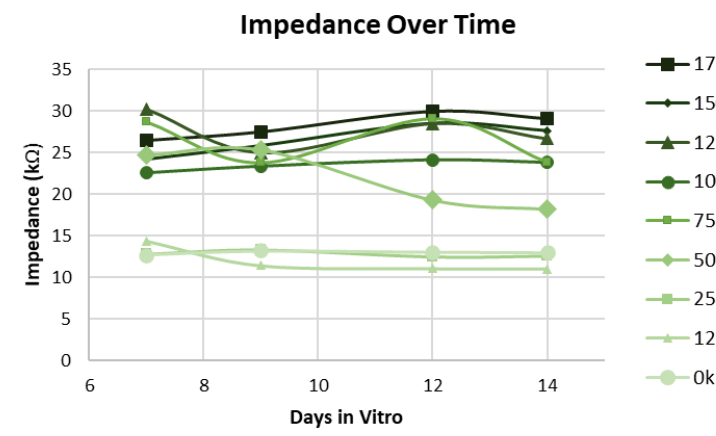
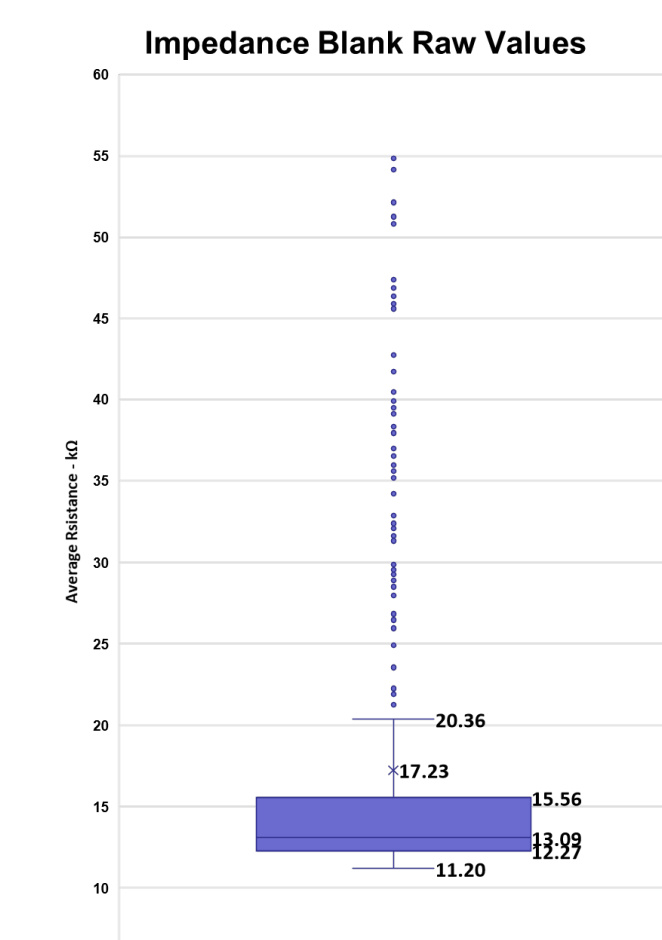
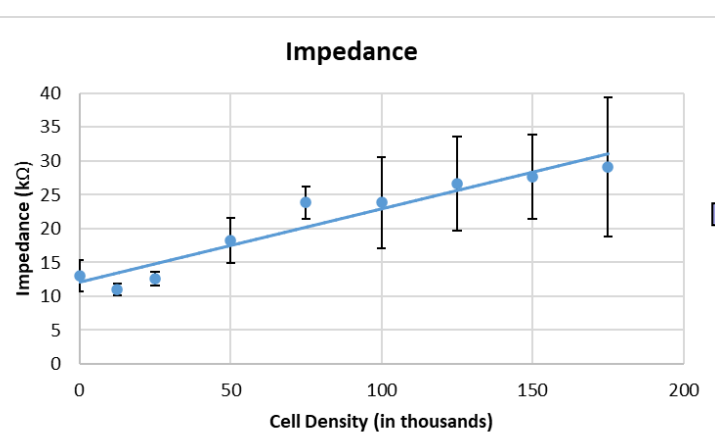
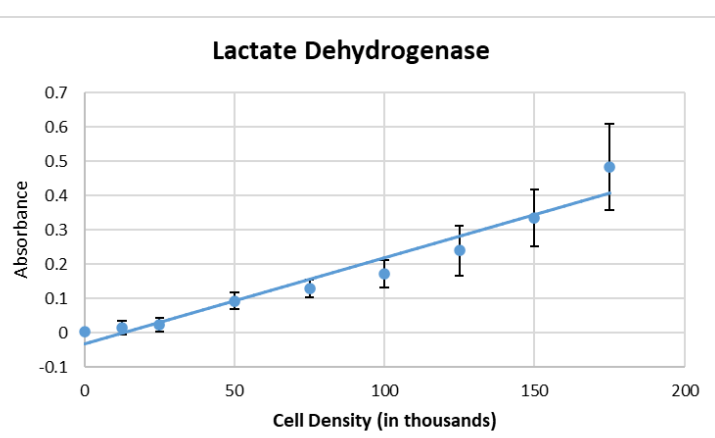
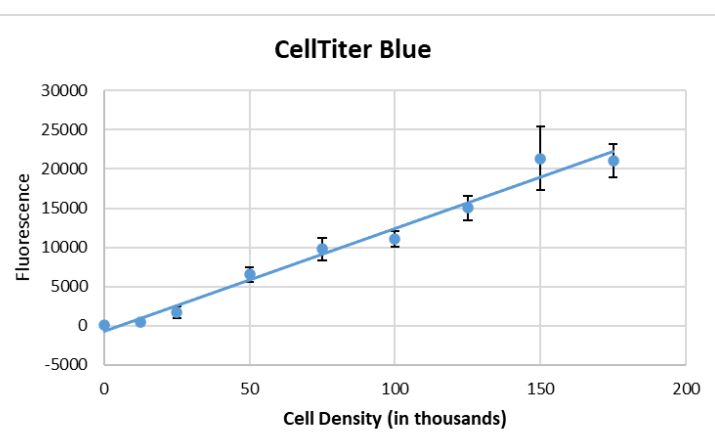
Impedance measurement 72 hour after acute treatment



Col 1: Solvent Control

Increasing concentration

Impedance, LDH and CTB signals all increase with increasing cell density



Method :

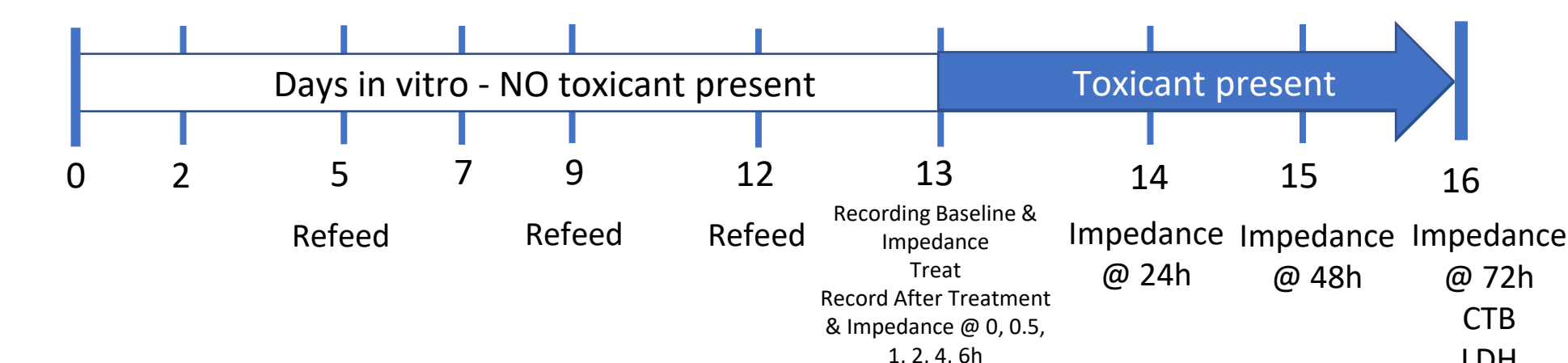
- MEA plate are seeded with different cell densities – 0k, 12.5k, 25k, 50k, 75k, 100k, 125k, 150k and 175k cells
- CTB and LDH performed on DIV14; Impedance measurements collected on multiple timepoints (DIV7, DIV9, DIV12, DIV14)
- To establish blank for impedance, measurements are taken from 3 MEA plates without cells
- The data is skewed, so the median – 13.09kΩ is set as blank.

Results :

- Impedance measurements are consistent over multiple days
- All assays show linear responses with cell densities up to 175k cells per well
- Higher impedance indicates higher cell density

Application of impedance following acute treatment in mature neural networks

Acute Assay

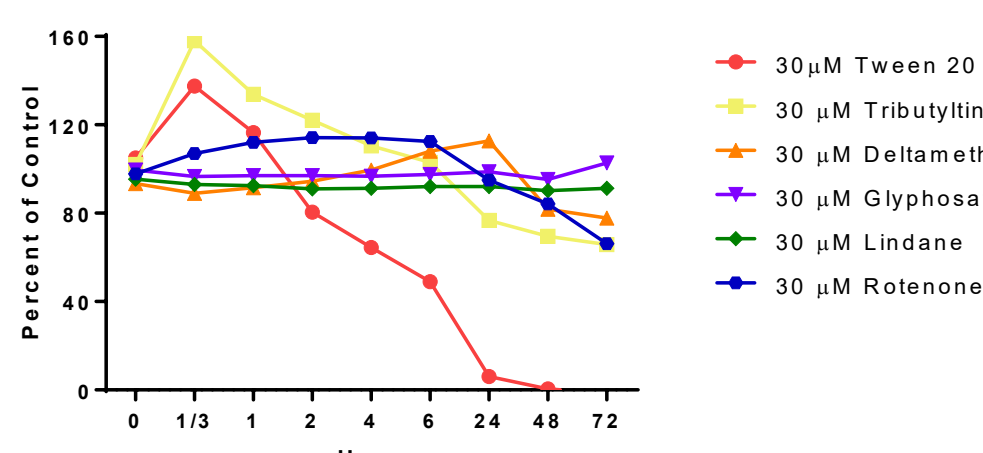


Method :

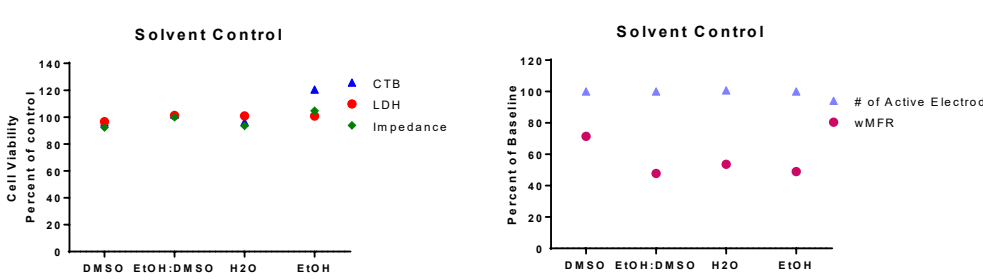
- Primary cultures from rat cortex plated on CytoView MEA 48-well plates
- Exposed to chemical treatment on DIV 13
- Impedance measurements taken before treatment and at 0, 0.5, 1, 2, 4, 6, 24, 48 and 72h post-treatment
- After the last impedance measurement, CTB and LDH assays were performed for comparison

Impedance increased then decreased over time after treatment

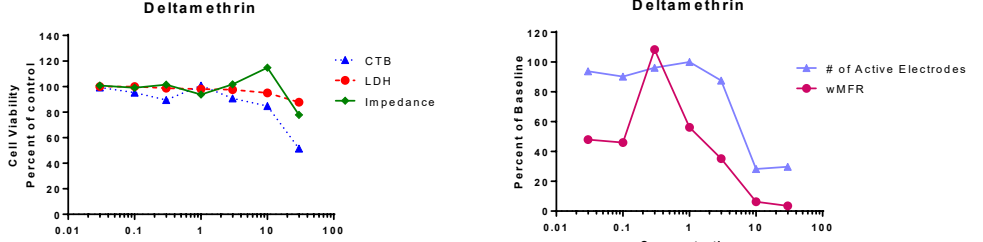
Impedance over Time After Treatment



Solvent Control



Cytotoxic at higher concentration

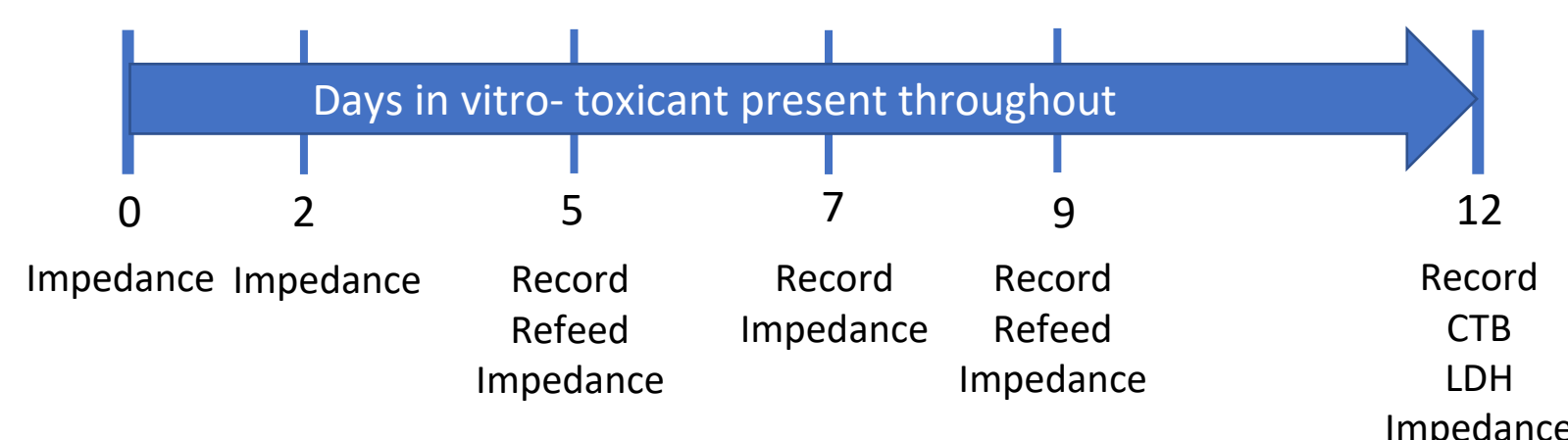


Results :

- Glyphosate and Lindane elicited no cytotoxic effect
- Tween 20, Tributyltin, Rotenone showed increasing cytotoxic effect with increasing concentration
- Deltamethrin showed some cytotoxic effect at higher concentration (>10μM)
- All treatments except for Glyphosate decreased neural network activity as reflected in # of active electrodes and weighted Mean Firing Rate (wMFR) at different concentrations (T20: >0.1μM, Tr: >0.03μM, D: >3μM, L: >30μM, R: >0.03μM)
- Deltamethrin and Lindane increased neural network activity at low concentrations (D: 0.3μM, L: 0.3 -10μM)
- For cytotoxic chemicals, impedance increased then decreased over time after treatment

Application of impedance during neural network formation

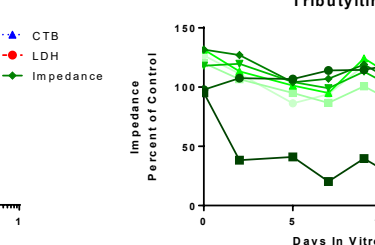
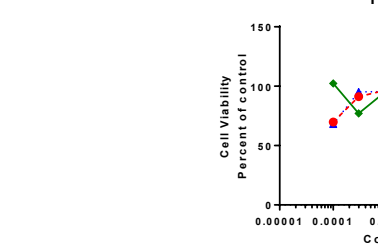
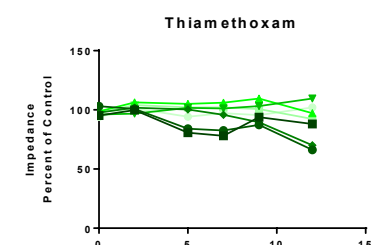
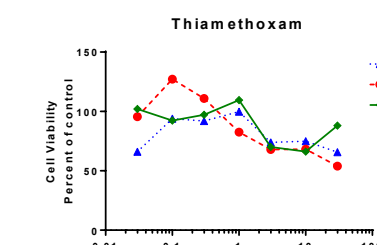
Network Formation Assay (NFA)



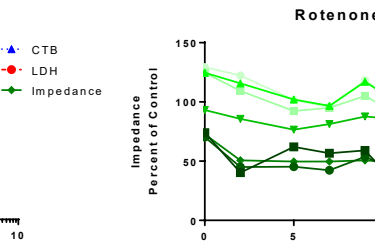
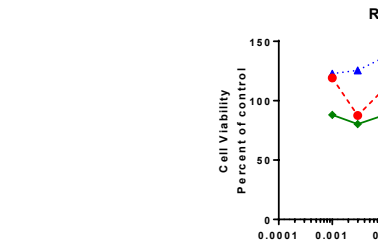
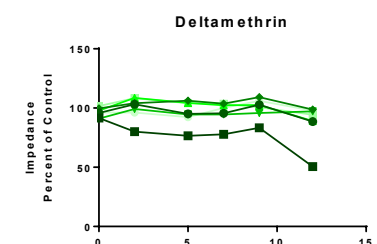
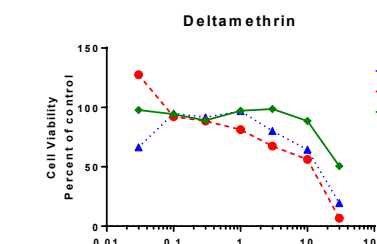
Method :

- Primary cultures from rat cortex plated on CytoView MEA 48-well plates
- Exposed to chemical treatment since DIV 0
- Impedance measurements taken on DIV 0, 2, 5, 7, 9 and 12
- Neural network activity recorded on DIV 5, 7, 9 and 12
- After the last recording on DIV 12, CTB and LDH assays were performed for comparison

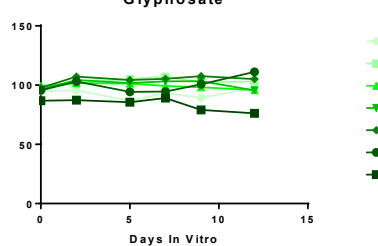
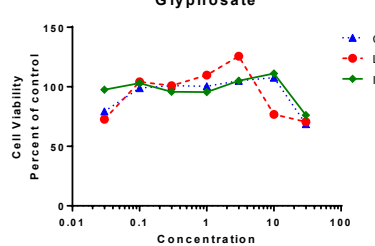
Impedance declined at later timepoints



Impedance declined at earlier timepoints



Impedance constant over time



Results :

- Thiamethoxam and Deltamethrin showed cytotoxic effects at later timepoints (~ DIV 9 to 12) at different concentrations (Th: >3μM, D: 30μM)
- Glyphosate and Lindane are generally non-cytotoxic; only slight decrease in impedance at 30μM
- Tributyltin and Rotenone showed great impact on cytotoxicity at low concentrations (>0.1μM) at early timepoints (~ DIV 0 to 2)

Summary

- Impedance measurements are comparable to LDH and CTB measurements
- These results demonstrated impedance as a reliable cell viability measure
- Impedance measurements can be decreased by technical variability from uncentered cell seeding within the well, which can artificially resemble low cell viability (not shown)
- Impedance measurements allow non-invasive, multi-timepoint monitoring of cell viability in longer exposure assays such as the network formation assay