Screening for Neurotoxicity Adverse Outcome Pathway (AOP) Perturbations in Water Quality Monitoring

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Aim: Propose specific in vitro assays to include in Effect Directed Monitoring (EDM) batteries that cover potential toxic effects not yet screened for by water quality monitoring test facilities

Why in vitro bioassays?

• 3R principle compliant
• Non targeted screening → effects of undefined mixtures
• More hazard-oriented
• Ameenable to High Throughput Screening (HTS)

Approach

1. Literature review → database of currently applied in vivo bioassays
2. Comparison with database of representative chemicals of emerging concern and their related human-relevant AOPs → identify KE’s not yet screened for in water quality monitoring
3. Selection of an assay gap → Neurotoxicity
4. Literature review → database of available in vitro bioassays measuring perturbations of KE’s in human neurotoxicity AOPs
5. Selection of an assay to evaluate its potential for use in water quality monitoring

Criteria for the selection of micro-electrode array assay (MEA)

• Endpoint related to a KE commonly found in neurotoxicity AOPs → Network Activity (e.g. Mean Firing Rate)
• High Throughput potential
• Ability to detect wide range of neurotoxicants
• Overlay with chemicals that have already been tested with EMERCHE chemical list

Criteria for the selection of chemicals

• Different neurotoxicity AOPs covered
• Chemicals that have been found negative and positive in vitro and are or are not known neurotoxicants (3rd column of Table 1)
• Toxicokinetic parameters available in EPA dashboard for relevant chemicals

Research questions

❖ Can the MEA assay be used in water quality monitoring to cover the gap of human neurotoxicity adverse outcome pathways?
❖ How much do toxicokinetic parameters affect the results of the assay?

Next Steps

Evaluate the assay by testing it in the lab with neurotoxicants found in water and/or use literature data (concentration-response curves)
Quantify toxicokinetic parameters (amount of chemical in media and cells) to establish whether or not they affect the result and if it is essential to quantify them when performing an assay

Take home message

There are perspectives to improve neurotoxicity screening in water quality monitoring, by implementing an assay that can detect a wide range of neurotoxic chemicals

Experimental procedure for MEA

Prepare MEA plate
Plate neurons
Maintain neurons until adequate activity is achieved
Record baseline
Process compound
Record post-treatment
Analyze spike trains

Figures 2 and 3

Figure 2

Experimental set-up and readouts from MEA

Figure 3a and 3b

Experimental setup for measurements of spontaneous electrical activity with MEAs and readout of field potentials in raster plot, activity heat maps and example traces of individual field potentials illustrating the degree and pattern of neuronal activity of the primary rat cortical culture.

Table 1

Chemicals of interest to derive or use from literature concentration-response relationships. The chemicals were selected in order to cover the scenarios mentioned in the 3rd column of Table 1. The logEC50 values as a measure of lipophilicity along with the NOAELs from mammalian neurotoxicity studies are also presented.

Abbreviations/Clarifications

1) KE: Key events
2) IVIVE: In vitro→in vivo extrapolation
3) MIEs: Molecular Initiating Events

* Oral doses estimated based on self reports of amount of commodities consumed, measured residual levels in commodities, and average body weights for given age and sex

References


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