ESTABLISHING A HUMANIZED IN VITRO MODEL FOR PESTICIDE-INDUCED NEUROTOXICITY: A COLLABORATIVE EFFORT BETWEEN NETRI AND ANSES

BOOTH #317

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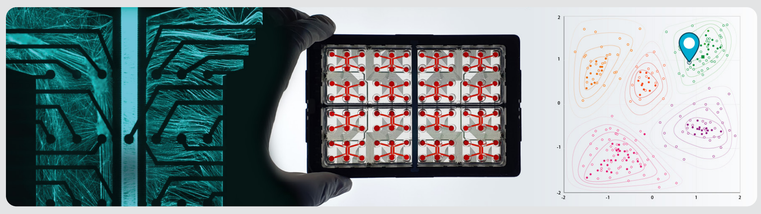
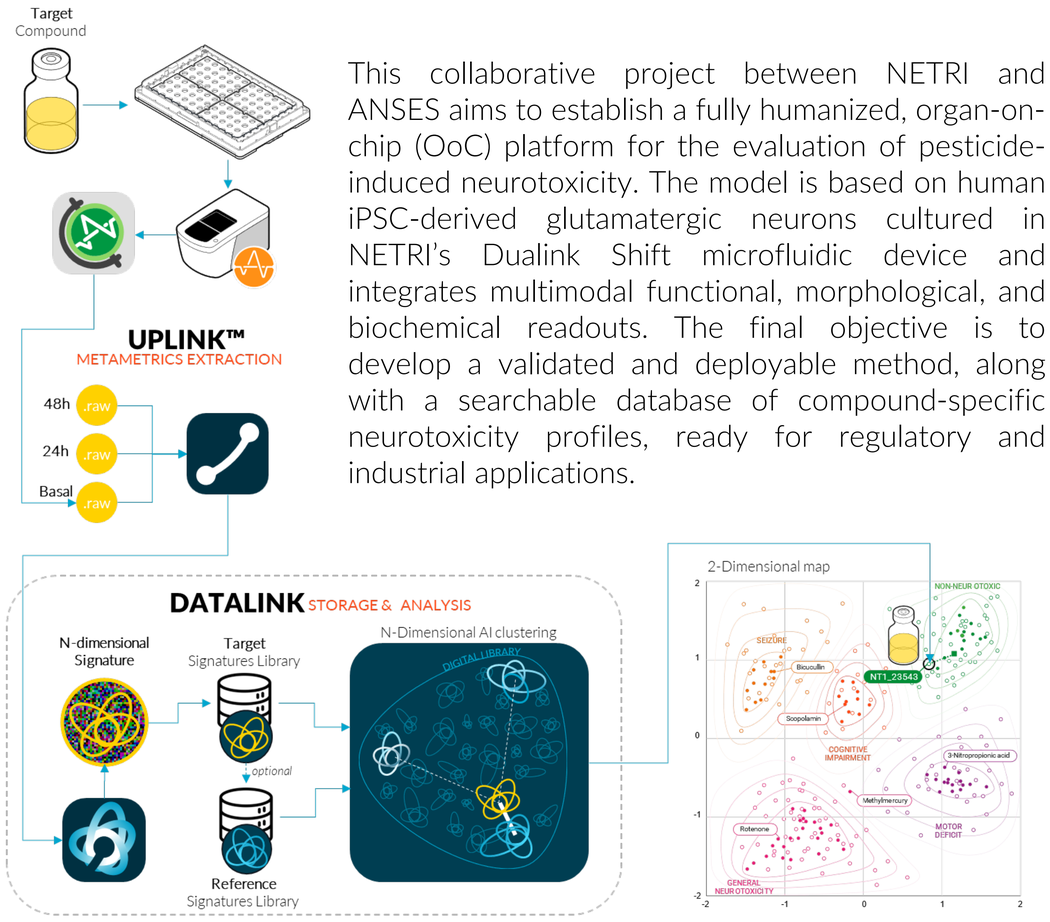
BACKGROUND



Concerns about the neurological impact of pesticide exposure are growing, particularly for sensitive populations such as children. Current toxicological methods often rely on animal models with limited human relevance and insufficient resolution for detecting subtle neurodevelopmental effects. To support improved chemical risk assessment and reduce animal use, New Approach Methodologies (NAMs) are being developed to deliver mechanistic and human-relevant data.

PROJECT AIM

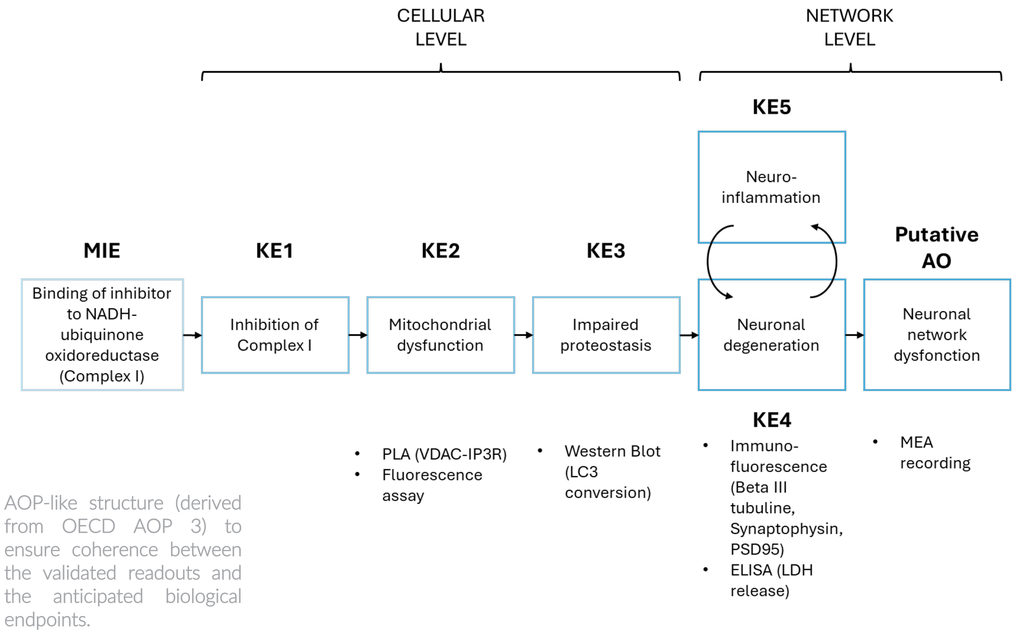


This collaborative project between NETRI and ANSES aims to establish a fully humanized, organ-on-chip (OoC) platform for the evaluation of pesticide-induced neurotoxicity. The model is based on human iPSC-derived glutamatergic neurons cultured in NETRI's Dualink Shift microfluidic device and integrates multimodal functional, morphological, and biochemical readouts. The final objective is to develop a validated and deployable method, along with a searchable database of compound-specific neurotoxicity profiles, ready for regulatory and industrial applications.

Schematic of the whole approach: from a test compound, all the way to the Digital Signature generation and comparison.

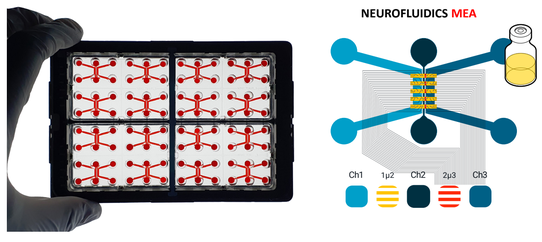
SCIENTIFIC RATIONALE AND AOP RELEVANCE

The approach is designed to address key mechanistic events identified in relevant Adverse Outcome Pathways (AOPs), such as mitochondrial dysfunction and synaptic impairment. By capturing early cellular and functional changes through multimodal analysis, this project contributes to the development of mechanistically anchored neurotoxicity assessment frameworks with high human relevance.



AOP-like structure (derived from OECD AOP 3) to ensure coherence between the validated readouts and the anticipated biological endpoints.

METHODOLOGICAL APPROACH

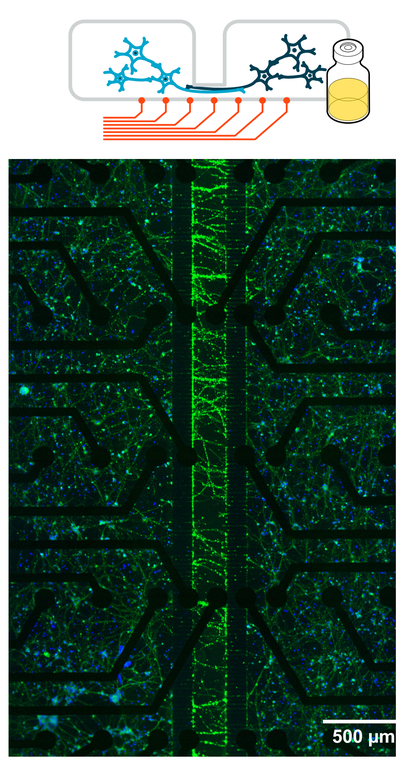


Cell Model: Human iPSC-derived glutamatergic neurons.

Multimodal Readouts:

- Electrophysiology via MEA to monitor neuronal activity.
- Imaging: Live imaging (JC-1) for mitochondrial integrity and immunostaining for neuronal/ synaptic markers (β3-tubulin, Synaptophysin, PSD95, VGlut1).
- Biochemical: LDH release (ELISA) to assess cytotoxicity.

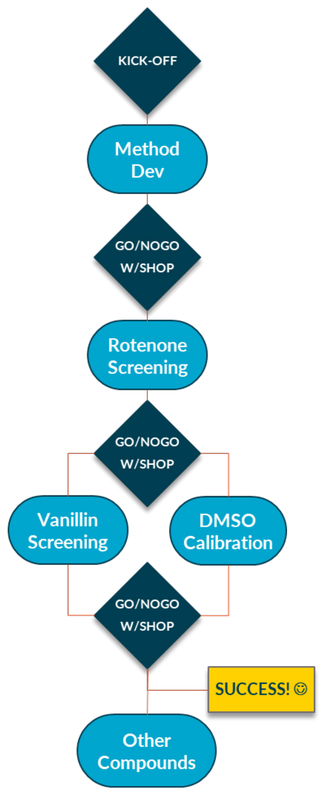
Data Integration: Digital Signatures combining functional, structural, and molecular data; supported by multivariate analysis and AI tools for compound classification.



Human iPSC-derived glutamatergic neurons culmtured in both channels 1 and 2, stained for β3-tubulin (green) and DAPI (blue).

PROJECT MANAGEMENT AND PLANIFICATION OF THE PILOT STUDY

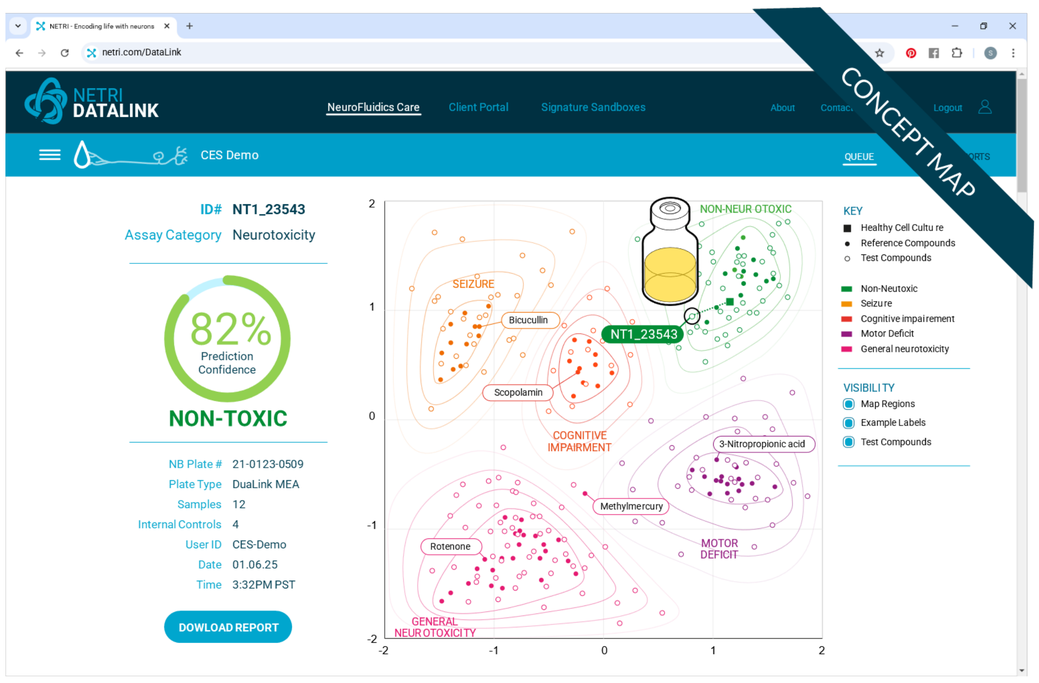
The experimental workflow begins with the characterization of rotenone, a well-known mitochondrial toxicant, across a range of concentrations to determine optimal dosing parameters. This first step will be conducted on non-MEA chips, using a combination of morphological, biochemical, and mitochondrial function assays. Once defined, selected concentrations will be assessed on MEA-integrated chips to evaluate functional impacts on neuronal network activity. In parallel, vanillin, a compound of interest with potential neurotoxic effects, will be explored in a similar manner to compare profiles. To ensure data robustness and control for solvent effects, a DMSO dose-response curve will be included following PARC (Partnership for the Assessment of Risks from Chemicals) recommendations. This tiered, multimodal strategy enables a comprehensive understanding of compound-specific effects and lays the groundwork for building a mechanistically anchored dataset for future classification and regulatory relevance.



CONCLUSION & PERSPECTIVES

This project will deliver a robust, reproducible, and mechanistically informative platform for assessing pesticide-induced neurotoxicity using a fully humanized organ-on-chip model. The integration of multimodal data—electrophysiological activity, cellular imaging, and biochemical markers—enables the construction of compound-specific Digital Signatures. These signatures will form the foundation of a dedicated neurotoxicity database, providing valuable insights for substance classification and risk assessment.

Advanced data analysis pipelines, including AI-based tools, will be developed as needed to support pattern recognition and mechanistic clustering of compounds. The platform and associated database are designed with regulatory application in mind, with the ultimate goal of supporting safer chemical design, improved regulatory decision-making, and reduced reliance on animal testing. Future efforts will focus on scaling the approach for higher-throughput screening and expanding the scope to additional neurotoxic mechanisms and compound classes.



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