Comparisons of Microelectrode Array Acute and Network Formation In Vitro Assays for Evaluating Neurotoxicity

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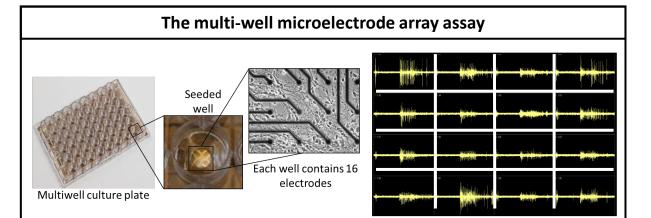
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Introduction and Background

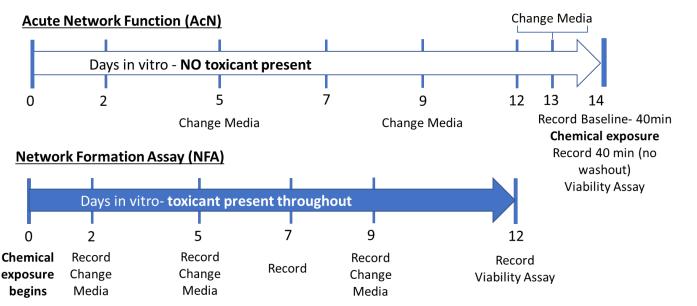**Need for new approach methodologies (NAMs):**

- Tens of thousands of chemicals have not been tested in guideline studies for neurotoxicity (NT) and developmental neurotoxicity (DNT).
- Current guideline studies, especially DNT studies are costly, time-consuming, use large numbers of animals and are subject to methodological and scientific uncertainties.
- New approach methodologies (NAMs) have been developed to address the existing need for information on the potential NT or DNT hazard for thousands of chemicals in the environment.

The multi-well microelectrode array (MEA) screening approach measures neuronal electrical activity following chemical exposure.

The multi-well microelectrode array assay

- The **acute network function (AcN)** and **network formation (NFA)** MEA assays are both included in the ToxCast data resource and use P0 dissociated rat cortical cells.
- The AcN assay measures potential acute hazard in a model of a developed neural network.
- The NFA measures potential developmental neurotoxicity hazard in a model of a developing network.

Acute Network Function (AcN)
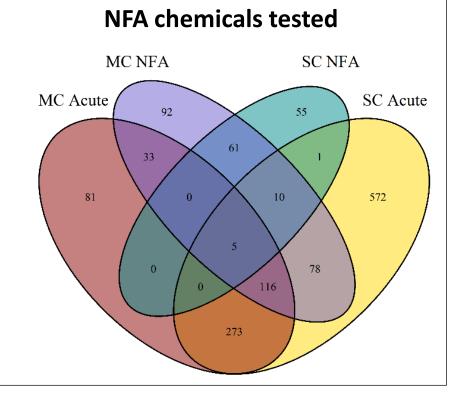
Network Formation Assay (NFA)

Hypothesis 1: Chemical activity (hits) in the AcN will be concordant with chemical activity in the NFA.
Hypothesis 2: Disruption of network formation occurs at lower concentrations than disruption of acute function.
Hypothesis 3: Chemicals with high selective activity in the AcN will also demonstrate high selective activity in the NFA.

Data analysis approach:

- A total set of 1377 chemicals were tested in these assays.
- A tiered screening strategy employed single concentration (SC) preliminary screening followed by multi-concentration (MC) response for a subset of chemicals.
- **154 chemicals were screened in** multi-concentration (MC) response in **both** the NFA and AcN.
- Computational methods were used to compare the chemical activity in the NFA and AcN for these 154 intersecting chemicals.
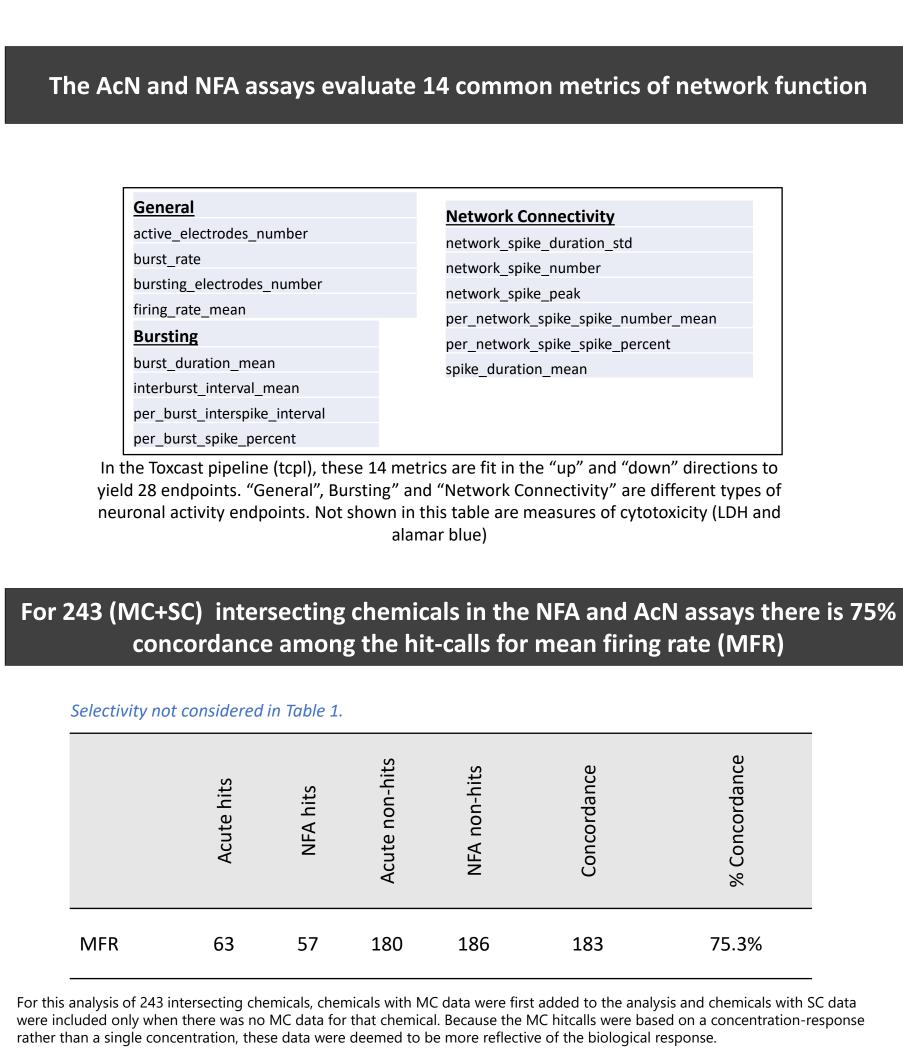
Overlap of MC and SC AcN and NFA chemicals tested

Hypothesis 1: Chemical activity in the AcN will predict (be concordant with) chemical activity in the NFA.

The AcN and NFA assays evaluate 14 common metrics of network function

General
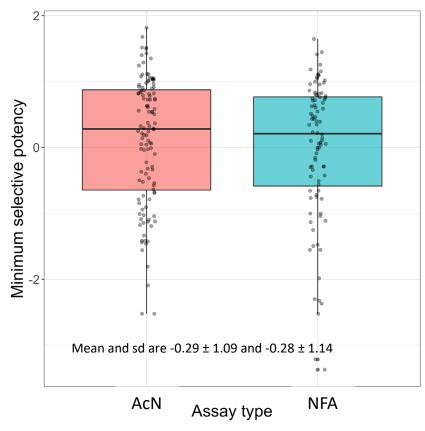active_electrodes_number
burst_rate
buristing_electrodes_number
firing_rate_mean

Bursting
burst_duration_mean
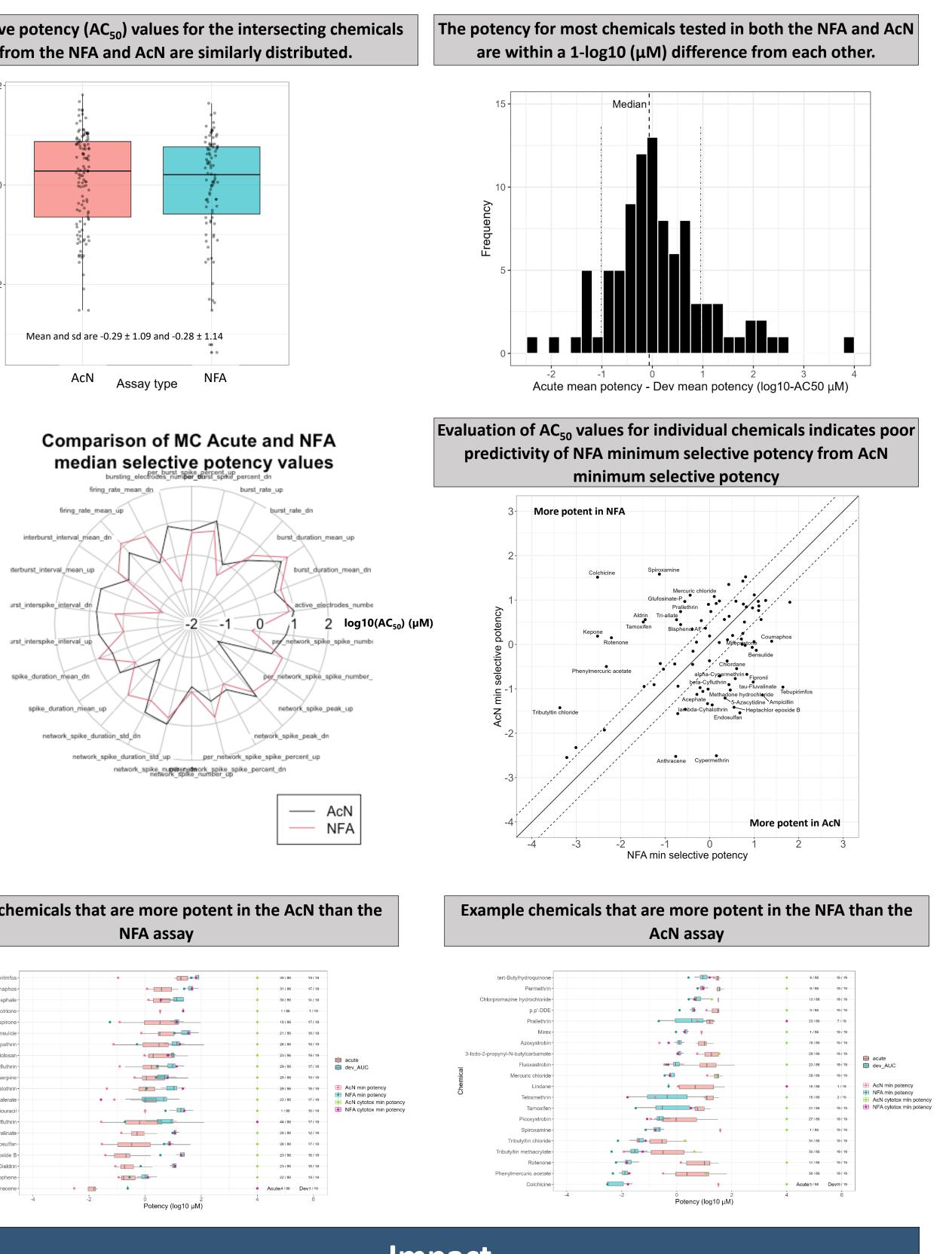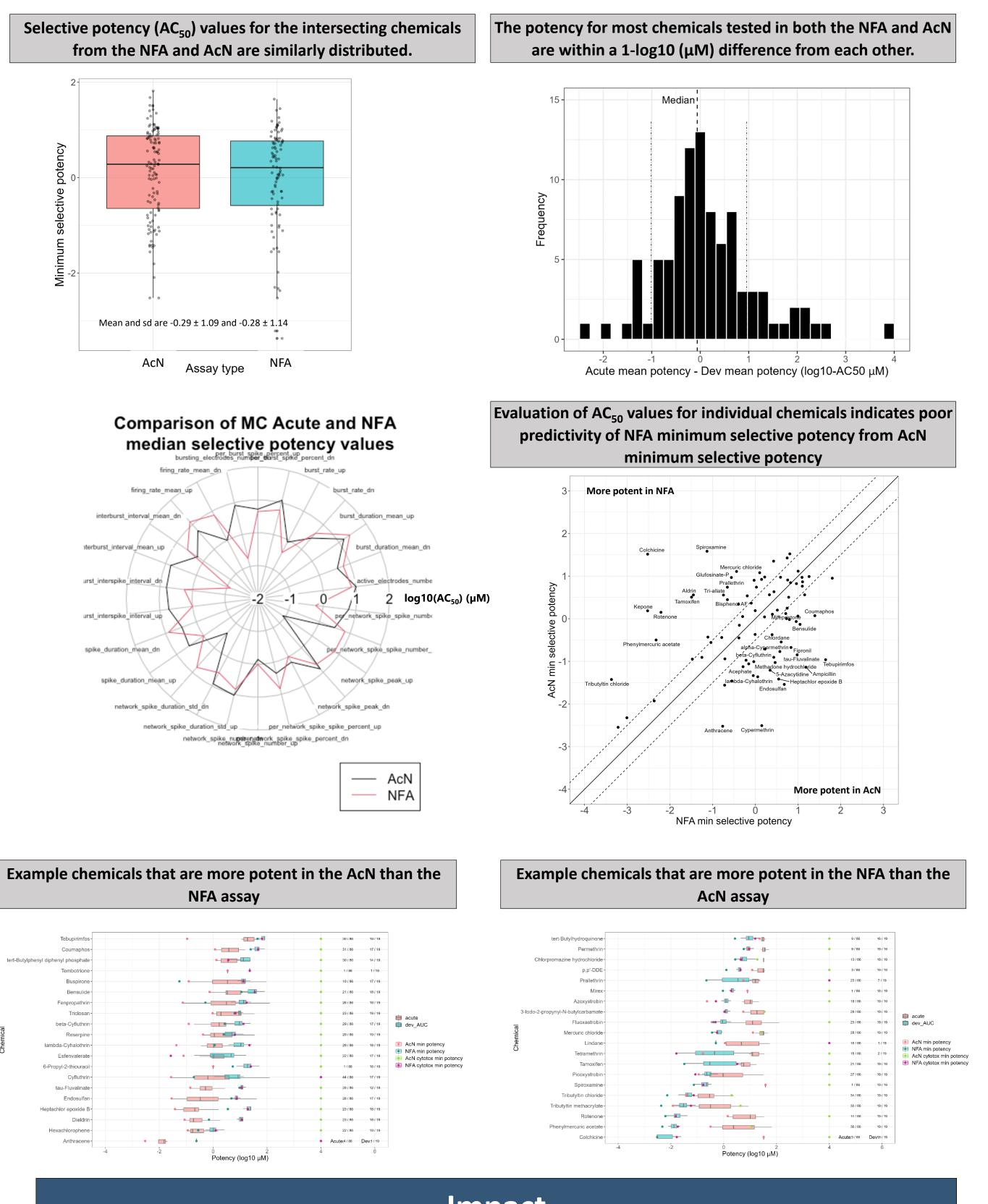interburst_interval_mean
per_burst_interspike_interval
per_burst_spike_percent

Network Connectivity
network_spike_duration_std
network_spike_number
network_spike_peak
per_network_spike_spike_number_mean
per_network_spike_spike_percent
spike_duration_mean

In the Toxcast pipeline (tcpl), these 14 metrics are fit in the "up" and "down" directions to yield 28 endpoints. "General", Bursting" and "Network Connectivity" are different types of neuronal activity endpoints. Not shown in this table are measures of cytotoxicity (LDH and alamar blue)

For 243 (MC+SC) intersecting chemicals in the NFA and AcN assays there is 75% concordance among the hit-calls for mean firing rate (MFR)

Selectivity not considered in Table 1.

	Acute hits	NFA hits	Acute non-hits	NFA non-hits	Concordance	% Concordance
MFR	63	57	180	186	183	75.3%

For this analysis of 243 intersecting chemicals, chemicals with MC data were first added to the analysis and chemicals with SC data were included only when there was no MC data for that chemical. Because the MC hitcalls were based on a concentration-response rather than a single concentration, these data were deemed to be more reflective of the biological response.

Concordance of the selective potency hit-calls for all endpoints (top) and for endpoints grouped into activity categories for the MC NFA and AcN assays (bottom) using the 154 intersecting chemicals and the 28 endpoints.

	Acute hits	NFA hits	Acute non-hits	NFA non-hits	Concordance	% Concordance
All endpoints	116	92	38	62	102	66.2%
General	89	70	65	84	101	65.6%
Bursting	99	60	55	94	93	60.4%
Network Connectivity	108	88	46	66	102	66.2%

Hypothesis 2: Disruption of network formation occurs at lower concentrations than disruption of acute function.

Selective potency (AC50) values for the intersecting chemicals from the NFA and AcN are similarly distributed.

Mean and sd are -0.29 ± 1.09 and -0.28 ± 1.14

The potency for most chemicals tested in both the NFA and AcN are within a 1-log10 (μM) difference from each other.

Comparison of MC Acute and NFA median selective potency values

— AcN
— NFA

Evaluation of AC_{50} values for individual chemicals indicates poor predictivity of NFA minimum selective potency from AcN minimum selective potency

More potent in NFA

More potent in AcN

Example chemicals that are more potent in the AcN than the NFA assay

Example chemicals that are more potent in the NFA than the AcN assay

Hypothesis 3: Chemicals with high selective activity in the AcN will also demonstrate high selective activity in the NFA.

Selective potency (AC_{50} values) informs differential bioactivity patterns of acute (AcN) vs developmental (NFA) exposure in the multi-concentration screens.

Assay endpoints

Cytotoxic Chemicals

3-Iodo-2-propynyl-N-butylcarbamate	tert-Butylphenyl diphenyl phosphate
Azoxystrobin	Picoxystrobin
Chlorpyrifos oxon	Pyraclostrobin
Chlorpyrifos-methyl	Resmethrin
Esfenvalerate	S-Bioallethrin
Etofenprox	Tembotrione
Flufenacet	Tetramethrin
Fluoxastrobin	Trichlorfon
Indoxacarb	Trifloxystrobin

Chemicals with bioactivity in the AcN but not the NFA

3,3',5,5'-Tetrabromobisphenol A	Disulfoton
Acephate	Endrin
Acetaminophen	Glufosinate-P ammonium
Acetamiprid	Glufosinate-ammonium
Acibenzolar-S-methyl	Lactofen
Aspartame	Perfluoroundecanoic acid
Atrazine	Selegiline hydrochloride
Butylated hydroxyanisole	Spirodiclofen
Caffeine	Tembotrione
Clotrimazole	Tetramethrin
Cymoxanil	Thiacloprid
	Tris(methylphenyl) phosphate

Chemicals with bioactivity in the NFA but not the AcN

- 1,1-Dimethylpiperidinium chloride
- 2-(Perfluorooctyl)ethanol
- 6-Propyl-2-thiouracil
- Aldicarb
- Cadmium chloride
- Chlorpyrifos-methyl
- Dimethoate
- Imidacloprid
- Metformin
- Methamidophos
- Mirex
- Perfluorooctanesulfonic acid
- Pravastatin sodium
- Simvastatin
- Spiroxamine

Impact

The NFA and AcN assays have different response profiles following chemical treatment. For a full understanding of the bioactivity of chemicals on neural networks, they should be tested in both assays.

Conclusions

Hypothesis 1: Weak support
- Concordance in hit calls for multiconcentration data between the AcN and NFA assays was between 60 and 66% .

Hypothesis 2: Not supported
- The minimum selective potency (AC_{50}) values were not significantly different. On average, the disruption of network formation in the NFA did not occur at lower concentrations than disruption of network function in the AcN.
- For chemical by chemical potency comparisons, no predictive relationship could be established

Hypothesis 3: Not supported
- Although there were many chemicals that were inactive across both assays, the biological activities of selective potency in the NFA and AcN were otherwise different.
  - There were compounds that were cytotoxic, some with more activity in the AcN, and others with more activity in the NFA
- Chemical activity in the AcN assay did not always predict activity in the NFA. Apart from cytotoxic chemicals, the NFA detected 15 chemicals that the AcN did not detect whereas the AcN detected 23 chemicals that the NFA did not detect (based on selective potency), many of which were associated with increased neuronal activity changes in the acute.