Evaluation of the ToxCast Assay Suite for the Detection of Neuroactivity

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PW-13
Poster Session I
October 19, 2022, 4:30 - 6:00pm
American Society for Cellular and Computational Toxicology
11th Annual Meeting

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Introduction

Background

- Previous studies identified potential gaps in the U.S. EPA's Toxicity Forecaster (ToxCast) assay suite for the detection of neuroactivity.[1,5,7,8]
- New nervous-system relevant assay endpoints have been added to ToxCast, including several whole-cell neuronal assays.
- **Can the new ToxCast assay suite…**
  - **detect bioactivity from neuroactive substances?**
  - **inform a protective point of departure for neuroactive substances?**
  - **substantiate differences between neuroactive and other substances?**

Data

477 neuroactive substances

with evidence of *in vivo* neuroactivity based on:

427
- Common knowledge in the field
- Manual curation of published literature
- Neurotoxicity data in the U.S. EPA's Toxicity Values database
- Neuroactive stereoisomers of one of the above

50
- Additional salts of the above

1,668 ToxCast assay endpoints

invitrodb v3.4, 2021, with 9 whole-cell neuronal assays taken from EPA's internal invitrodb (accessed March 2022)

426 nervous-system relevant (NSR) | **1,242 other**

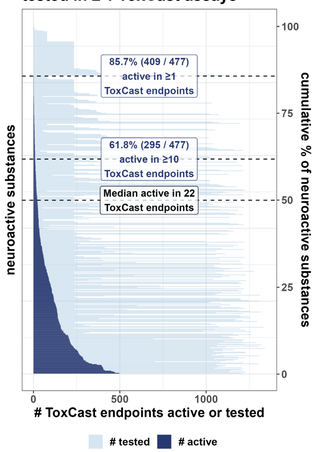
98 Derived from whole-cell neuronal assays

328 Derived from cell-free or non-neuronal cell assays with a neuro-relevant target (based on Human Protein Atlas,[2,3] expert-knowledge, or derivation from neuronal tissue)

ToxCast pipeline indicators used to exclude positive hit calls with:
- 3 or more caution flags
- Concentration that caused a 50% of maximal response (AC_{50}) < minimum concentration tested and model top < 20% above the cutoff
- cell viability assay with a gain-loss model fit

ToxCast detects bioactivity from 86% of neuroactive substances

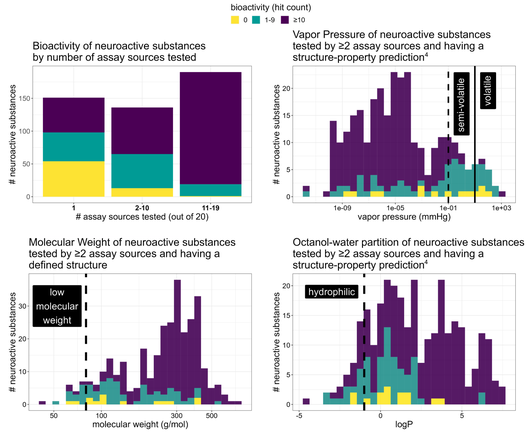
Number of active ToxCast endpoints for the 477 neuroactive substances tested in ≥ 1 ToxCast assays



- Neuroactive substances (86%) and other tested substances (79%) were active in ≥1 ToxCast assay endpoint.
- Substances are unequally tested across ToxCast assays.

Many neuroactive substances not active in ToxCast are semi-volatile to volatile or have not been screened thoroughly

Testing and physicochemical property values of neuroactive substances, by level of bioactivity in ToxCast

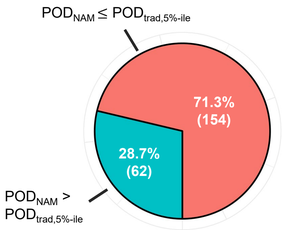


Other reasons for lack of activity:
- Insufficient concentration tested (e.g., phenobarbital)
- Stereoisomer tested may not be the most potent form (e.g., endosulfan II)
- Metabolic activation required (e.g., cyclophosphamide monohydrate)
- Not screened in assay(s) designed for putative molecular target and action (e.g., naloxone)

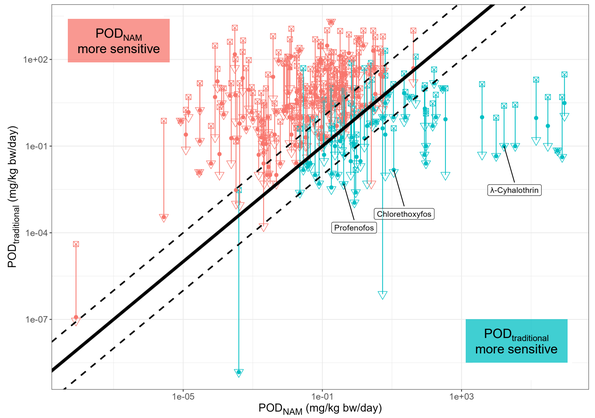
ToxCast appears to detect bioactivity at a sufficiently sensitive concentration for 71% of neuroactive substances

Traditional *in vivo* POD (POD_{trad}):
- Applied filters to the study PODs in U.S. EPA's Toxicity Values database:
  - ✓ Oral/gavage administration
  - ✓ Common species
  - ✓ Dose units convertible to mg/kg bw/day
  - ✓ POD type such as BMDL, LEL, or NEL, etc.
  - ✗ Acute studies
- Collapsed across POD types, species, and study types to obtain traditional *in vivo* PODs (POD_{trad}) for each substance:

quantile: ▽ min ● 5%-ile ⊠ 50%-ile



$POD_{traditional}$ (from *in vivo* studies) vs. POD_{NAM} (derived from ToxCast *in vitro* bioactivity) for 216 neuroactive substances



New Approach Methodologies POD (POD_{NAM}):

$$\frac{in\ vitro\ \text{POD}}{(\mu M)} * \frac{\text{oral dose}\ (mg/kg\ bw/day)}{1(\mu M)} = \frac{\text{POD}_{NAM}}{(mg/kg\ bw/day)}$$

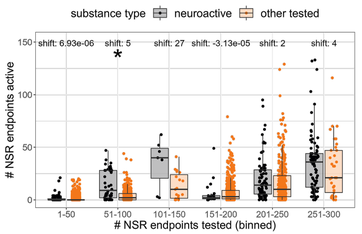
Lower 5th%-ile of ToxCast AC_{50}s (bounded by lowest concentration tested)

Oral dose required to reach 1 μM steady-state plasma concentration for 95%-ile most sensitive human (estimated with httk[6] 2.2.1)

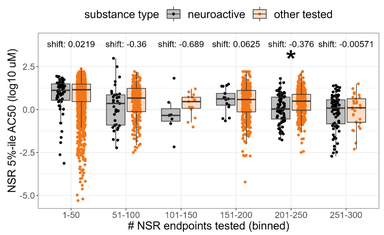
In vitro POD converted to an administered equivalent dose

Promiscuity of substances in NSR ToxCast assays indicate additional information is needed to differentiate neuroactive from other substances

Qualitative activity in NSR assays is similar for neuroactive and other tested substances



Quantitative sensitivity in NSR assays is similar for neuroactive and other tested substances

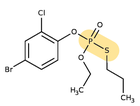


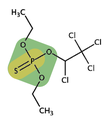
- Mann-Whitney tests indicate only slight shift in qualitative or quantitative activity for some groups of neuroactive and other substances tested in a similar number of NSR assay endpoints (* = Bonferroni-adjusted p-value < 5%).

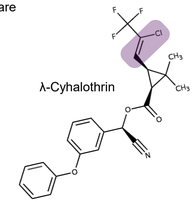
Neuroactive substances with any of 3 "enriched" structural features have higher odds of lacking quantitative sensitivity in ToxCast (POD_{NAM} > $POD_{trad,5\%-ile}$)

ToxPrint chemotype (CT) odds ratio = (neuroactives **with** CT: # POD_{NAM} > $POD_{trad,5\%-ile}$ / # POD_{NAM} ≤ $POD_{trad,5\%-ile}$) / (neuroactives **without** CT: # POD_{NAM} > $POD_{trad,5\%-ile}$ / # POD_{NAM} ≤ $POD_{trad,5\%-ile}$)

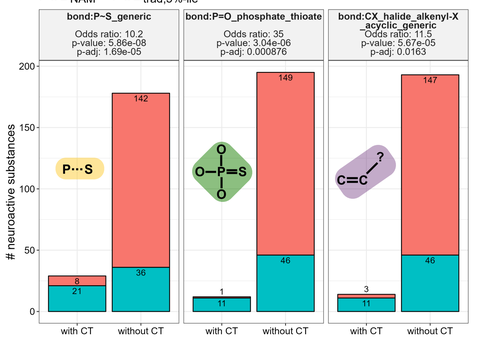
- 3 / 288 ToxPrint CTs represented are "enriched" based on:
  (adapted from Paul Friedman et al., 2020)[5]
  - ≥3 substances with POD_{NAM} > $POD_{trad,5\%-ile}$ contain the CT
  - odds ratio ≥ 3
  - ≤5% Bonferroni-adjusted probability that true odds ratio ≤ 1
- All substances containing an enriched CT are organophosphates or pyrethroids.


Profenofos


Chlorethoxyfos


λ-Cyhalothrin

ToxPrint chemotypes enriched among neuroactive substances with POD_{NAM} > $POD_{trad,5\%-ile}$



- 52% (32 / 62) neuroactive substances with POD_{NAM} > $POD_{trad,5\%-ile}$ contain an enriched CT.

Conclusions

Qualitative sensitivity
- ToxCast assays can detect most neuroactive substances that have been adequately screened, except for volatiles/semi-volatiles.
- Further screening of chemicals may reveal missed neuro-relevant molecular targets of concern.

Quantitative sensitivity
- POD_{NAM} is more sensitive than $POD_{trad,5\%-ile}$ for most (71%) neuroactive substances.
- Three structural features found in some organophosphates and pyrethroids are enriched among neuroactive substances with POD_{NAM} > $POD_{trad,5\%-ile}$.
  - Assay improvements or safety factor adjustments may be needed to achieve sufficient quantitative sensitivity for these substances.

Specificity of NSR ToxCast assays
- NSR ToxCast assays detect toxicodynamic activity from many neuroactive as well as other tested substances.
- Additional toxicokinetic information, such as blood-brain barrier and brain compartment modelling, will likely be needed to predict neuroactivity *in vivo*.

Works Cited

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This poster does not reflect US EPA policy. A.F.C. was supported by appointment to the Research Participation Program of the U.S. Environmental Protection Agency, Office of Research and Development, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. EPA.