*Bartmann et al.:*

# A Human iPSC-Based *In Vitro* Neural Network Formation Assay to Investigate Neurodevelopmental Toxicity of Pesticides
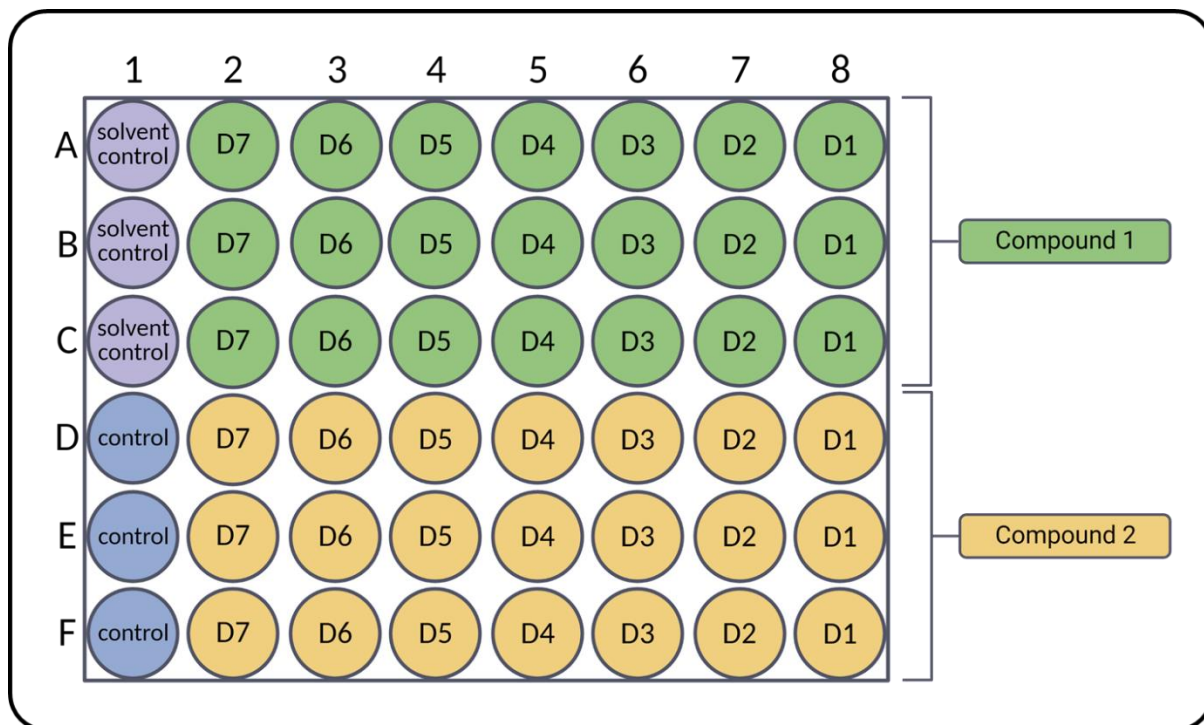
## Supplementary Data

**Tab. S1: Chemical compounds used for toxicity testing on MEAs**
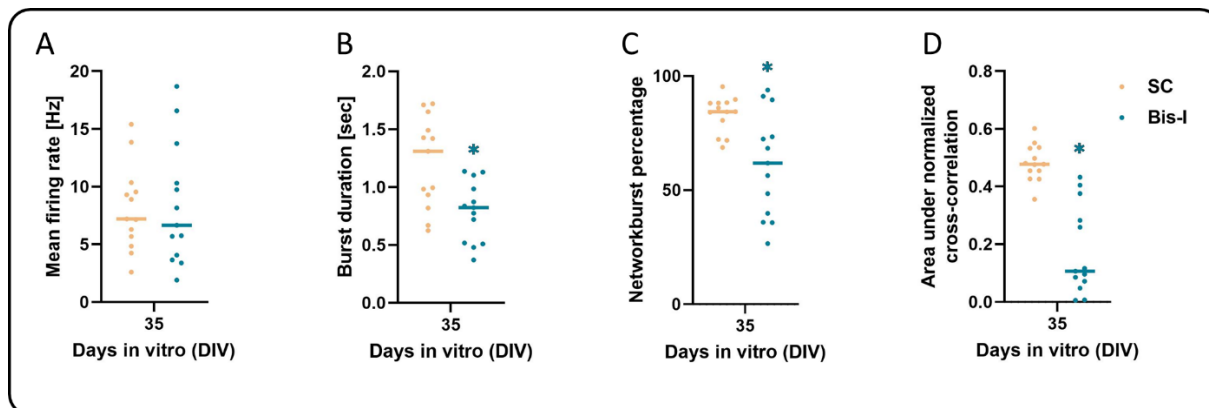CASRN, CAS registry number; DTXS ID, DSSTox substance identifier

| Compound | CASRN | DTXS ID | Supplier | Highest tested concentration [µM] | Solvent | Conducted experiments |
|---|---|---|---|---|---|---|
| Acetaminophen | 103-90-2 | DTXSID2020006 | ToxCast | 20 | DMSO | 2 |
| Acetamiprid | 160430-64-8 | DTXSID901015148 | Sigma-Aldrich | 20 | DMSO | 2 |
| Acibenzolar-S-methyl | 135158-54-2 | DTXSID1032519 | ToxCast | 20 | DMSO | 2 |
| Aldicarb | 116-06-3 | DTXSID0039223 | Sigma-Aldrich | 20 | DMSO | 2 |
| Alpha-Endosulfan | 959-98-8 | DTXSID9037539 | Sigma-Aldrich | 20 | DMSO | 2 |
| Beta-Cyfluthrin | 1820573-27-0 | DTXSID8032330 | Sigma-Aldrich | 20 | DMSO | 3 |
| Beta-Cypermethrin | 1224510-29-5 | DTXSID6052871 | LGC Standards | 20 | DMSO | 3 |
| Bisindolylmaleimide I | 133052-90-1 | DTXSID50157932 | Merck | 10 | DMSO | 1 |
| Carbaryl | 63-25-2 | DTXSID9020247 | Sigma-Aldrich | 20 | DMSO | 3 |
| Chlorpyrifos | 2921-88-2 | DTXSID4020458 | ToxCast | 20 | DMSO | 2 |
| Chlorpyrifos-methyl | 5598-13-0 | DTXSID6032352 | Sigma-Aldrich | 20 | DMSO | 3 |
| Clothianidin | 210880-92-5 | DTXSID2034465 | Sigma-Aldrich | 20 | DMSO | 2 |
| Deltamethrin | 52918-63-5 | DTXSID8020381 | Sigma-Aldrich | 20 | DMSO | 3 |
| Diazinon | 333-41-5 | DTXSID9020407 | Sigma-Aldrich | 20 | DMSO | 3 |
| Dimethoate | 60-51-5 | DTXSID7020479 | Sigma-Aldrich | 20 | DMSO | 2 |
| Dinotefuran | 165252-70-0 | DTXSID7034549 | Sigma-Aldrich | 20 | DMSO | 3 |
| Disulfoton | 298-04-4 | DTXSID0022018 | ToxCast | 20 | DMSO | 3 |
| Etofenprox | 80844-07-1 | DTXSID9032610 | ToxCast | 20 | DMSO | 3 |
| Fipronil | 120068-37-3 | DTXSID4034609 | ToxCast | 20 | DMSO | 3 |
| Flufenacet | 142459-58-3 | DTXSID2032552 | Sigma-Aldrich | 20 | DMSO | 3 |
| Imidacloprid | 138261-41-3 | DTXSID5032442 | Sigma-Aldrich | 20 | DMSO | 3 |
| Metaflumizone | 139968-49-3 | DTXSID6040373 | ToxCast | 20 | DMSO | 3 |
| Methamidophos | 10265-92-6 | DTXSID6024177 | ToxCast | 20 | DMSO | 3 |
| Penthiopyrad | 183675-82-3 | DTXSID6058005 | Sigma-Aldrich | 20 | DMSO | 2 |
| Rotenone | 83-79-4 | DTXSID6021248 | Santa Cruz | 0.3 | DMSO | 2 |
| Spirodiclofen | 148477-71-8 | DTXSID6034928 | ToxCast | 20 | DMSO | 3 |
| Thiacloprid | 111988-49-9 | DTXSID7034961 | Sigma-Aldrich | 20 | DMSO | 3 |
| Thiamethoxam | 153719-23-4 | DTXSID2034962 | Sigma-Aldrich | 20 | DMSO | 2 |
| Tri-allate | 2303-17-5 | DTXSID5024344 | ToxCast | 20 | DMSO | 2 |

**Fig. S1: Exposure scheme of the hNNF assay**
Solvent control: 0.1% DMSO. D7-1: Dilution 7 (lowest concentration) – dilution 1 (highest concentration). Controls include Bis-I exposure (5 µM) during the whole assay duration, as well as acute treatment with BIC and CNQX at DIV 21. For each experimental run at least three wells for each control were used.



**Fig. S2: Bisindolylmaleimide I (Bis-I) inhibits neural network development on 48-well microelectrode arrays (MEA) after 35 days of exposure, described by evaluation of specific network parameters**
Starting at DIV 7, networks were treated with 5 µM Bis-I and compared to the solvent control (SC) of the respective plate. Data are represented as single experiment values (median of 3 wells each) of 13 independent experiments and merged by median (colored bar). Statistical significance was calculated using two-tailed Student's t-tests. A p-value below 0.05 was termed significant. *, significant compared to the respective SC.