



## Data Article

## Disease network data for the pesticide fipronil in rat dopamine cells



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## ABSTRACT

Transcriptome data were collected in rat dopamine cells exposed to fipronil for 24 h using microarray analysis. Fipronil is a phenylpyrazole pesticide that acts to inhibit gamma-aminobutyric acid (GABA), blocking inhibitory synaptic transmission in the central nervous system. Transcriptome data were subjected to pathway analysis and subnetwork enrichment analysis. We report that 25  $\mu$ M fipronil altered transcriptional networks in dopamine-synthesizing cells that are associated with Alzheimer's Disease, Huntington Disease, and Schizophrenia. Data analysis revealed that nerve fibre degeneration, nervous system malformations, neurofibrillary tangles, and neuroinflammation were all disease processes related to the transcriptome profile observed in the rat neuronal cells. Other disease networks altered by fipronil exposure at the transcript level were associated with the mitochondria, including mitochondrial DNA depletion syndrome and mitochondrial encephalomyopathies. These data, along with those presented in Souders et al. (2021), are significant because they increase understanding into the molecular mechanisms underlying human disease following exposures to neuroactive pesticides. These data can be reused to inform adverse outcome pathways for neurotoxic pesticides.

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Specifications Table

Subject	Biological Sciences, Omics: Transcriptomics
Specific subject area	Pesticide, central nervous system, neurotoxicity
Type of data	Table, figure, and supplemental file of network data in excel
How data were acquired	Microarray processing was performed according to manufacturer's protocols (Agilent Low RNA Input Fluorescent Linear Amplification Kit and Agilent 60-mer oligo microarray processing protocol, Agilent). The Agilent-074036 SurePrint G3 Rat GE v2 8 × 60K Microarray (G4858A, Series GSE118738, BioProject PRJNA486656) was used to probe samples. Microarray slides were scanned by Agilent DNA Microarray Scanner. Raw expression data along with tiff images were extracted by Agilent Feature Extraction Software (v10.7.3.1) which was used to extract spot intensity.
Data format	Raw, filtered and analyzed
Parameters for data collection	Cells were treated without (control) or with the pesticide. Transcriptome response was measured.
Description of data collection	Microarray analysis was performed on dopamine cells treated with 25 µM fipronil for 24 h. Biological replicates included <i>n</i> = 6 control and <i>n</i> = 6 fipronil. Microarray processing was performed according to manufacturer's protocols (Agilent Low RNA Input Fluorescent Linear Amplification Kit and Agilent 60-mer oligo microarray processing protocol, Agilent).
Data source location	Not applicable.
Data accessibility	With the article and in repository Repository name: Gene expression Omnibus Data identification number: GSE118738 Direct URL to data: <a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE118738">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE118738</a>
Related research article	Souders CL 2nd, Rushin A, Sanchez CL, Toth D, Adamovsky O, Martyniuk CJ. 2021. Mitochondrial and transcriptome responses in rat dopaminergic neuronal cells following exposure to the insecticide fipronil. <i>Neurotoxicology</i> . 85:173-185. <a href="https://doi.org/10.1016/j.jneuro.2021.05.011">https://doi.org/10.1016/j.jneuro.2021.05.011</a> .

Value of the Data

- Data are useful as molecular biomarkers of pesticide neurotoxicity.
- Environmental regulators and toxicologists will find data useful in assessing human exposure routes.
- Society can benefit from data in terms of contributing to risk assessment for pesticides.
- Data can be used to determine novel associations between pesticide exposure and disease.
- Data presents new molecular mechanisms associated with neurodegeneration.

1. Data Description

Fipronil is an agricultural and residential pesticide that is used to control unwanted pests by acting to inhibit GABA receptors in the central nervous system. This leads to overexcitation and death of the unwanted pests, but exposures can lead to adverse effects in non-target organisms such as wildlife and humans. In this study, we treated undifferentiated N27 DA neuronal cells, derived from SV40 immortalized rat fetal midbrain neurons, for 24 h to 25 µM fipronil. Following this, samples were processed for microarray analysis to determine the response of the transcriptome. Data that accompany this Data in Brief article are presented in Souders II et al. [1].

**Table 1**

Sub-networks related to brain disease and mitochondria following exposure to fipronil. The gene set seed, total number of neighbors in the network, number of measured neighbors, median fold change, and *p*-value and provided in the table.

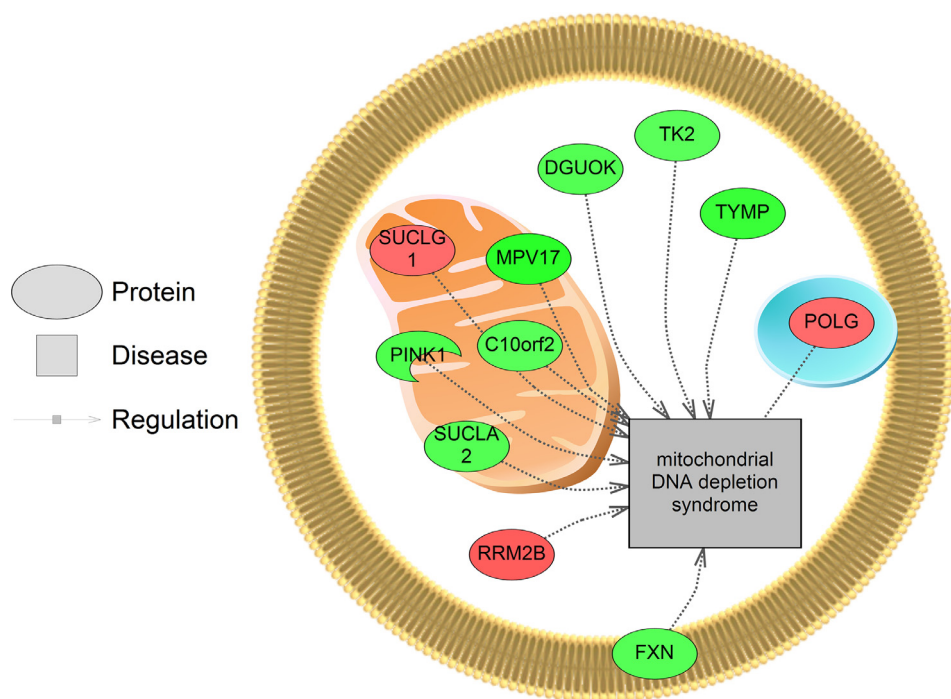
Gene Set Seed	Total # of Neighbors	# of Measured Neighbors	Median change	<i>p</i> -value
Alzheimer Disease	841	785	1.002	0.010
Amyloid Neuropathies, Familial	10	10	1.094	0.021
amyloid plaque	117	111	1.093	0.049
Brain Injuries	372	361	-1.028	0.016
Genotoxic Stress	135	131	-1.096	0.006
Huntington Disease	202	197	-1.021	0.047
mitochondrial DNA depletion syndrome	11	11	-1.326	0.033
Mitochondrial Encephalomyopathies	10	9	-1.385	0.007
nerve fiber degeneration	169	163	-1.110	0.003
Nervous System Malformations	78	72	1.017	0.021
Neurofibrillary Tangles	97	93	1.031	0.029
Neurofibroma	19	17	-1.331	0.031

Disease network analysis using Pathway Studio (v12) (Elsevier) was conducted with the Name + Alias feature. A sub-network enrichment analysis was conducted in the program to identify diseases associated with fipronil-induced toxicity in the dopamine cells. The enrichment *p*-value for all queries was set at  $p < 0.05$ . All disease networks identified as significantly affected by fipronil are provided in Supplemental Data 1. Table 1 highlights many of these diseases, with a focus on neurodegeneration and mitochondrial dysfunction. The table presents the gene set seed, total number of neighbors related to the disease or disease process, the number of measured neighbors in the network, the median change of the network based upon the median value of all the genes in present and measured, and the *p*-value.

Examples of down-regulated diseases and disease-related processes included Vitamin K Deficiency, Polyradiculoneuropathy, Chronic Inflammatory Demyelinating, Mitochondrial Encephalomyopathies, Primary Dystonia, DNA Virus Infections, Neurofibroma, Mitochondrial DNA depletion syndrome, and Myxoid Liposarcoma among others. Examples of up-regulated diseases and disease-related processes included Upper Respiratory Tract Infections, Fragile X-associated tremor/ataxia syndrome, Inflammatory Breast Neoplasms, Monogenic disorder, Inflammatory Pseudotumor, Hyper-IgM Immunodeficiency Syndrome, X-linked lymphoproliferative disease, Cerebrovascular Trauma, Idiopathic Membranous Glomerulonephritis, and Myoepithelioma among others. Neuroinflammation is a common occurrence with many diseases and pesticide exposures and we identified transcriptome changes that were associated with Inflammatory Breast Neoplasms, Inflammatory diseases, and Inflammatory Pseudotumors. Fipronil affects the central nervous system and we point out there were several diseases related to the central nervous system including nerve fiber degeneration, Nervous System Malformations, Neurofibrillary Tangles, Neurofibroma, Neurofibroma, Plexiform, and Neurologic Manifestations. Lastly, there is evidence that fipronil can perturb the mitochondria, resulting in mitochondrial stress and redox imbalance. Mitochondrial DNA depletion syndrome (Fig. 1) was a disease network identified by our analysis and included transcripts such as frataxin, ribonucleotide reductase M2 B (TP53 inducible), thymidine phosphorylase, succinate-CoA ligase, alpha subunit, succinate-CoA ligase, ADP-forming (beta subunit), polymerase (DNA directed) gamma, PTEN induced putative kinase 1, chromosome 10 open reading frame 2, and Mpv17 mitochondrial inner membrane protein.

## 2. Experimental Design, Materials and Methods

Rat primary immortalized mesencephalic dopaminergic cells (N27) were maintained in RPMI-1640 (1X) with L-glutamine media (Gibco, Carlsbad CA USA) in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C. Media was supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) antibiotic-antimycotic (100x; Invitrogen, Carlsbad CA USA). N27 cells were grown to ~70%



**Fig. 1.** Disease network for mitochondrial DNA depletion syndrome in dopamine cells following exposure to 25  $\mu$ M fipronil. Red indicates that the gene is increased in expression relative to the control and green indicates a down-regulation of the transcript. Abbreviations of genes and their fold changes are reported in Supplemental Data 1 (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

confluency in 75 cm<sup>2</sup> Corning CellBIND culture flasks (Corning Incorporated, Corning, NY, USA). Microarray analysis was performed on cells exposed to 25  $\mu$ M fipronil: The groups were as follows: DMSO-treated cells ( $n = 6$ ) and fipronil-treated cells ( $n = 6$ ).

RNA was extracted from cells using 1 mL TRIzol<sup>®</sup> Reagent (Life Technologies) and RNA purified using the Qiagen RNeasy<sup>®</sup> Mini Kit (Qiagen) as per manufacturer's protocols. The NanoDrop-2000 (Thermo Scientific, Wilmington, DE, USA) and 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) were used to assess the quantity and quality of the RNA from all samples. Each sample had RNA Integrity Numbers greater than 7. Microarrays (Agilent-074036 SurePrint G3 Rat GE v2 8  $\times$  60K Microarray) were processed as per Cowie et al. [2]. Raw expression data, along with tiff images were extracted using Agilent Feature Extraction Software (v10.7.3.1). All microarray data adhered to established guidelines "Minimum Information About a Microarray Experiment (MIAME)" (<http://www.ncbi.nlm.nih.gov/geo/info/MIAME>).

Differentially expressed genes (DEGs) were identified by first importing raw intensity data into JMP<sup>®</sup> Genomics (v8). Quantile Normalization was used to normalize data. The arrays were quality control checked using a distribution analysis that plots the intensity distributions of each microarray slide to ensure these distributions are relatively equal. Normalized intensity data were filtered based on the limit of detection of the microarrays which was set at 1.5 for signal intensity based upon control probe intensity and the standard curve from the "RNA Spike-In mix". An ANOVA followed by an FDR correction was used in JMP<sup>®</sup> Genomics to identify transcripts differentially expressed by fipronil (FDR corrected  $P$ -value < 0.05). Fold changes for all genes on the microarray were imported into Pathway Studio for processing to identify differentially expressed networks following fipronil exposure. Pathway Studio conducted gene set en-

richment analysis using the nonparametric Kolmogorov-Smirnov test with an enrichment  $P$ -value set at  $P < 0.05$ .

## Ethics Statement

All experimental data were conducted and analysed within ethical and biosafety guidelines outlined by the University of Florida. A commercial cell line was used solely for all studies (Rat primary immortalized mesencephalic dopaminergic cells (N27)).

## CRediT Author Statement

**Christopher L. Souders:** Supervision, Formal analysis, Data curation; **Anna Rushin:** Formal analysis, Methodology; **Christina L. Sanchez:** Formal analysis, writing; **Darby Toth:** Formal Analysis, Methodology; **Ondrej Adamovsky:** Formal analysis, Methodology; **Christopher J. Martyniuk:** Writing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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## Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.dib.2021.107299](https://doi.org/10.1016/j.dib.2021.107299).

## References

- [1] C.L. Souders II, A. Rushin, C.L. Sanchez, D. Toth, O. Adamovsky, C.J. Martyniuk, Mitochondrial and transcriptome responses in rat dopaminergic neuronal cells following exposure to the insecticide fipronil, *Neurotoxicology* 85 (2021) 173–185.
- [2] A.M. Cowie, K.I. Sarty, A. Mercer, J. Koh, K.A. Kidd, C.J. Martyniuk, Molecular networks related to the immune system and mitochondria are targets for the pesticide dieldrin in the zebrafish (*Danio rerio*) central nervous system, *J. Proteom.* 157 (2017) 71–82.