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Data Article

Dataset for diseases associated with exposure to broflanilide, a novel pesticide, in larval zebrafish (*Danio rerio*)

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ABSTRACT

Broflanilide is a novel pesticide that can antagonize ion channels and disrupt neurotransmitter systems in the brain. Zebrafish larvae were exposed to either 0, 1 or 10- µg/L broflanilide in the water for a period of 7 days during early development. RNA extraction was conducted on larval zebrafish for RNA-seq analysis using the Illumina NovoSeq 6000. Raw sequence data were processed through fastp and clean reads obtained by removing adapter and poly-N sequences. Alignment and differential gene expression analysis was conducted using HISAT2, StringTie assembler, and FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced). Subnetwork enrichment analysis (SNEA) revealed that exposure to 1 µg/L broflanilide altered gene networks associated with axonal injury, depression, neuroinflammation, and traumatic brain injury while exposure to 10- µg/L broflanilide resulted in changes in gene networks associated with brain infarction and ischemia, excitotoxicity, and neurogenic inflammation. In addition, genes related to MPTP-induced neurotoxicity were al-

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tered by broflanilide which has relevance for Parkinson's disease. Several transcripts were identified as being associated with a disease network link to neurodegeneration and included phospholipase A2 activating protein, calpain 1, ATPase Na+/K+ transporting subunit alpha 2, glia maturation factor beta, sphingomyelin phosphodiesterase 1, leucine rich repeat kinase 2, glutamate ionotropic receptor NMDA type subunit 2C, lysosomal associated membrane protein, and calcium/calmodulin dependent protein kinase II alpha among others. Data presented here include disease biomarkers for a novel pesticide and can be reused to refine models that describe adverse outcome pathways for neurotoxicity.

Specifications table

Subject	Environmental toxicology
Specific subject area	Pesticides, disease, aquatic toxicology
Type of data	Table Image Figure Supplemental Data
How the data were acquired	Embryos were randomly placed into 25 mL beakers containing 10 mL of sterile embryo rearing media (ERM). Experimental groups included: Solvent control ($n = 5$), 1 µg/L broflanilide treatment ($n = 4$), or 10- µg/L broflanilide treatment ($n = 3$). On day 7 post fertilization (dpf), larvae were collected from each beaker and RNA was extracted using TRIzol (Thermo Fisher Scientific, Waltham, MA USA). RNA integrity was measured using the Agilent 2100 Bioanalyzer. Libraries were generated for 12 samples and were sequenced by the Novogene Corporation. The NEBNext Poly(A) mRNA Magnetic Isolation module (New England Biolabs, catalog # E7490) was used for library preparation. RNA library construction followed instructions outlined in the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (New England Biolabs, catalog #E7760). Libraries were sequenced using the Illumina NovoSeq 6000 followed methods outlined in [1].
Data format	Raw Analyzed Filtered
Description of data collection	Fold changes and p-value for all transcripts were generated using and mapped into Pathway Studio 12.0 (Elsevier). Subnetwork enrichment analysis (SNEA) for disease networks was conducted. A figure was generated to describe relationships between transcripts altered in response to broflanilide.
Data source location	Institution: University of Florida Aquatic Toxicology Laboratory City/Town/Region: Gainesville, Florida Country: USA
Data accessibility	In repository Repository name: NCBI Gene Expression Omnibus Data identification number: GSE236484 Direct URL to data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE236484

1. Value of the data

- Data represents biomarkers for diseases associated with broflanilide exposure.
- Data can be used by government and industrial regulators to inform the adverse outcome pathway framework.
- Society benefits because molecular data informs us about the potential risks associated with chemical exposures in our environment.

- Data can be used to predict disease outcomes associated with pesticide exposure.
- Data present biomarkers of diseases linked to pesticides used in agriculture.

2. Objective

Broflanilide is a recently registered pesticide used to mitigate unwanted pests in agriculture. Broflanilide acts to disrupt gamma aminobutyric acid (GABA) receptors in the brain of insects [2,3]. Indiscriminate use of pesticides like broflanilide can lead to unwanted negative effects in aquatic species like fish. The objective of the study was to measure the transcriptome response in zebrafish larvae following exposure to the pesticide at a low and high concentration over a seven-day period. Zebrafish larvae (AB × Tu strain) were exposed to either 0, 1, or 10- µg/L broflanilide and several endpoints related to toxicity were measured including transcriptomics in whole larvae. Differential gene expression profiles were determined using an RNA-seq approach and the data were subjected to an *in-silico* analysis using Pathway Studio (v12) (Elsevier) to uncover diseases related to the transcript response.

3. Data description

Twelve libraires were sequenced using the Illumina NovoSeq 6000 following methods outlined in [1]. A bioinformatics pipeline described in the methods below identified transcripts differentially expressed in response to broflanilide. Transcripts were mapped using the Name + Alias feature in Pathway Studio (v12). A sub-network enrichment analysis (SNEA) was performed to reveal disease biomarkers associated with pesticide exposure. The enrichment value was $p < 0.05$.

Table 1 presents diseases associated with the central system that were revealed in both datasets (1 and 10 µg/L exposure groups) ($p < 0.001$). The gene set seed, total number of neighbors in the network, number of measured neighbors, median fold change, and *p*-value can be

Table 1
Top disease networks related to neurotoxicity in larval fish treated with broflanilide (1 or 100 µg/L) ($P < 0.001$). The table presents the gene set seed, total number of neighbors in the network, number of measured neighbors, median fold change, and *p*-value. Transcriptome data are located in NCBI GEO Accession (GSE236484).

Concentration	*Gene set seed*	*Total # of neighbors*	*# of measured neighbors*	*Median change*	*p-value*	*Activation score*
Low (1 µg/L)	Axonal injury	30	24	1.365	1.706E−04	1.604
	Behavioral manifestation	44	35	1.153	3.259E−04	2.357
	Depression	211	138	1.062	1.834E−04	2.100
	Depressive symptoms	46	34	1.271	3.798E−04	2.183
	Neuroinflammation	320	210	1.080	3.860E−04	3.713
	Neurological deficit	174	120	1.020	1.018E−05	1.206
	Neuropathic pain	228	159	1.094	3.266E−06	2.142
	Post-traumatic stress disorder	28	20	1.143	7.807E−04	0.000
	Traumatic brain injury	185	128	1.009	5.245E−05	2.610
High (10 µg/L)	Brain edema	126	81	−1.032	7.240E−04	0.928
	Brain infarction	62	43	−1.108	1.906E−04	−0.471
	Brain ischemia	144	103	−1.030	2.277E−04	1.947
	Demyelination	164	114	−1.061	1.822E−04	−0.781
	Excitotoxicity	131	103	−1.049	7.759E−04	−0.707
	Neurogenic inflammation	27	19	−1.208	8.472E−05	−0.632
	Neuropathic pain	228	156	−1.095	5.866E−07	−0.272
	Traumatic brain injury	185	126	1.027	9.929E−04	0.649

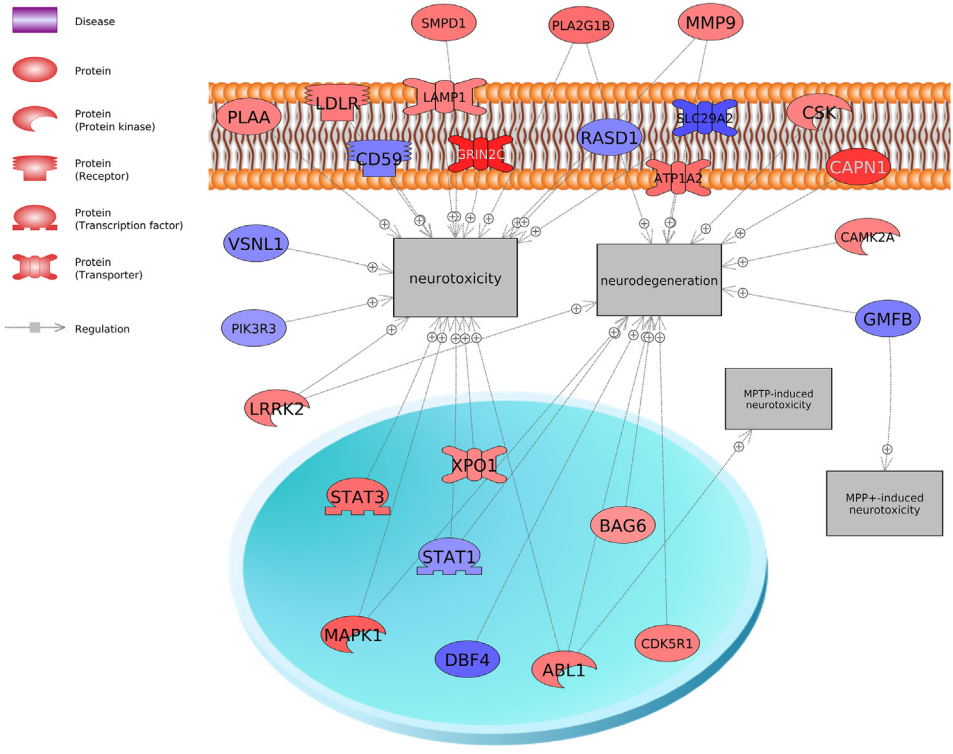

Fig. 1. Predicted disease network following exposure to 1 µg/L broflanilide. Red indicates increased expression of transcript relative to the control and blue indicates down-regulation of transcript relative to the control. Abbreviations are as follows: ABL1 (ABL proto-oncogene 1), non-receptor tyrosine kinase, ATP1A2 (ATPase Na+/K+ transporting subunit alpha 2), BAG6 (BAG cochaperone 6), CAMK2A (calcium/calmodulin dependent protein kinase II alpha), CAPN1 (calpain 1), CD59 (CD59 blood group), CDK5R1 (cyclin dependent kinase 5 regulatory subunit 1), CSK (C-terminal Src kinase), DBF4 (DBF4 zinc finger), GMFB (glia maturation factor beta), GRIN2C (glutamate ionotropic receptor NMDA type subunit 2C), LAMP1 (lysosomal associated membrane protein 1), LDLR (low density lipoprotein receptor), LRRK2 (leucine rich repeat kinase 2), MAPK1 (mitogen-activated protein kinase 1), MMP9 (matrix metallopeptidase 9), PIK3R3 (phosphoinositide-3-kinase regulatory subunit), PLA2G1B (phospholipase A2 group IB), PLAA (phospholipase A2 activating protein), RASD1 (ras related dexamethasone induced 1), SLC29A2 (solute carrier family 29 member 2), SMPD1 (sphingomyelin phosphodiesterase 1), STAT1 (signal transducer and activator of transcription 1), STAT3 (signal transducer and activator of transcription 3), VSNL1 (visinin like 1), XPO1 (exportin). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

found in the table. SNEA uncovered that exposure to 1 µg/L broflanilide altered gene networks associated with axonal injury, depression, neuroinflammation, and traumatic brain injury while exposure to 10 µg/L broflanilide resulted in changes in gene networks associated with brain infarction and ischemia, excitotoxicity, and neurogenic inflammation (Table 1). Overall, in the low dose treatment, SNEA revealed 713 disease networks that represented transcripts preferentially regulated by broflanilide ($p < 0.05$) while in the high dose treatment, there were 868 disease networks identified ($p < 0.05$). Of these, there were 492 gene networks for disease that overlapped between the two concentrations of broflanilide, which equates to a 45.2% overlap in common diseases.

Within the top 20 based on *p*-value, the common diseases among both concentrations were hyperalgesia, inflammation, neutrophil infiltration, mechanical hyperalgesia, pneumonia, respiratory hypersensitivity, cardiac remodeling, pain, and neuropathic pain. In Fig. 1, the genes related to MPTP-induced neurotoxicity are presented, and these are important for their role in

degenerative diseases like Parkinson's disease. Several transcripts were identified as being associated with a disease network link to neurodegeneration and included phospholipase A2 activating protein, calpain 1, ATPase Na+/K+ transporting subunit alpha 2, glia maturation factor beta, sphingomyelin phosphodiesterase 1, leucine rich repeat kinase 2, glutamate ionotropic receptor NMDA type subunit 2C, lysosomal associated membrane protein, and calcium/calmodulin dependent protein kinase II alpha among others. The pathway connects genes together based upon the literature and database that describes gene-protein interactions among entities.

4. Experimental design, materials, and methods

The OECD guideline 236 [4] for the embryonic acute toxicity test was followed with slight modification. Fertilized, viable embryos ($n = 20$) were randomly distributed into 25 mL beakers containing 5 mL embryo rearing media (or ERM). Exposure groups included ERM, 1 µg/L, and 10-µg/L broflanilide. An 80–90% media change was performed each day and fish were anesthetized and flash frozen using liquid nitrogen on 7 dpf. Further description for the methods exposure is presented in [5]. After 7 days, larval fish were extracted in TRIzol (Thermo Fisher Scientific, Waltham, MA USA) as per manufacturer's protocol. RNA quantity and quality were determined using the Qubit® 2.0 Fluorometer (ThermoFisher, Grand Island, NY, USA) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.), respectively. Libraries and sequencing were conducted under contract by an independent company using a NovoSeq 6000 instrument (Novogene Corporation, Beijing, China) and proceeded as per methods in [1]. Twelve samples were used for RNA-seq library construction (RINs > 7). Sample sizes included DMSO solvent control ($n = 5$), 1 µg/L broflanilide ($n = 4$), and 10 µg/L broflanilide ($n = 3$).

Analysis of transcriptome data is outlined in [1]. Briefly, raw reads (FASTQ format) were first processed through fastp and clean reads were obtained by removing reads containing adapter and poly-N sequences as well as those of low quality. HISAT2 was used for RNA-seq data alignment [ensembl_danio_rerio_grcz11_gca_000002035_4]. Mapping information from all samples was combined as input into StringTie assembler [6]. FPKM (Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced) was used as an estimate of gene expression. Read counts were first normalized and FDR values for each transcript generated. To identify the disease networks, transcripts were mapped to Pathway Studio (Elsevier, V12) [7] using the official gene names. Subnetwork enrichment analysis identifies diseases associated with the gene list using known relationships (i.e., expression, binding, etc.) derived from a knowledge base. A distribution of expression values is determined [1]. A $p < 0.05$ indicates enriched diseases in the query dataset relative to what is expected by random chance.

Ethics statement

We confirm that experiments complied with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). Institutional Animal Care and Use Committee of University of Florida approved all experiments (UF IACUC#201708562). The sex of larval fish is unknown.

CRediT author statement

Sarah J. Patuel: experimentation, data analysis, writing, editing; Cole D. English: experimentation, data analysis; Victoria Lopez-Scarim: experimentation, data analysis; Isaac Konig: experimentation, data analysis; Chris L Souders: II: supervision, experimentation, data analysis; Emma Ivantsova: experimentation, data analysis, writing, editing; Chris J. Martyniuk: Conceptualization, supervision, writing, reviewing, data analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Disease networks identified in larval fish treated with broflanilide (1 and 10 µg/L exposure groups) (P⟨0.001) (Original data) (Gene Expression Omnibus NCBI).

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