Dysregulation of gene expression in fathead minnow larvae exposed to contaminated groundwater

ecotoxchip

BACKGROUND INFORMATION

Assessment of legacy contaminated industrial sites represents a key challenge in environmental risk assessment. In particular, exposure to complex mixtures at such sites is often hampered by the limitations inherent to traditional hypothesis-based toxicity tests. In this study we applied a short-term fathead minnow (FHM) embryo assay to identify specific toxicity pathways using V1 EcoToxChips.

OBJECTIVE

Characterize the effects of short-term exposures to incremental concentrations of environmental complex mixtures on relative gene expressions in FHM embryos/larvae using EcoToxChips (V1) and compare effects to behavioral outcomes such as photo-dependent swimming responses.

METHODS & RESULTS

Freshly fertilized FHM embryos were exposure to incremental dilutions of raw groundwater samples (CTR, Reference [REF], 0.625%, 1.25%, 2.5%, 5% and 10%) in quadruplicates. At 7 days post fertilization (dpf) a sub-set of whole embryos from each replicate group were pooled (10 fish/replicate) and immediately flash frozen in liquid N2. RNA was extracted, cDNA run on the V1 FHM EcoToxChip. A second sub-set of fish were subjected to behavioral analyses (light-dark transition test). The data were analyzed with EcoToxXplorer.ca and other statistical tools as appropriate with key findings noted below.

Table 1: Number of genes with altered expression and relative changes (|log2FC|) of select genes in FHM embryos exposed to groundwater compared to negative controls. (|log2FC| threshold = 1; n = 3; Statistical test = Anova, p<0.05; red = significantly upregulated, green = significantly downregulated)

Expression compared to CTR	Ref-10%	Mod-5%	Mod-10%	Hig-5%	Hig-10%
Number of genes					
Upregulated	0	5	16	3	11
Downregulated	1	1	7	1	10
Fold changes of select genes					
ahrrb	1.64	3.09	9.25	4.75	3.99
cyp3c3	1.14	3.65	4.68	1.88	2.65
sod1	0.41	1.26	0.44	0.71	1.27

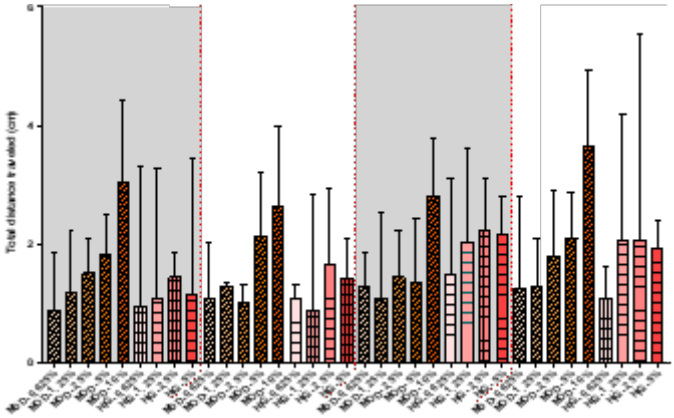

Figure 1: Total distance traveled (cm) of fathead minnow larvae after 168 hours of exposure to groundwater mixtures) (values = median with 5-95 percentile, n = 4, statistic test = ANOVA, significantly different = *, p<0.01)

TAKEAWAYS

FHM embryo EcoToxChip analysis revealed molecular key events associated with four Adverse Outcome Pathways (AOPs), informing potential adverse outcomes. Impaired behavior partially could be associated with mitochondrial dysfunction in nigrostriatal neurons AOP.

	Key Event	Adverse outcome pathway	Adverse outcome	Source on AOP Wiki
ahrrb	Activation, AhR	AhR Activation	Uroporphyria + ELS mortality	aopwiki.org/aops/131 aopwiki.org/aops/21
cyp3c3	Altered gene expression specific to CAR activation, Hepatocytes + Activation, Androstane receptor	CAR Activation	Hepatocellular adenomas	aopwiki.org/aops/107
sod1	Mitochondrial dysfunction 1	Mitochondrial dysfunction in nigrostriatal neurons	Parkinsonian motor deficits	aopwiki.org/aops/3

Notes

Gasque-Belz L, Siciliano S, Hogan N, Weber L, Campbell P, Peters R, Hanson M, and Hecker M. 2021. Presentation to: 42nd SETAC North America Annual Meeting, Nov. 14-18, 2021; Virtual Conference.