Using the new R package "ToxMixtures" to evaluate potential biological effects of pesticide mixtures in Great Lakes tributaries

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Humans create and use hundreds of thousands of chemicals, leading to the widespread presence of diverse chemical mixtures in aquatic ecosystems that can be difficult to assess for ecotoxicity.

The new R package—**ToxMixtures**—assists in analyzing, visualizing, and interpreting measured concentration data for individual chemical compounds and mixtures of chemicals identified in the same sample as they relate to chemical potency across multiple biological targets.

Data Sources

ToxMixtures begins with the output of the R package toxEval, and further analyzes the chemical mixtures after exposure activity ratios (EARs) have been computed.

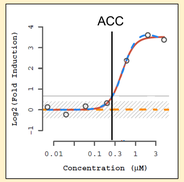
ToxMixtures and toxEval organize information from online resources on chemical potency, biological functions, gene ontologies, and pathways to offer guidance as to *how* chemicals may interact biochemically at the concentrations detected in environmental samples.

Data	Description	Example
ToxCast	Bioactivity measurements (i.e., endpoints) for thousands of chemicals that cover a range of cellular responses and molecular signaling pathways.	"ATG_AR_TRANS_up": Measure of mRNA for gain-of-signal activity can be used to understand the reporter gene at the transcription factor-level as they relate to the Androgen Receptor (AR) gene.
AOPs	Each Adverse Outcome Pathway (AOP) includes a nested sets of key events that describe measurable steps along a pathway from one or more molecular perturbations to an adverse outcome for an organism or population.	"AOP #23": AR agonism leading to reproductive dysfunction in repeat-spawning fish.
DAVID	The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a comprehensive set of functional annotation tools to investigate biological meaning of genes.	The AR gene codes for a protein that functions as a steroid-hormone activated transcription factor. Androgens (e.g., testosterone) are involved in reproduction, sexual differentiation, and many important physiological processes.
PANTHER	The Protein Analysis Through Evolutionary Relationships (PANTHER) Classification System combines genomes, gene function classifications, pathways, and statistical analysis tools.	Gonadotropin releasing hormone receptor pathway.

Workflow

Compute EARs for each sample, chemical, and endpoint:

$$\mathbf{EAR} = \frac{\textbf{Measured concentration}}{\textbf{Activity concentration at cutoff}}$$



Example dose response curve. The activity concentration at cutoff (ACC) is determined at the intersection of the preferred active model (blue/red curves) and the significance threshold of the assay (horizontal black line).

For each sample, sum EARs across common biological targets:

$\mathbf{EAR_{mixture}} = \sum \mathbf{EAR}$ for multiple chemicals active on the same endpoint.

$\mathbf{EAR_{gene}} = \sum \mathbf{EAR}$ for multiple chemicals active on endpoints with common gene targets and directions.

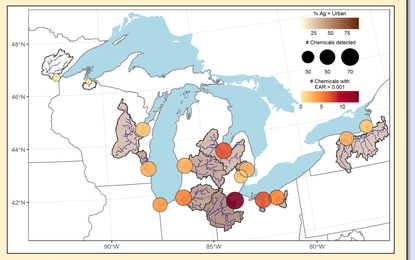
$\mathbf{EAR_{AOP}} = \sum \mathbf{EAR}$ for multiple chemicals active on endpoints with common AOPs.

Define EAR and site thresholds to prioritize chemical mixtures and summarize information about targets exceeding thresholds.

Pesticides in Great Lakes Tributaries

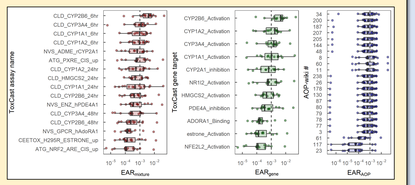
Between 16 and 81 pesticide and pesticide transformation products were detected in passive samples in 15 tributaries of the Great Lakes.

Endpoints, genes, and AOPs exceeding the user-defined thresholds are plotted and available information from online sources are summarized (see tables below).



Chemical detections and EARs in passive samplers collected in 15 Great Lakes tributaries in June-July 2016.

The chemical mixtures analysis indicated potential activity on biological pathways related to a range of cellular processes including, xenobiotic metabolism, extracellular signaling, endocrine function, and protection against oxidative stress.



Endpoints (left), genes (middle), and AOPs (right) prioritized using an EAR threshold of 10^{-3} and site threshold of 2.

Summary tables

Example output tables from ToxMixtures analysis of pesticide mixtures in Great Lakes tributaries.

Gene summary: Genes exceeding the EAR threshold, the endpoints and chemicals included in the EAR summations, and an abbreviated Entrez gene summary.

Site summary: The genes, endpoints, and AOPs above the user-defined EAR threshold and the chemicals included in the mixtures for each site.

Tables organize available information to help interpret the types of biochemical processes linked to chemical mixtures.

Gene Symbol	Endpoints	Chemicals	Entrez Gene Summary (abbreviated)
CYP2B6	CLD_CYP2B6_24hr CLD_CYP2B6_48hr CLD_CYP2B6_6hr NVS_ADME_hCYP2B6	Acetochlor, Atrazine, Metolachlor, Diuron, Metalaxyl, Sulfentrazone, Simazine, 2,4-D, Ametryn	This gene, CYP2B6, encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and its expression is induced by phenobarbital. The enzyme is known to metabolize some xenobiotics, such as the anti-cancer drugs cyclophosphamide and ifosphamide…

Site	Chemicals	Gene Symbols	Endpoints	Abbreviated AOP Titles
Fox	2,4-D Metolachlor	CYP2B6 CYP3A4 HMGCS2	CLD_CYP2B6_6hr CLD_CYP3A4_6hr CLD_HMGCS2_24hr	Mitochondrial dysfunction and neurotoxicity (3); Calcium-mediated neuronal reactive oxygen species production and energy imbalance (26); Liver X receptor activation leading to Liver Steatosis (34); Ionotropic glutamatergic receptors and cognition (48); nAChR activation contributes to colony death (77-80, 87, 178); Phospholipase A inhibitors lead to hepatotoxicity (130); Lysosomal uptake induced liver fibrosis (144); Vitamin K epoxide reductase inhibition resulting in coagulopathy (187); Estrogen receptor activation leading to breast cancer (200); Basal cytotoxicity (205); NADPH oxidase activation leading to reproductive failure (207); Reactive oxygen species production leading to population decline (238)