

### Background

We are conducting research with our partners to address the following questions:

- What constitutes reliable endogenous metabolite-based markers of effective exposures for ecologically relevant species?
- What are the temporal aspects of these exposures?
- How are key initiating events linked to whole-organism adverse outcomes?
- Are these linkages conserved across species (small fish, rats, etc.)?
- What are the defining characteristics of exposures to which organisms can compensate?

We seek to answer these questions in regard to numerous applications. For example:

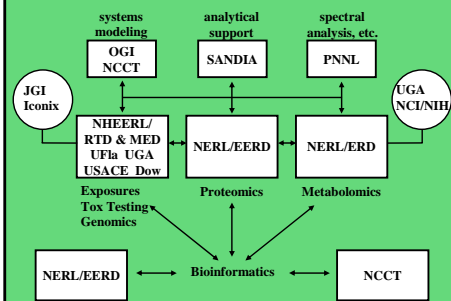
- Understanding and linking the exposure and effects of endocrine disrupting chemicals (EDCs) within the hypothalamic-pituitary-gonadal (HPG) axis of small fish models.
- Defining metabolite-based markers of exposure for triazole pesticides, and differentiating the impacts of different triazoles.

### Results / Conclusions

- NMR-based metabolomics has proven to be an effective tool for investigating the biologically relevant impacts of exposure to important environmental chemicals like triazole fungicides and EDCs.
- Metabolomics has also proven very useful for defining the temporal and compensatory aspects of exposures. Data of this type is informative when conducting chemical exposure assessments.

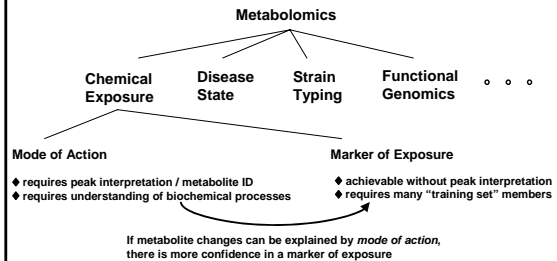
### Partners

Partnerships with researchers in academia, industry, national labs, and various Federal agencies (in addition to other EPA labs) has been crucial for the early success of NERL's metabolomics program. There are now numerous collaborations that have been established, and many active projects are underway.



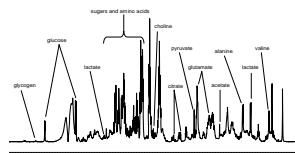
### What is NMR-Based Metabolomics?

Metabolomics is becoming a widely used 'omic tool in a variety of fields. Within chemical risk assessment, it can be used to generate markers of exposure, and/or to investigate mode-of-action.



### NMR Spectroscopy

NMR spectroscopy is an analytical technique that allows one to observe the levels of metabolites in a variety of tissues and biofluids for metabolomic analyses.



### NMR-based Metabolomics for Chemical Exposure Studies

#### Advantages:

- no need for sequenced genome
- open-ended (no preselection of metabolites)
- high throughput < 15 min per sample run in most cases
- low per sample cost < \$2.00 USD
- high level of structural information
- non-invasive (i.e., urine)
- non-destructive
- little or no sample preparation
- excellent reproducibility (e.g., cross-instrument)

#### Disadvantages:

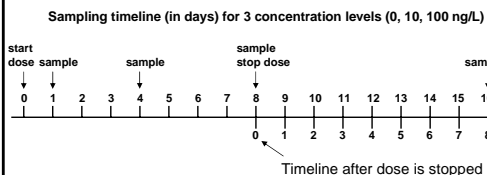
- need for metabolite peak assignment
- observed metabolites "preselected" by abundance
- when metabolites of interest in low abundance > 1 hr per sample run
- high initial cost for instrumentation > \$750K USD
- "overlapping" information

\* Disadvantages that may be mitigated by complementary use of other analytical techniques and/or chemometrics

### Methodology / Data / Analysis

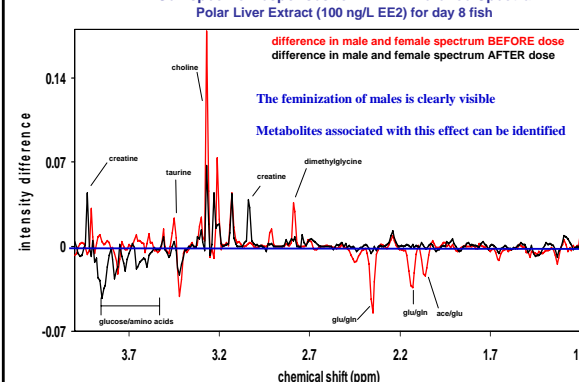
#### Understanding Fathead Minnow Exposure to Endocrine Disrupting Chemicals

##### An Exposure Conducted with the Model EDC 17 $\alpha$ -ethinylestradiol (EE2)



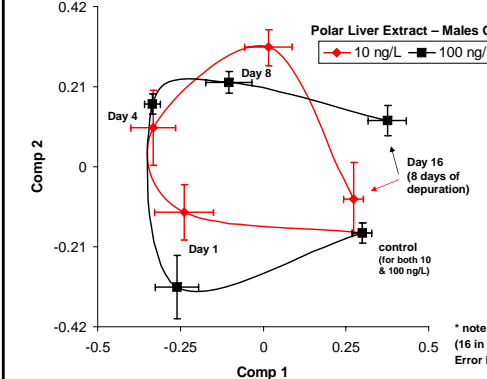
- Fish (both sexes) were sampled at three time points (1, 4, and 8 days) after exposure, with an additional collection after eight subsequent days (i.e. day 16) of residence in uncontaminated water.
- For a given "class" (where class = sex, exposure level, and sampling-time point), 8 fish were sampled (total of 192 fish).
- Plasma, liver, brain, and gonadal tissues were collected.

##### Sex-specific Responses to EE2 – Difference Spectra



- Difference spectra (generated from the average of all individual fish within each class) reveal sex-specific responses to EE2 exposure.
- Exposed fish (males and females) are much more similar metabolically than controls (i.e. exposure to EE2 induces feminization in male fathead minnow).

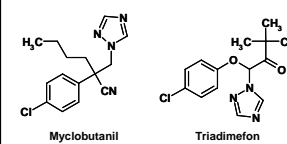
##### Temporal and Compensatory Aspects of Exposure Scores Plots from PLS Models



- Two partial least squares (PLS) models were built for all time points (one for the low concentration and one for the high concentration). An overlay of the two scores plots for these two models is shown, using mean values for each class.
- The 8-day depuration (16-day classes) does not provide a full recovery of the effects of EE2. However, there is clearly a significant movement toward the controls. Fish exposed to the low concentration of EE2 display a greater recovery than those exposed to the high concentration.

\* note: A marker is the mean of 7 or 8 class members (16 in the case of controls). Error bars represent the standard error of the mean.

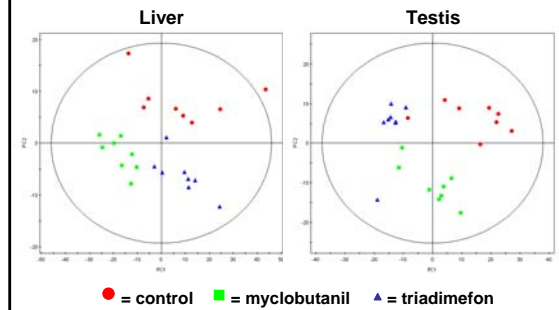
#### Assessing Exposure to Triazole Fungicides



A goal of metabolomics here is to "differentiate" impacts of exposure

- 14-day rat exposure (daily) to either myclobutanil or triadimefon
- Liver, testis, and blood serum collected at the end of two weeks

#### Triazole-Specific Responses Evident in Liver and Testis Metabolite Profiles



#### Testicular Toxicity of Myclobutanil Suggested by Metabolite Profiles

