

A Proteomic Approach to Estrogen Agonist Screening

Authors: Kimberly Salinas, Michael Hemmer, Calvin Walker
U.S. EPA/Office of Research and Development (ORD)/National Health and Environmental Effects Research Laboratory (NHEERL)/Gulf Ecology Division (GED)

Keywords: ecotoxicoproteomics, SELDI-TOF-MS, MALDI-TOF-MS, sheepshead minnow, estrogen-responsive proteins

As part of a tiered approach, new molecular techniques are being developed to assist the U.S. Environmental Protection Agency in evaluating the toxic potential of chemicals and, if necessary, setting the priority for their further testing. Screening methods for use in chemical risk assessments should be high throughput, have low animal requirements, and be adaptable across many platforms and species. In this study, we describe a validated method for screening chemicals for estrogenic activity using a small fish assay and either surface enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS) or by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). Adult male sheepshead minnows (*Cyprinodon variegatus*) were exposed to the native ligand 17- β -estradiol (E2), and blood plasma was analyzed for differential expression of proteins in control versus E2 treated fish. Differential expression of estrogen-responsive protein biomarkers was determined by comparing the mass spectra of E2 treated fish and untreated control fish. Protein biomarkers determined to be diagnostic of E2 exposure demonstrated 100 percent sensitivity and specificity for fish exposed to the known estrogen agonists, methoxychlor, bisphenol-a and 4-tert-pentylphenol as compared to the solvent control and to the non-estrogenic chemicals, endosulfan and chlorpyrifos. One of the estrogen-responsive biomarkers was identified by tandem mass spectrometry as zona radiata fragment 1, an egg envelope protein that is normally produced in gravid female fish or male fish which have been exposed to estrogenic chemicals. The techniques suggest protein expression profiling can be adapted for other chemical mode of actions and would provide a cost-effective tool for screening and prioritization of existing chemical inventories.

Point of Contact:

Kimberly Salinas
Biologist
U.S. EPA/ORD/NHEERL/GED
Gulf Breeze, FL 32561
850-934-9207
salinas.kimberly@epa.gov