DNA Damage Index: A New Tool for Assessing Toxic Effects of Contaminants

Fish that inhabit polluted environments are continuously exposed to soluble and suspended contaminants. Metals and organic compounds are readily absorbed across the gill and have the potential to alter gill physiology. Dr. Donald C. Malins (Pacific Northwest Research Institute; University of Washington SBRP) and Dr. John Stegeman (Woods Hole Oceanographic Institution; University of Washington SBRP) are working to develop a model to monitor marine contamination at Superfund sites using non-destructive gill biopsies of fish.

The cytochrome P450 (CYP) multigene family of enzymes plays a key role in the oxidative biotransformation of a variety of xenobiotics. Induction of CYP1A has been identified in fish gills and is critical for the oxidation of a variety of aquatic contaminants including polynuclear aromatic hydrocarbons (PAHs) and planar polychlorinated biphenyl (PCB) congeners. CYP1A expression can result in reactive oxygen production that can elicit subtle radical-induced alterations in base and backbone structures of DNA. Malins and Stegeman are using these changes as biomarkers to assess toxicological damage to fish resulting from habitat contamination. A significant advantage arising from this research is the development of a biopsy method for obtaining DNA from fish that does not involve sacrificing the fish being studied.

Malins and Stegeman use principal components analysis (PCA) of data from Fourier-transform-infrared (FTIR) microscope spectroscopy to identify structural differences in gill DNA from fish collected at contaminated and reference sites. PCA of FTIR spectra is based on approximately a million correlations between spectral absorbances per wavenumber over the entire spectrum. Various properties of the spectra (e.g., peak heights and peak locations) are reduced to independent principal component scores. These scores can be used to construct two- or three-dimensional plots where each spectrum is represented by a single point whose location is a highly discriminating measure of DNA structure. The separation of the samples into different clusters of points signifies that the groups are structurally dissimilar.

The researchers applied this methodology to compare DNA from gill samples collected from two groups of English sole from Puget Sound, WA. One group of fish was from the industrialized Duwamish River (DR) in Seattle and the other from the relatively clean Quartermaster Harbor (QMH). The DR was placed on the National Priorities List due to sediment contamination and the Washington State Department of Health has issued an advisory warning of a possible adverse health risk from eating English sole and other bottom fish from the lower DR. Site history and earlier analysis of sediments from QMH supported its use as a reference site.

Sediment analyses in this study determined that concentrations of PCBs, dioxins, furans and PAHs were substantially higher in DR sediments than in QMH. The gills of the DR English sole had a relatively high degree of CYP1A expression, while the QMH fish gills had little or no CYP1A expression, consistent with the levels of contaminants at the two sites.

Statistical analyses of FTIR spectra of gill DNA identified significant structural differences between the fish groups. Marked structural damage was found in the gill DNA of the DR fish as reflected in differences in base functional groups and conformational properties. Three-dimensional plots of the principal component scores revealed a distinct separation between the two groups, suggesting that the nature and degree of contaminant exposure contributed to the formation of unique modifications in the

DNA structure of the DR group. These findings imply that environmental chemicals contribute to the DNA changes in the gill.

In addition, logistic regression analysis allowed Malins and Stegeman to develop a *DNA Damage Index* to assess the effects of contaminants on the gill. The DNA Damage Index represents a substantial basis for assessing biological effects of pollution and for the responsible control of environmental contamination. The index could also be used to evaluate the effects of remediation, by monitoring the reduction or elimination of differences in the spectral biomarkers between the groups, thus signaling progress toward restoring the health of the fish in the contaminated environment.

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To learn more about this research, please refer to:

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Malins, D.C., J.J. Stegeman, J.W. Anderson, P.M. Johnson, J. Gold and K.M. Anderson. 2004. Structural Changes in Gill DNA Reveal the Effects of Contaminants on Puget Sound Fish. *Environmental Health Perspectives* 112(5):511-515.