# DEVELOPMENT OF TWO SUBTRACTIVE cDNA LIBRARIES FROM WINTER FLOUNDER.

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Chromium is a major contaminant at several Superfund sites including Shipyard Creek in Charleston, SC, Pacific Sound Resources in Elliott Bay and Puget Sound, Seattle, WA, as well as Portsmouth Naval Shipyard in Kittery, ME. These marine and estuarine areas contaminated with chromium are primarily contaminated with the trivalent or hexavalent forms, both of which tend to accumulate in the sediments. Therefore, winter flounder (*Pseudopleurinectes americanus*) was used as the sentinel species to examine the effects of chromium on alterations in gene expression in liver. Furthermore, winter flounder is a carnivorous species that may also biomagnify chromium from its food, as well bioconcentrate chromium from the water and sediments. Humans also consume large quantities of flatfish, including winter flounder and halibut, which are sediment dwellers.

Three winter flounder per group were injected with either 0.15N saline, 400μg/kg Cr(III), or 25μg/kg Cr(VI) in 0.15N saline. Each flounder weighed between 300 and 360 grams. Twenty-four hours following injections, winter flounder were euthanized with MS-222 and the livers were excised. Half of the livers were used to make cytosol and microsomes and the other half was extracted for RNA using TriZol Reagent (Life Technologies, Gaithersburg, MD). Total RNA was used to obtain mRNA for subtractive hybridization, which was done using the Clontech PCR-Select™ cDNA subtraction Kit (Palo Alto, CA). Subtractive clones were obtained and individual cDNA clones were amplified by PCR and spotted onto nylon filters to make 96-well microarrays. Microarrays have demonstrated several potentially differentially expressed genes due to Cr(VI) and a few that may be differentially expressed due to Cr(III).

For Cr(VI), we have partially sequenced several genes that are putatively differentially expressed, including glutathione S-transferase alpha3, a non-selenium glutathione peroxidase, protein elongation factor-2, carboxypeptidase, cysteine proteinase inhibitor, and complement regulatory plasma protein (ApoH). Several other genes (ESTs) have no or considerably weaker similarities based on GenBank. Primers have been made to ApoH, elongation factor-2, GSTα and glutathione peroxidase. RT-PCR indicates that the GST is down-regulated about 40% and glutathione peroxidase is down-regulated more than 80%. GSH levels are doubled, indicating that exposure to Cr(VI) causes oxidative stress. RT-PCR has shown only a slight change in the regulation of elongation factor-2 by Cr(VI). Furthermore, data from our arrays recently confirmed by real-time PCR, indicates that an EST fragment isolated during subtractive hybridization is reduced 90%.

For Cr(III), we have partially sequenced two putative cysteine proteases, and a putative oxidoreductase/periplasmic zinc transporter, however, we have not confirmed differential expression of any of these genes. Interestingly, Cr(III) treated HepG2 human liver hepatoma cells have demonstrated alterations in cysteine proteases and several proteinase inhibitors using cDNA and confirmed by RT-PCR. Further work may demonstrate similar results in vivo with flounder liver. (Supported in part by the CMTS grant P30 ES03828-16).