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Short communication

Alterations in hepatic gene expression by trivalent chromium in *Fundulus heteroclitus*

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Abstract

Cr(III) is the dominant toxicant at some Superfund sites within the United States and therefore we are interested in its effects. Cr(III)s mechanisms are not well studied or understood because of its low bioavailability. We have attempted to characterize the effects of Cr(III) on gene expression in *Fundulus heteroclitus* (mummichog) liver. The NOEC and LOEC were determined at 32 and 64 mg/L, respectively, by measuring growth and mortality after exposing juveniles for 30 days. Secondary adult male exposures were performed at 32 mg/L, livers excised, and RNA extracted. Arrays were probed with cDNA from untreated or Cr(III)-exposed adult fish and gene expression was quantified. Cr(III) at 32 mg/L altered the expression of five genes, including GSTτ, GSTα, and ALDH4. Ultimately, we anticipate using this gene expression information to ascertain whether Cr(III) is bioavailable at potentially adverse concentrations in contaminated sites.

1. Introduction

Chromium stably exists in the environment as Cr(III) or Cr(VI). Cr(VI) is more toxic because it is bioavailable and produces radicals during reduction to Cr(III). In turn, the Cr(III) binds macromolecules, forms adducts with thiol groups on proteins, creates DNA adducts, and causes DNA strand breakage (Dayan and Paine, 2001). However, while Cr(III) may be the ultimate toxicant following Cr(VI) exposure, Cr(III) is not as toxic as Cr(VI) because of its low bioavailability (Dayan and Paine, 2001).

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Chromium is a primary toxicant at 8% of the Superfund sites, including estuarine sites, such as Shipyard Creek in Charleston, SC and the Piscataqua River in Kittery, ME. Typically both forms of chromium are found at Superfund sites, but one form dominates. Chromium partitions to the sediments. Therefore sediment-associated organisms such as mummichogs are exposed to high levels, as they are in direct contact with contaminated sediments and ingest other sediment dwelling organisms. However, not much is known about the molecular effects of chromium on aquatic organisms.

cDNA arrays were developed for mummichogs following subtractive hybridization or differential display of livers excised from Cr(VI), Cr(III), arsenic, or polycyclic aromatic hydrocarbon (PAH) exposed mummichogs. Thus, the array is targeted for determining differential expression caused by chromium, arsenic, or PAHs in mummichogs. Overall, the array contains 270 duplicate spots, including 13 blanks or plasmid controls. A hybridized array is shown in Fig. 1. Information on the individual genes found on the array can be found on the Gene Expression Omnibus (GEO-GPL2535) or www.mummichog.org.

We used the mummichog arrays developed in our lab to determine Cr(III)-induced changes in hepatic gene expression. The information generated by the array may help determine the mechanism, or bioavailability of Cr(III). In this study, we treated adult mummichogs at the NOEC (32 mg/L), and used cDNA arrays to determine alterations in gene expression. In the future, we anticipate using the gene expression profile to determine the dominant bioavailable chromium species at estuarine Superfund sites.

2. Materials and methods

2.1. Exposure of mummichogs to Cr(III)

All animal research was performed in accordance with IACUC approved protocols. Mummichogs were housed at 25 °C in a 14/10 light/dark cycle at 18‰ salinity. Juvenile

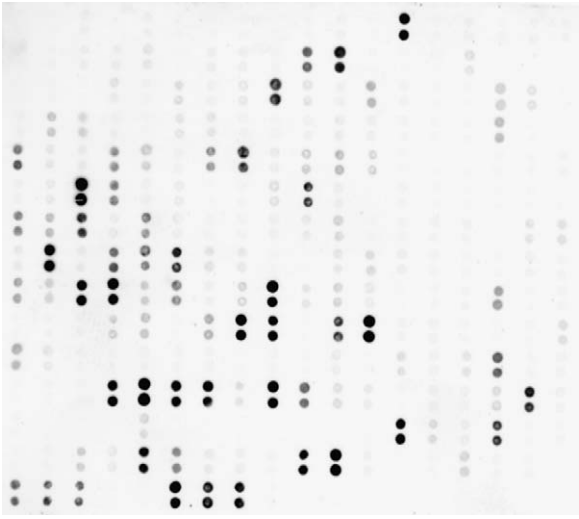

Fig. 1. A mummichog cDNA array probed with ^{33}P lableled hepatic cDNA from mummichogs exposed to Cr(III).