Investigations Into Age-Related Susceptibility to Arsenic Carcinogenicity

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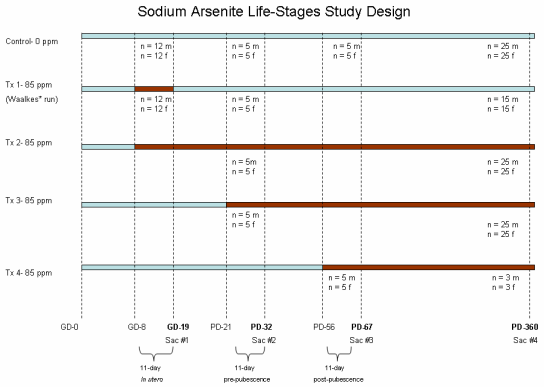
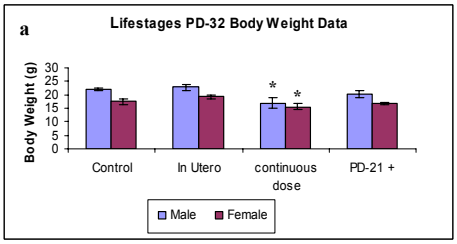
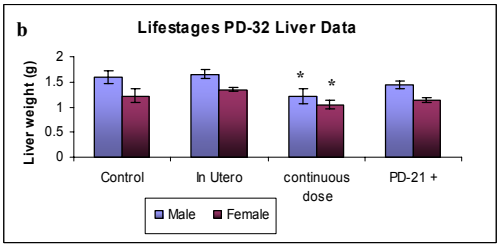
Environmental Issue: Arsenic compounds are ubiquitous in the environment and are a major health problem in many parts of the world. Human exposure to arsenic has been identified from both natural and anthropogenic sources. Most chronic exposure comes from drinking water, particularly in underdeveloped regions of the world such as Chile, India, Bangladesh, and other Asian countries. Epidemiology studies suggest that chronic exposure to inorganic arsenic and its metabolites are associated with large increases in cancer of the skin, bladder, and lung as well as more moderate increases in the kidney and liver. However, until recently, few useful animal models have been developed to study arsenic carcinogenesis experimentally. Recent findings suggest that *in utero* exposure to inorganic arsenic increases the susceptibility of mice to liver and lung cancer (Waalkes, 2003). This data suggests that investigation into age-related susceptibility to arsenic carcinogenicity is necessary for accurate estimation of human cancer risk.

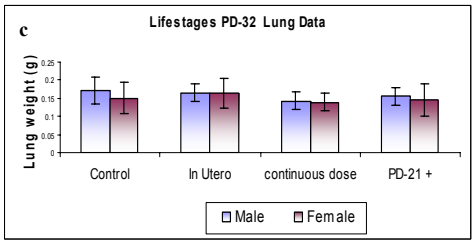
Approach and Study Design: This National Health and Environmental Effects Research Laboratory (NHEERL), multi-divisional (ECD, ETD, and RTD) research study is designed to fill known data gaps associated with arsenic cancer risk, particularly the influence of age on cancer susceptibility from arsenic exposure. To do this, C3H mice were exposed to 85 ppm inorganic arsenic in their drinking water for 11 days during three stages of development: *in utero* (gestation day 8–19), pre-pubescence (postnatal day 21–32, and post-pubescence (postnatal day 56–67). Target tissues were (will be) taken and fractionated for analysis of protein profiles by 2-D gel electrophoresis (DIGE) and mass spectrometry, gene regulation by microarray and DNA methylation analysis, and genetic toxicology by micronucleus induction. Additional groups of animals will be continuously exposed for an additional 52 weeks to determine increased susceptibility to tumor/foci risk. Actual arsenic concentrations in milk and drinking water will be chemically determined by Atomic Absorption Spectroscopy.



Preliminary results: Although there was no difference between the birth and tissue weights of C3H pups exposed to 85 ppm sodium arsenite *in utero* versus control mice, earlier results show an increase in tumors later in life (Waalkes 2003). Mice that were continually dosed from GD-8 to PD-32 were significantly lower in both total body and liver weight. Water consumptions of dosed mice were also significantly less than those not receiving sodium arsenite, however there was no significant difference in food consumption between all groups. Differences in water consumption may suggest a palatability issue related to the dosing solutions and/or differential susceptibility to general arsenic toxicity through time. Preliminary results showed that the differences in body and liver weight observed at PD-32 are no longer significant at PD-67, but that the differences become apparent again as the animals age beyond PD-100 (data not shown). No treatment-related differences in micronucleus induction were observed.



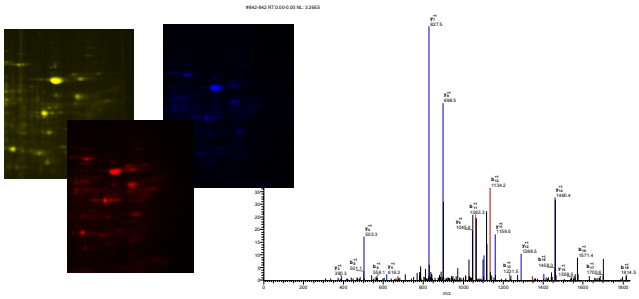




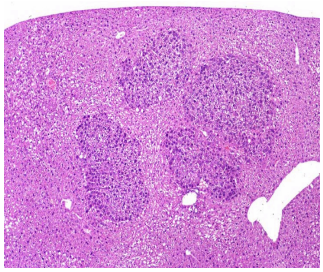
Comparison of mouse body[a], liver[b] and lung[c] weight at 32 days of age (postnatal day 32, PD-32) after water vehicle exposure (**Control**), 11-days of 85 ppm sodium arsenite exposure *in utero* only from gestation day 8 (GD8) to GD19 (**In Utero**), prolonged 85 ppm exposure from GD8 to PD-32 (**continuous dose**), and 11-days of 85 ppm exposure from PD-21 through PD-32 (**PD-21+**). * indicates significance (p<0.05).

On-Going Biological Endpoints Analysis: Tissues collected at GD-19, PD-32, and PD-67 will undergo genomic and proteomic profile evaluation. Tissues collected at PD-67 and PD-360 will also undergo histopathological evaluation of preneoplastic and neoplastic lesions. Affymetrix Mouse Genome 430A 2.0 arrays will be used to evaluate differential gene expression, and 2-D DIGE with LC/MS/MS will be used to identify differentially expressed proteins. Biological pathway determination will be performed using the Database for Annotation, Visualization and Integrated Discovery 2.1 (DAVID). Tumor incidence, latency and multiplicity parameters will be evaluated in mice after 360 days of 85 ppm sodium arsenite exposure. Hepatic foci will also be evaluated by immunohistochemical analysis (GST-Pi positive foci) after the 360 day exposure.

Affymetrix Mouse Genome 430A 2.0 Arrays



Fluorescence 2-D Differential Gel Electrophoresis (DIGE) with LC/MS/MS



Mouse Hepatic Foci (Preneoplasia)

Impact: These studies support regulatory human health requirements through characterizing age-related susceptibilities to tumors induced by environmental chemicals. Results of these studies might be used to more accurately set environmental exposure standards for arsenic that do not rely on default correction factors.

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