

1453 BERYLLIUM BLOOD LYMPHOCYTE PROLIFERATION TEST (BLPT): VARIABILITY AND POSITIVE PREDICTIVE VALUE.

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Lymphocyte proliferation assays are widely used as biomarkers for exposures to various metals including beryllium. Results are typically categorized as: abnormal (positive), normal (negative) or borderline. This study evaluated nearly 5000 blood lymphocyte proliferation test (BLPT) results collected since 1992 from three labs during periodic surveys at two beryllium plants in Tucson, AZ and Elmore, OH. Single split blood samples were sent to two independent laboratories. If both labs report negative BLPT-the person is considered not sensitized. Discrepancies between the two labs or uninterpretable results warrant a repeated testing. Two positive BLPTs, either from the two independent labs or from repeat testing at the same lab are sufficient to consider a person beryllium sensitized. Analysis included examination of inter- and intra-laboratory variability by calculating a kappa statistic, and assessment of positive predictive value of BLPT with respect to forecasting chronic beryllium disease (CBD). The level of agreement between the first and the second test for all three labs (intra-lab variability) was fair to moderate with kappa ranging between 0.3 and 0.5. The inter-lab agreement depended on the survey site ranging from poor (kappa=0.1) in Elmore to good (kappa=0.8) in Tucson. An abnormal BLPT test was approximately 50% predictive of CBD as detected on subsequent bronchoscopy. The BLPT has a high degree of variability, its positive predictive value is acceptable, but the sensitivity and specificity are still unknown. BLPT may serve as a useful medical surveillance tool, however it does not meet the criteria for a screening test.

1454 BIOMONITORING FOR BERYLLIUM: EXPERIENCE WITH A U.S. WORK FORCE.

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Experiments in animals indicate that beryllium (Be) has a very low bioavailability, a moderate to long half-life, and that urine is the primary route of excretion for absorbed Be. The feces is the primary route of excretion of unabsorbed Be following oral or inhalation exposure (via mucociliary escalator). Studies conducted in the 1960s indicated that Be workers had elevated levels of urinary Be but a correlation between levels of Be in urine or blood and airborne concentrations of Be in work areas could not be established. There have been suggestions that workers may be absorbing Be by routes other than inhalation. Therefore, the purpose of this study was to determine whether a biomarker approach (using urine or blood) could be used to evaluate the extent and route of exposure using more modern analytical techniques. If so, the method would be useful in assessing whether workplace behaviors or exposure interventions are effective in limiting human exposures to beryllium in the workplace. The population evaluated consisted of individuals working with beryllium metal, beryllium oxide, and alloys. A control population of administrative personnel from buildings that are separate from the manufacturing facility were included in the survey. Of the 193 urine samples collected, only 18 had detectable levels of Be (detection limit of 0.2 µg/L). Based on a comparison to nationwide data collected as part of the National Health and Nutrition Examination Survey (NHANES III), the data indicated that workers exposed to different forms of beryllium in the workplace had urinary levels that were indistinguishable from background levels in the United States. These preliminary results suggest that a more sensitive method for detecting beryllium in biological media is needed. Therefore, studying blood or urine to identify Be exposure will only be appropriate when the quantitative technique is sensitive enough to distinguish Be concentrations in exposed and unexposed populations.

1455 DEVELOPMENT OF METHODS FOR ANALYSIS OF BIOMARKERS OF BENZENE EXPOSURE USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) AND MATRIX ASSISTED LASER DESORPTION IONIZATION (MALDI).

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Because benzene is used extensively in industry and is a widespread industrial pollutant, it is important that biological monitoring accurately reflect the

history of exposure. To provide more information on previous exposures than a single biomarker can provide, it is desirable that a battery of biomarkers be used. This work describes the development of assays for longer and shorter-term biomarkers of benzene exposure. Recent biomonitoring studies have focused on assessment of urinary levels of S-phenylmercapturic acid (S-PMA) and trans,trans-muconic acid (t,tMA) as they are specific to benzene and are biomarkers of recent exposure. Methods to assay a specific longer lived biomarker, S-phenylcysteine adducts, in albumin and hemoglobin have also been published, but require extensive preparatory work. Previous studies of the urinary metabolites have largely extracted and analyzed S-PMA and t,tMA separately, by splitting the samples prior to analysis. In this study, methods were developed for the combined extraction, derivatization and analysis of S-PMA and t,tMA from urine via liquid extraction, followed by derivatization using hydrochloric acid/methanol and GC/MS analysis. Recoveries were 90% for S-PMA and 78% for t,tMA from spiked water samples. The limit of detection for t,tMA was 10 ng/mL and 20 ng/mL for S-PMA. The best sensitivity for the combined analytes was obtained using positive ionization, although negative ionization was more sensitive for t,tMA alone. MALDI analysis of S-phenylcysteine adducts in albumin was accomplished using tryptic digests and provided sensitivity comparable to current literature methods. (Supported by U.S. EPA Assistance Number R826249-01-0.)

1456 ANALYSIS OF URINARY METABOLITES OF BUTADIENE.

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The toxicity of 1,3-butadiene (BD) varies greatly in rodent species and raises the question of whether the risk of BD exposure in humans is more like that of the sensitive species, the mouse, or more like that of the resistant species, the rat. Numerous studies have indicated that one reason for the species differences in response to BD is that the blood and tissues of BD-exposed mice contain high levels of both the mono- and the diepoxide metabolite of BD, while the tissues and blood of exposed rats contain very little of the diepoxide. The diepoxide is far more mutagenic than the monoepoxide, and it is reasonable that the diepoxide plays a major role in tumor induction in the mouse. A major question is the extent to which humans metabolize BD to the diepoxide, but evidence for diepoxide formation in humans is difficult to obtain. The diepoxide would be expected to clear rapidly from the blood, as is seen in BD-exposed mice. Currently we are testing the hypothesis that the diepoxide along with MI and MII, which are water soluble, would be excreted in the urine and could be used as markers of exposure. To extend this research to humans, diepoxide, MI and MII were assayed for in urine. Diepoxide was studied by monitoring diepoxide protein-binding in urine using a matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method. Briefly, the method involves a protein affinity capture step followed by mass spectrometric detection. The protein that we chose to assay for was β_2 -microglobulin (B2M) which is a urinary protein common to primates and rodents and contains many possible adduct sites for diepoxide. Agarose beads are crosslinked with an antibody towards the B2M and this then becomes our affinity column. Results from rat and mouse exposures indicate the presence of diepoxide in the urine of mice but not rats. Urines from workers exposed to BD are being analyzed by electrospray ionization mass spectrometry (LC/MS/MS) for MI and MII. The urine was first cleaned-up by a solid phase extraction method, then simultaneously analyzed for the two metabolites by LC/MS/MS. (Research supported by HEI 97-1, NIH Grants ES-06015 and ES-09401, and CMA Research Agreement OLF-16.0-RES-LRRI.)

1457 AN EMPIRICAL MODEL OF BENZENE EXPOSURE BASED ON MULTIPLE BIOMARKERS.

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To improve human health risk assessment for benzene exposure, an empirically based model using a battery of biomarkers from blood, exhaled air, and urine samples is being developed. For development, testing and validation, the model is being formulated based on experimental measurements from controlled exposure conditions in mice. The critical factors in the development of the model are the use of biomarkers that: 1) can be measured non-invasively, 2) have different clearance patterns, and 3) are relatively specific to benzene. Benzene in exhaled breath, benzene in blood, and phenylcysteine adducts on albumin and on hemoglobin will be used in the model. Although