study is part of a multicenter study sponsored by the German "Hauptverband der gewerblichen Berufsgenossenschaften." The study has not yet been completed; after all subjects have been evaluated, further differences in the concentrations of these oncogene/tumor suppressor gene products might be observed between radon-induced lung cancer and those with lung cancer of other etiology. In the framework of the complete study, we are evaluating p53 mutations in tumor tissue, bronchoalveolar lavage, and sputum as well as other biomarkers so that we will be able to compare these findings with those of serum concentrations of p53 and anti-p53 antibodies. Therefore, we may observe a relation between the biomarkers evaluated, type of cancer, individual patient diagnosis, and health and occupational history.

References


**Increased Bladder Cell Micronuclei in Two Populations Environmentally Exposed to Arsenic in Drinking Water, Lee E. Moore, Allan H. Smith, Claudia Hopenhayn-Rich, Mary Lu Biggs, Marcella L. Warner, David A. Kalman, and Martyn T. Smith** (1 Univ. of California School of Public Health, Berkeley, CA; 2 Univ. of Washington School of Public Health and Community Med., Seattle, WA; 3 address correspondence to this author: 140 Warren Hall, School of Public Health, University of California, Berkeley, CA 94720; fax 510-843-5539; e-mail lemur@ucf.berkeley.edu)

Inorganic arsenic (As) is a known human carcinogen and a powerful clastogen in vitro. To date, results of epidemiological biomarker studies comparing genetic damage in As-exposed and unexposed lymphocytes have been inconsistent and do not correlate well with findings in vitro. The lack of significant findings may indicate that lymphocytes are not primarily affected by As, which is consistent with the lack of evidence for hematopoietic cancer among As-exposed populations. Several studies do, however, link As exposure with bladder cancer. To further examine this relation, we have used a new exfoliated cell micronucleus assay incorporating fluorescent in situ hybridization (FISH) and a centromeric probe as a measure of As-induced genotoxicity in exfoliated bladder cells. Micronuclei are extranuclear bodies in the cell cytoplasm that form when acentric fragments or whole chromosomes are left behind the main nucleus at telophase. An increase in the prevalence of micronuclei within a population of cells indicates that chromosome damage has resulted from exposure to a genotoxic agent. We conducted two cross-sectional biomarker studies of individuals chronically ingesting As in drinking water to test the hypothesis that ingestion of inorganic As results in an increase in the frequency of micronucleated bladder cells. In a preliminary study in Nevada, we examined the exfoliated bladder cells of 18 (10 female, 8 male) highly exposed (mean 1311 μg/L As in water) individuals (mean age 37.5 years, range 14–74) and 18 sex- and age-matched controls (16 μg/L As; mean age 37.0 years, range 16–70). Later, a larger study of 71 highly exposed males (mean 600 μg/L As in water and 616 μg/L As in urine; ages 19–75, mean 41.8 years) and 55 males with low exposure (mean 15 μg/L As in water and 66 μg/L As in urine; ages 19–75, mean 42.4 years) was conducted in Northern Chile to confirm these findings.

The study population in North America included residents of a county of Nevada with private water supply wells. Exposed subjects were defined as individuals with well water As concentrations >500 μg/L, i.e., >10 times the World Health Organization’s permissible concentration for drinking water and the US Maximum Contaminant Level (50 μg/L As). All exposed individuals were matched for age, sex, and smoking status to individuals in the low-exposed group. In Chile, study subjects included individuals from two towns who drank water from sources containing high (600 μg/L) and low (15 μg/L) concentrations of inorganic As.

Exposure was assessed in urine samples collected and analyzed for inorganic As and its methylated metabolites by the method of Crecceans. Exfoliated bladder cells were isolated from urine specimens within 1 h of collection via centrifugation, and the cell pellet was washed with 9 g/L NaCl. After centrifugation, cells were dropped onto coded slides, air-dried, fixed in 800 mL methanol at 0 °C, and stored in a container filled with nitrogen gas at −20 °C until used for the micronucleus assay.
We used a new version of the micronucleus assay incorporating the fluorescent dye propidium iodide and FISH with biotin-labeled probes specific for all human centromeres (2). Briefly, slides were preheated for 30 min on a slide warmer at 63 °C to fully attach cells to the slide. They were then treated with 300 mg/L pepsin (Sigma Chemical Co., St. Louis, MO) for 30 min to permeabilize the cells (3). The slides were rinsed twice in phosphate-buffered saline and fixed in buffered 40 g/L paraformaldehyde (Sigma) for 20 min at 0 °C. After washing, the slides were baked for 20 min at 63 °C and then hybridized with a biotin-labeled α-satellite probe for all human centromeres (Oncor, Gaithersburg, MD) as previously described (2). Propidium iodide (Sigma), 1 mg/L in Vectashield solution (Oncor), was used to counterstain the DNA. Slides were scored as previously described (4).

Mean urinary As concentrations and micronucleated cell (MNC) frequencies are presented in Table 1. In both studies, there was a marked contrast in exposure between the high-exposure and low-exposure groups. In Nevada, the high-exposure group had an average urinary As concentration of 751 μg/L compared with 68 μg/L in the low-exposure group. In Chile, the high- and low-exposure groups had average urinary As concentrations of 616 and 66 μg/L, respectively.

MNC frequencies from both studies showed small yet consistent differences between high-exposure and low-exposure groups. In the Nevada study, MNC frequencies in the high-exposure group were 2.79/1000 cells compared with 1.57/1000 cells in the low-exposure group, giving a frequency ratio (FR) of 1.8 (P = 0.09) (4). After stratification by sex, the MNC frequency was increased among highly exposed males compared with low-exposed males, and to a lesser extent between highly exposed and low-exposed females. The FR was greater for males than for females, 2.34 (P = 0.07) vs 1.42 (P = 0.38), respectively (4).

The larger South American study, which was confined to men, yielded results similar to those of the North American study. The MNC frequencies in the high-exposure group (n = 71) compared with the low-exposure group (n = 55) were 3.14 and 2.60/1000 cells, respectively (FR = 1.21; P = 0.08). Because some individuals in the low-exposure group had high urinary As concentrations, we limited the comparison to include only those with concentrations of <100 μg/L (n = 38). The mean micronucleus frequency for those with urinary As concentrations of <100 μg/L was 2.45/1000 cells (P = 0.05) (data not shown). When the group was limited to include only individuals with urinary As concentrations of <50 μg/L (n = 21), the MNC frequency was 1.59/1000 cells, resulting in a greater contrast in MNC frequency (FR = 1.97; P < 0.001).

In both studies, a small increase in micronucleated exfoliated bladder cells was seen. The increase was consistent although small, compared with other studies in which the exfoliated micronucleus assay is used as a biomarker of genotoxicity to the bladder urothelium. Low-exposure groups from both studies include some As-exposed individuals. In the South American study, when the low-exposed group was limited to subjects with urinary As concentrations of <50 μg/L, the mean MNC frequency was reduced to 1.59/1000 cells. This suggests that As may exert some genotoxic effects at low concentrations, but additional analysis must be completed before a clear dose–response relation can be determined.

Micronucleus formation is a rare event, and frequencies found in healthy, unexposed populations are generally very low (0.05–0.2%) (3). In the Nevada study, we scored at least 500 cells/person to reduce the potential for MNC frequency fluctuations that can occur when <1000 cells are scored for each subject (2). In the Chile study, 12 samples (23%) contained <500 normal intact cells. When these individuals were excluded from the analysis, many of the high-frequency individuals disappeared from the low-exposure group but remained in the high-exposure group, suggesting that the high frequencies found in the low-exposure group were artifacts caused by scoring small numbers of cells. For example, in the highly exposed group, when all individuals and those with <500 cells/slide are included in the analysis, 9% of individuals exhibit micronuclei frequencies >1%, a level similar to that found in radiation-exposed epithelial cells (5, 6). In the low-exposure group, when all individuals are included in the analysis, 2.6% exhibit micronuclei levels >1%. When we include only individuals for whom >500 cells were scored, no individuals are found with MNC frequencies >0.8%. The appearance of several high-frequency individuals in the high-exposure group (with only a small difference in the mean MNC frequencies for each group) could suggest that the genotoxic effect is limited to a few presumably sensitive individuals. Similarly, in Nevada, some high-frequency individuals were found only in the exposed group; however, these individuals were also the most highly exposed (4).

In conclusion, results from both the North and South

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**Table 1. Comparison of exposure indices and micronucleated cell frequencies in North and South American study populations.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Exposure</th>
<th>As conc, μg/L (range)</th>
<th>Mean (range) MNC frequency/1000 cells</th>
<th>Fold increase (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevada: male</td>
<td>Very high (n = 8)</td>
<td>1300 (220–5200)</td>
<td>855 (171–2500)</td>
<td>5.00 (0.49–13.10)</td>
</tr>
<tr>
<td>Nevada: male</td>
<td>Low (n = 8)</td>
<td>17 (5–100)</td>
<td>79 (11–284)</td>
<td>2.14 (0.00–4.86)</td>
</tr>
<tr>
<td>Nevada: female</td>
<td>Very high (n = 10)</td>
<td>1321 (220–5200)</td>
<td>661 (114–1842)</td>
<td>1.82 (0.00–6.22)</td>
</tr>
<tr>
<td>Nevada: female</td>
<td>Low (n = 10)</td>
<td>15 (5–100)</td>
<td>58 (12–350)</td>
<td>1.28 (0.00–3.00)</td>
</tr>
<tr>
<td>Chile: male</td>
<td>High (n = 71)</td>
<td>300 (580–670)</td>
<td>616 (84–1893)</td>
<td>3.14 (0.00–18.2)</td>
</tr>
<tr>
<td>Chile: male</td>
<td>Low (n = 55)</td>
<td>15 (12–17)</td>
<td>66 (4–267)</td>
<td>2.60 (0.00–14.6)</td>
</tr>
<tr>
<td>Chile: male</td>
<td>Very low (n = 21)</td>
<td>15 (12–17)</td>
<td>35 (4–49)</td>
<td>1.59 (0.00–8.2)</td>
</tr>
</tbody>
</table>

* We attempted to include only individuals with well water As concentrations <10 μg/L; however, two individuals had concentrations of 100 μg/L.

* Five individuals had urinary As concentrations >50 μg/L.

* Includes some individuals with occasional exposure to higher concentrations.
American studies provide evidence that As is genotoxic to human bladder epithelium. The micronucleus assay, however, may not be the most sensitive biomarker to reflect such damage, because As does not appear to behave primarily as a strong clastogen or aneuploiden in vivo. Possibly, cytotoxicity and decreased cell proliferation may negatively influence the micronucleus frequencies found in exfoliated bladder cells exposed to As concentrations as high as those found in these studies (6).

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References

Chromosomal Aberrations and Sister-Chromatid Exchanges as Biomarkers of Exposure to Pesticides, Elisabet Carbonell, Noel Xamena, Adameu Cresu, and Ricardo Marcos1 (Unitat de Genètica, Dept. de Genètica i de Microbiol., Edifici Cn, Univ. Autònoma de Barcelona, 08193-Bellaterra, Spain; 1 author for correspondence: fax (3) 581 23 87, e-mail IBGE@EBCUAB1.eearn)

Despite the beneficial effects of pesticides, many of these biologically active chemicals reportedly produce a wide range of toxic effects in different biological systems and represent, therefore, a potential hazard to humans and to nature. Among the harmful effects induced by pesticides, genotoxicity is of concern because of its proven relation with genetic diseases, cancer, reproductive effects, and ageing (1–3). This has prompted the search for adequate biomarkers measuring genotoxic exposures and effects. Several markers in humans for detecting genotoxicity are now available, including chromosome aberrations (CA) and sister-chromatid exchanges (SCE).

Structural chromosome alterations have been widely used as a genetic end point to study the mutagenic effects of ionizing radiation and chemicals. Many tumors are associated with CA (4), emphasizing the importance of studying these aberrations as a relevant biological end point to assess the risk involved in exposure to mutagenic/carcinogenic agents. A recent study of a large cohort has proved the relation existing between CA induction and cancer risk (5).

SCE is a cytological manifestation of DNA double-strand breaking and rejoining at apparently homologous sites between two chromatids of the same chromosome. Although the molecular basis of SCE is not completely understood, the efficient induction of SCE by numerous mutagenic and carcinogenic agents, especially those that form DNA adducts, has extended their use as relevant biomarkers to detect genotoxic agents or risk exposures.

CA and SCE are widely accepted as good biomarkers for genotoxicity; they are now used in the biomonitoring of human populations environmentally or occupationally exposed to suspected genotoxic agents. Biomonitoring studies of occupational groups exposed to pesticides often present conflicting results that could be explained by the complex mixture of the compounds used or by the variability in the degree of exposure of each individual or group. To add more information on the genetic risk associated with pesticide exposure, we present a biomonitoring data for a population occupationally exposed to pesticides. CA and SCE in peripheral blood lymphocytes were used as risk biomarkers, and blood samples were obtained in both high- and low-exposure periods.

The exposed group consisted of 29 men working in flower and (or) fruit cultivation in El Maresme (Barcelona, Spain). Two blood samples were taken from each individual in a follow-up study: the first in a period of high exposure to pesticides (spring–summer), the second in a period of lower exposure (autumn–winter). Concurrent control studies of unexposed people included 29 healthy men during spring–summer and 24 healthy men during autumn–winter. Control individuals were not directly exposed to any particular environmental agent.

Before the cytogenetic study, all subjects filled in a questionnaire about personal data, smoking and drinking habits, diet, drugs and medication consumption, health status and medical history, and exposure to known genotoxic agents. The agricultural workers completed another specific questionnaire in which the type of working activity, duration of contact with pesticides, kind of pesticides used, and protective measures were recorded.

Venous blood from each subject was placed in heparinized Vacutainer Tubes (Becton Dickinson, Rutherford, NJ). Before culturing, 0.5 mL of whole blood and 2 mL of chromosome medium (RPMI-1640) were centrifuged, and the supernate was removed to eliminate the blood plasma. Lymphocyte cultures were set up by adding 0.5 mL of this “clean” blood to 4.5 mL of chromosome medium supplemented with 200 mL/L heat-inactivated fetal bovine serum, antibiotics (penicillin and streptomycin), and glutamine. Lymphocytes were stimulated by 10 g/L phytohemagglutinin.

Two replicates for each assay were prepared from each blood sample. The cultures were incubated at 37 °C for 54 h to measure CA and for 72 h to measure SCE. For SCE studies, 24 h after the initiation of cultures, 5-bromodeoxyuridine (15 mg/L) was added. At 2 h before harvesting, demecolcine (Colcemid) was added to arrest cells in metaphase. The cells were collected by centrifugation, resuspended in a prewarmed hypotonic solution (0.075 mol/L KCl) for 20 min, and fixed in acetic acid:methanol (1:3, by vol). Air-dried preparations were made, and the slides were stained with Giemsa. For each donor, we examined 100 well-spread metaphases (50 per replicate) containing...