Population monitoring: experience with residents exposed to uranium mining/milling waste

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Abstract

More emphasis should be placed upon using biomarkers to address potential health risk among populations exposed to high concentrations of environmental toxicants. Among these studies, those which integrate exposure measurements with analyses of validated biomarkers may provide more reliable information for risk assessment and disease prevention. We have used a multidisciplinary approach to elucidate potential health hazards in a population living around uranium mining/milling facilities. The study included 24 target and 24 control residents who were matched for age and gender and selected based on time of residence in the study areas and proximity to mining/milling sites. Environmental samples were analyzed for uranium-238 ($^{238}\text{U}$) concentrations and lead isotope ratios using inductively coupled plasma-mass spectrometry (ICP-MS) procedures, and blood samples were collected for cytogenetic analysis. We found that the $^{238}\text{U}$ concentrations in soil samples were significantly higher than those in the control areas. In addition, the concentrations in the surface soil were significantly higher than in the subsurface soil ($p < 0.05$) from target areas indicating environmental contamination by the mining/milling activities. Lead isotope data from soil samples taken near a railroad transfer location was significantly different from those of other sites, indicating contamination by non-native ore transported from sources outside of the region to local milling facilities for processing. Therefore, local residents have been exposed to low levels of radioactive contamination from the mining/milling activities on a daily basis for many years. From our cytogenetic analysis, the target population had more chromosome aberrations than the controls, although the differences were not significant ($p < 0.05$). However, using our challenge assay, cells from the target population had a significantly abnormal DNA repair response, compared to cells from the same control population. In conclusion, the observed environmental contamination by uranium is consistent with the observed genotoxic effects in the target residents. Therefore, the residents have increased health risk and some of the health problems will most likely be related to exposure to the radioactive contaminants. Since the chromosome aberration frequency revealed increased, but not significant differences between the exposed and the control populations, we

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conclude that the health risk among the exposed residents is similar to those among nuclear workers. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Populations exposed to catastrophic environmental accidents, such as the Chernobyl nuclear accident in the former Soviet Union, suffer tragic consequences. Consequently, such events attract instant attention and tremendous resources are provided to them to alleviate their sufferings. Much attention is also placed on understanding the health effects in these exposed populations. Although such accidents can be devastating to a large number of people, their impact on a long-term health basis and in the global scale may not be enormous compared with the potential effects from continuous exposure to high concentrations of environmental toxicants. Furthermore, with continued improvements on safeguards against such accidents, the impact on global health will continue to diminish.

It is generally accepted that exposure to high concentrations of environmental toxicants is hazardous to health. However, the extent of such health hazards is difficult to assess. Development of procedures which can be used to precisely identify health hazards in the exposed public is probably a single most significant approach towards establishing effective programs for disease prevention. These exposed populations can be found readily in the United States and around the world, e.g., residents living around hazardous waste sites (HWS) [1].

Due to previously ineffective environmental laws to regulate disposal of hazardous toxicants, there exists many HWS in the United States (US). Many sites are well documented but numerous sites are undoubtedly unaccounted for. In the US it has been estimated that 275 million tons of hazardous wastes are produced annually [2]. There now exists an estimated 40,000 sites on the US Environmental Protection Agency’s inventory of uncontrolled waste sites. Out of this number, approximately 1296 have been added or are proposed to be included on the National Priority List (NPL), representing sites of greatest concern to the public’s health [3]. In terms of waste site constituents, more than 2000 unique chemicals have been identified so far, with over 275 of these representing an extreme threat to human health. The classes of chemicals most often encountered at HWS during health assessments conducted by the Agency for Toxic Substances and Disease Registry (ATSDR) include volatile organic compounds (74% of assessments), inorganic substances (71%), halogenated pesticides (37%), polyaromatic hydrocarbons (25%), phenols/phenoxyc acids (23%), phthalates (22%), nitrosamines/esters/alcohols (15%), and organophosphates (14%) [4].

While the statistics on HWS are impressive, of primary concern is the fact that there are an estimated 41 million people in the US living within a 4-mile radius of 1134 NPL sites [5]. Lichtveld et al. [6] estimated that approximately 2000 people are most exposed to a typical HWS in the US. The serious health concerns from these citizens on environmental contamination and their long-term health are well documented [7]. Furthermore, the percentage of HWS that ATSDR has categorized as urgent public health hazards has increased over time, suggestive of a trend, or possibly due to more rigorous assessments [4]. Whichever the case, there is little question that multitudes (men, woman, and children of all ages) living in proximity to HWS are exposed chronically to chemical and radioactive wastes with undetermined consequences.

After the breakup of the former Soviet Union, previously censored information became available to the public suggesting a relationship between disastrous environmental contamination and human health problems [8]. Such indications are supported by epidemiologic studies which show that the cancer rate in Russia has doubled during the last 20 years (to 1.6 million/year), whereas the rate of cancer in Western Europe has declined [9]. These authors associate the
increased cancer outcome to the release of industrial waste into the environment. The impact of the environmental contaminant on the population can be further revealed by the evidence that 85% of the urban population in Russia lives in territories where environmental pollution exceeds permissible standards [9].

Attention to long-term environmental problems on a global scale is necessary because this may alert countries around the world to the excessive human price paid for short-term economic gain and political complacency. It is also timely to do so because scientists are gaining the necessary tools to precisely identify such environmental problems.

2. Studies to address environmental health problems

Although the citizens and the scientific communities are concerned about long-term environmental disease in the general populations, few studies have been conducted to address the problem. It should be emphasized that results from studies using accidentally and occupationally exposed populations are not suitable for use to assess health risk of the exposed public. The main reason is that the exposure conditions for the three groups are so significantly different that extrapolation of observed effects from one to the other can be complicated by profound uncertainties. Well designed studies on the health risk of the exposed public will reduce the mentioned uncertainty and will provide a better handle for use in disease prevention.

There exists an obvious need for studies to address the question of possible long-term health consequences from the described exposures. While a few epidemiological studies have been useful in identifying health effects of residents to hazardous wastes [10,11] and they are the classical approach to identifying at-risk populations, most community based exposures are not conducive to this type of investigation. This is largely due to small sample sizes commonly associated with these types of exposures and infrequency and long latency of the disease events, e.g., cancer, and therefore these studies tend to suffer from low statistical power. As previously mentioned, a typical hazardous site has approximately 2000 at-risk individuals which is too few for most epidemiological study designs [12–14]. In addition, traditional epidemiological studies rely on disease as the endpoint, reducing the impact of disease prevention. However, with the advent of sophisticated and precise laboratory techniques, it seems promising that associations between exposure and human disease can be elucidated prior to clinical manifestation.

A new field of population study has been initiated by integrating laboratory measurements of internal dose, biologically effective dose, biologic effect, and susceptibility with epidemiologic methodologies, hence linking individual exposure with a relevant biological event [15]. This new initiative is known as molecular epidemiology. The biological component is evaluated with biological markers, or biomarkers, which are observable endpoints that indicate events in the process leading up to disease [16]. Biomarkers are typically arranged into three groups: (1) biomarkers of exposure/dose, e.g., DNA adduct; (2) biomarkers of effect, e.g., chromosomal aberration; and (3) markers of susceptibility, e.g., genetic polymorphism. So far, studies which have employed these techniques and have applied them to residential population based exposure scenarios are relatively few [17–19].

Perera et al. [20] utilized a battery of markers and found genetic damage associated with extreme environmental pollution in the industrialized Silesian region of Poland. A total of 88 individuals were enrolled in the study; 39 representing the exposed group and 49 from the chosen control site in a rural section of the country. Individuals were selected to minimize confounding factors and were administered a questionnaire for obtaining personal information such as age and smoking habits. The markers utilized in the study were chosen based on exposure to polycyclic aromatic hydrocarbons (PAHs), which was the most prevalent and widespread mutagenic and carcinogenic class of chemicals in the area. The markers included PAH–DNA adducts, sister chromatid exchange (SCE) with high-frequency cells, and standard chromosomal aberrations, as well as expression of the ras oncogene. In the regression model of the study, after adjusting for age and smoking, exposure to environmental pollution was
significantly related to PAH–DNA adduct levels, to SCEs, and to chromosomal aberrations.

3. An investigation of environmental causes for neural tube defects (NTD)

In some cases, the urgency of the situation dictates what can be done. Nevertheless, properly designed studies should be emphasized. For example, we have investigated the possible causes of a significant surge in prevalence of babies having NTD in Brownsville, Cameron County, TX. NTDs are the most common congenital anomalies afflicting humans [21]. Although no known cause has been established, environmental factors appear to play a role. The latter is suggested by the geographic variations in NTD rates, and associations with environmental toxins and nutrition [11,21–24]. For the years 1990–1991, a significantly increased number of babies with NTD (33.6 per 10,000 live births), particularly anencephaly, in Cameron County, TX was reported by the Texas Department of Health and the Center for Disease Control [25]. The record also indicates that the prevalence of anencephaly was very high in the previous years: 15.1 and 20.4 cases per 10,000 live births for 1990 and 1991, respectively, compared with 6 cases per 10,000 in the general US population. Our research team was involved with addressing whether exposure to environmental toxicants could have been a cause of the health problems. The major source of environmental toxicants in the area was suspected to be from the tremendous build up of industrial plants across the Texas/Mexico border. This suspicion is supported by the detection of a variety of industrial chemicals in ambient air samples and biological specimens [25,26]. In addition to industrial chemicals, the population is exposed to pesticides used for agriculture as well. A summary of our cytogenetic and epidemiologic studies is provided here.

In our cytogenetic study, blood samples were collected from 19 mothers who had delivered babies with NTD. These mothers were healthy and not exposed to any known genotoxic agents through work or through clinical procedures. They were matched by age, gender and ethnicity with 14 mothers in Corpus Christi, TX, who had delivered normal babies. The blood samples were analyzed using the standard cytogenetic assay and a challenge assay [27] for documentation of chromosome aberrations and abnormal DNA repair response (Table 1). Our data indicates that the two populations did not differ in genotoxic outcome as determined by the two assays [28].

In the epidemiologic study, the previous NTD report [25] which was based on review of death certificates was confirmed by a population survey (Fig. 1). The prevalence of anencephaly stands out compared with other birth defects in Cameron County and in the other two neighboring control counties (Fig. 2). In addition, the prevalence of anencephaly in Cameron county is significantly correlated with the number of industrial plants and employees in the Mexican side across the Texas/Mexico border (Table 2). As the industrial activities waxed and waned

Table 1
A summary of the chromosome effects in exposed populations compared with concurrent controls

<table>
<thead>
<tr>
<th>Study</th>
<th>Cases/controls</th>
<th>Conditions</th>
<th>% Changes from controls</th>
<th>Aberrant cells</th>
<th>Deletions</th>
<th>Dicentrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural tube defects*</td>
<td>19/14</td>
<td>Background</td>
<td>+15.4</td>
<td>N.A.*</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Challenged</td>
<td>−13.9</td>
<td>N.A.</td>
<td>−4.7</td>
<td></td>
</tr>
<tr>
<td>Uranium residents*</td>
<td>24/24</td>
<td>Background</td>
<td>+22.9</td>
<td>N.A.</td>
<td>N.A.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Challenged</td>
<td>+19.4*</td>
<td>+33.0*</td>
<td>+4.6</td>
<td></td>
</tr>
</tbody>
</table>

*Data obtained from Ref. [27]. A total of 200 metaphases were analyzed for the background aberration frequency and 100 for the challenged frequency. The challenged frequency was based on the effect after exposing lymphocytes in vitro to 100 cGy gamma-rays.

Not applicable as background and the frequencies were extremely low to be meaningful.

*Data obtained from Ref. [18]. A total of 600 metaphases were analyzed for the background aberration frequency and 200 for the challenged frequency.

Significantly higher compared with controls, $p < 0.05$. 
Throughout the years, so has the anencephaly rate increased and decreased in Cameron but not in the other two counties [29].

In conclusion, the high prevalence of anencephaly was unique to Cameron County and certain localities within Cameron County. The high prevalence of this defect does not appear to extend to other types of birth defects. The data suggest that the birth defect was associated with environmental pollution from industrial activities. However, no genotoxic effects could be detected using blood samples from the affected population [28]. The lack of detectable genotoxicity could be due to several factors. One of them is that the exposure has changed and/or that the effect has disappeared.

Based on experience from our studies, several future directions can be recommended. Environmental surveillance of air, water and soil needs to be conducted to evaluate potential impact on health problems. When health problems are suspected, a sensitive and relevant biomarker study should be conducted to provide early warning signal for health outcome. In addition, future efforts need to be directed toward obtaining information on pregnancy termination practices along the border (and other areas as well), better understanding of migration patterns to and from the border region, and deliveries that occur out of as well as in hospital and birthing center facilities.

### Table 2

<table>
<thead>
<tr>
<th>County</th>
<th>Coefficient</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cameron</td>
<td>0.61</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Hidalgo</td>
<td>0.28</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Nueces</td>
<td>0.11</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

* Spearman's rank order correlation coefficient.

4. Limiting factors in population studies

Frequently, studies that have employed the application of biomarkers have done so with an improper or ineffective experimental design. For instance, a study may have incorporated markers of exposure, estimating internal dosimetry, but neglected the use of markers to gauge responses or consequences from such an exposure, e.g., measuring the wrong DNA adduct or investigating chromosomal aberrations from exposure to non-genotoxins. The most common problems appear to be the use of small sample sizes and the lack of appropriate control populations. Careful and complete coupling of markers is essential in order to maximize the sensitivity of the application and minimize errors in interpretation of biomarker based data.

In most studies attempting to correlate environmental exposure with human disease there exists a paucity of data concerning the exposure assessment, particularly estimates of personal exposure. In the process of risk characterization, the exposure assessment is an imperative and fundamental step in illuminating associations between exposure/risk and an
estimate of the dose/response relationship. Without valid exposure data inferences concerning the exposure/risk relationship remains incomplete. Study designs addressing a current exposure scenario should make every effort to include the sampling of all relevant exposure pathways for the chemicals of concern and include personal monitoring if possible.

We have an ongoing study to elucidate health risk of residents living around uranium mining and milling activities. In this study, we integrate environmental data with genetic monitoring for determination of the cause and effect relationship. A summary of our current data is presented here.

5. Health risk among residents exposed to uranium mining/milling waste

During the last few years, we have been conducting studies to elucidate potential health risk among residents living around uranium mining/milling areas in Karnes County, TX. Uranium has been mined and milled extensively around the northern part of the County for over 30 years. From 1978 to 1985, for example, approximately 5,211,000 tons of ore have been processed by one milling plant alone in the area [30]. After the removal of high grade uranium in the milling process, a large volume of hazardous residue, called tailings, is left behind. The tailings which contain most of the radionuclide species in the original ore are disposed of in open-air tailing piles. Through the tailings, radionuclides and toxic chemicals become more readily available for dispersal through the hydrologic and atmospheric processes than in the original underground ore [30]. In addition, the tailings release radioactive radon gas into the environment (9.54 × 10^2 Ci/year) [31]. Therefore, the residents have been concerned that their health may be compromised by the environmental contamination problem. This condition is typical of many communities around hazardous waste areas.

The Texas Department of Health has conducted a standard epidemiological study to evaluate the cancer mortality and adverse reproductive outcome of the uranium-exposed population. Like many such exposed populations, the sample size of the immediately exposed individuals is too small to be used for a typical epidemiologic study. Therefore, the residents of the entire county was used as the exposed population and their health outcome was compared with those of standard population data. From this investigation, only non-significant increases in some health problems such as leukemia and spina bifida were detected [32]. However, there are limitations in this study. First, the health effects were ascertained only from death certificates. Second, the residents from the entire county was used as the exposed population. Therefore, the documented results must be considered a substantial underestimate of the real problem.

We initiated our study by conducting a geographic and background survey to identify the active mining/milling operations, unclaimed mine/mill sites, and sites that have been closed for less than three years. Households that were located within 1 mile and downwind from the identified operations/sites were considered potentially exposed. Households that are located within 0.5 miles and in the other directions were also considered exposed. We have also chosen the southern part of the County which is approximately 10 miles south of the mining/milling areas as the location for selection of unexposed households. The identified households from both the exposed and control areas were visited by a member of our field team and interviewed using our 37-page questionnaire. We interviewed 173 residents: 73 exposed and 81 controls. The selection criteria for inclusion of volunteers into our study were: no occupational experience in uranium industry, non-cigarette smokers, and no exposure to genotoxic agents from occupation and from clinical procedures. The acceptable individuals from the exposed group were matched with those from the control group based on age (± 5 years) and gender. Environmental samples from the selected households and blood samples from the selected individuals were collected for environmental and cytogenetic analysis.

An environmental exposure assessment was conducted near two mining/milling sites (Panna Maria and Susquehanna) and a mining-only area. Soil samples (n = 61) were collected from the yards of exposed homes within 1 mile of the sites at the surface and at 30 cm subsurface. From each home, carpet vacuums, filter entrapments and surface dust swabs
settled dusts on furniture, appliances, etc.) were collected \((n = 15)\) as were water well samples when available \((n = 7)\). Similar types of samples were collected from the control households. Environmental samples were analyzed for the quantity of uranium-238 \(^{238}\text{U}\) and lead isotope ratios using the inductively coupled plasma-mass spectrometry (ICP-MS) technique \([33, 34]\). \(^{238}\text{U}\) was found to have a statistically higher \((p = 0.006)\) concentration in the surface soil from all mining/milling areas compared to corresponding subsurface samples (Fig. 3). Additionally, surface soil from the exposed sites were statistically higher \((p = 0.001)\) compared to the control site surface soil as were subsurface soils \((p = 0.02)\). Indoor dust samples from homes in the exposed area were found to have significantly higher \((p = 0.003)\) levels of uranium than samples from homes in the control area (Fig. 4). Water samples collected from potable water wells (150–300 ft) revealed leaching from the Panna Maria mining/milling complex as \(^{238}\text{U}\) concentrations were up to six times higher \((31.5 \mu g/l)\) compared to other study areas and the control sites. Furthermore, lead isotope ratio analysis of selected soil samples revealed a significant shift in \(^{206}\text{Pb} / ^{207}\text{Pb}\) and \(^{206}\text{Pb} / ^{208}\text{Pb}\) ratios in samples from the Panna Maria study area, indicating contamination with both native and non-native ore. The Panna Maria samples were collected from the yard of a home near a railroad terminal used for transporting uranium ore from out of state. The ore was off-loaded onto trucks and transported to the Panna Maria mill for processing.

Blood samples were collected from 24 exposed and 24 matched controls. The samples were used for determining the existence of chromosome aberrations using the standard cytogenetic assay and of abnormal DNA repair response using a challenge assay \([27]\). From the analysis of 600 metaphase cells from each individual, we found that cells from the exposed individuals had more chromosome aberrations than those from the controls (Table 1). However, the difference was not significant \([19]\). After the cells were challenged by an in vitro exposure to gamma-rays, cells from the exposed population had significantly more chromosome-type aberrations, especially dicentrics, than cells from the controls \([19]\) indicating abnormal DNA repair response. Therefore, the results from both assays were consistent with each other.

The results from our environmental and cytogenetic investigations indicate that the target population has been exposed excessively to uranium through contamination from the mining/milling industries.
The exposure is most likely the cause of the observed increase in chromosome aberrations and the significantly abnormal DNA repair response. Therefore, it seems reasonable that the exposed population is at greater risk for developing health problems as a result of living near the uranium mining/milling facilities. The level of increase in chromosome aberrations using the standard cytogenetic assay suggests that the target population may have been exposed to less than 10 cGy ionizing radiation [35], a level similar to that of nuclear workers (5 rem/year). This level of exposure will cause these residents to have health risk equivalent to those of nuclear workers.

6. Conclusions and future recommendations

It should be emphasized that the future of using biomarkers in population studies hinges upon their usefulness for providing reliable risk evaluation. By providing risk assessment data, these studies will have impact on regulatory policies and on establishing disease prevention strategies. In order to do so, biomarker studies need to be conducted using acceptable protocols, such as adequate population sizes, integration of exposure assessment with biomarker measurement, appropriate biomarkers which can detect relevant biological effects and appropriate biomarkers which can indicate health outcome. While catastrophic events are unfortunate occurrences in our recent history, they do provide investigators with the opportunity to validate current and evaluate newly developed biomarkers for specificity and sensitivity in predicting health outcomes in exposed populations. Thus far, cytogenetic endpoints have the largest population data base amassed and have shown to be relevant to disease outcome. Balanced chromosome translocations have the utility of being stable over time so that a protracted effect can be measured and evaluated. The formation of chromosomal aberrations has been shown to be dose dependent for both chemical and radiation exposures [36,37]. Furthermore, a large-scale Nordic prospective study, which evaluated cytogenetic endpoints and environmental exposure, found a positive correlation between chromosomal aberration frequency and risk for developing cancer (two-fold increase comparing high CA frequency with low CA frequency) [38]. The Nordic study, in addition to other independent investigations, seems to confirm that chromosomal aberrations are reliable biomarkers for predicting future cancer risk [39,40]. Emphasis should be placed on characterizing the precise relationship between biomarkers and the disease process. Otherwise, our participation in national health programs will be limited.

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