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Biodosimetry of Chernobyl Cleanup Workers from Estonia and Latvia Using the Glycophorin A In Vivo Somatic Cell Mutation Assay


Center for Environmental and Occupational Health and Toxicology, University of Pittsburgh, and Molecular Carcinogenesis Program, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania 15235; Department of Laboratory Medicine, University of California, San Francisco, California 94143; Department of Epidemiology and Biostatistics, Institute of Experimental and Clinical Medicine, EE0016 Tallinn, Estonia; Latvian Health Department, Latvian Oncological Centre, LV-1079 Riga, Latvia; Finnish Cancer Registry, FIN-00179 Helsinki, Finland; Unit of Cancer Epidemiology, Karolinska Institute, S-171 76 Stockholm, Sweden; Finnish Center for Radiation and Nuclear Safety, FIN-00880 Helsinki, Finland; and Extramural Programs Branch and Radiation Epidemiology Branch, National Cancer Institute, Rockville, Maryland 20852

INTRODUCTION

The April 26, 1986, steam explosion that destroyed reactor number 4 of the Chernobyl, Ukraine, nuclear power plant resulted in a massive contamination of the surrounding environment with radioactive fission products (1, 2). Subsequent to initial measures to control the reactor accident that took the lives of 31 emergency personnel and resulted in clinical symptoms of acute radiation syndrome in approximately 200 others, a large population of individuals, conscripted from each of the 15 Republics of the former Soviet Union, participated in an extensive cleanup of radioactive materials and restoration of the immediate and surrounding area and the construction of a sarcophagus to isolate the damaged reactor from the environment. This effort involved an estimated 25,000 cleanup workers who worked at the site beginning shortly after the accident in 1986 through early 1987 and who may have received as much as 70 cGy of whole-body exposure to external γ radiation, and as many as 600,000 individuals who worked on subsequent cleanup operations and who received an estimated mean dose of approximately 10 cGy (3).

Among these participants were residents of the three Baltic countries, Estonia, Latvia and Lithuania, where 4,833, 6,002 and more than 7,000 individuals have been identified, respectively, to date. We have begun a comprehensive cohort study of cancer occurrence among these well-defined populations of Chernobyl cleanup workers using record-linkage techniques beginning with feasibility studies of thyroid cancer and leukemia incidence (4; Rahu et al., unpublished results) and an evaluation of radiation exposures to be reconstructed from physical dosimetry records, extensive questionnaire data and biological dosimetry methods. A detailed description of the identification and demographic characterization of the Estonian pop-
ulation, the cohort design and results of a comprehensive questionnaire regarding work experience at Chernobyl will be described elsewhere (Tekkel et al., manuscript submitted for publication). The experimental design of the Latvian study paralleled that of the Estonian cohort and was conducted in a similar manner. In addition to this study of cleanup workers from the Baltic countries, pilot studies documenting radiation exposures and case-control studies of leukemia incidence among Chernobyl cleanup workers from Belarus and the Russian Federation have also been conducted within the framework of the Experimental Collaboration Project 7 (ECP-7) between the Confederation of Independent States and the European Commission and two case-control studies of the risk of leukemia and of thyroid cancer among 1986–1987 entrants included in the State Chernobyl Registries of Belarus and Russia who worked within the 30-km zone around the Chernobyl reactor are planned using similar designs (3).

The GPA locus mutation assay used to assess the biological effect of radiation exposure in these populations employs immunolabeling and flow cytometry to enumerate peripheral blood erythrocytes expressing GPA allele-loss variant phenotypes (5). The frequency of these variants reflects the level of inactivating somatic mutations at this locus in erythroid progenitor cells of the bone marrow (6). Several GPA-based investigations of human populations with acute whole-body exposures to ionizing radiation have been conducted. These include studies of Hiroshima atomic bomb survivors (7–11), individuals accidentally exposed to $^{137}$Cs in Brazil and Estonia (12, 13), and Chernobyl accident victims (14). These studies have demonstrated increases in and long-term persistent elevation of the frequency of GPA allele loss variants that are associated with radiation dose in these populations. Linear regressions of the observed GPA allele-loss variant frequencies as a function of estimated radiation doses yield a slope of approximately 25 radiation-induced allele-loss erythrocytes/10⁹ cells/Gy. Since erythrocytes have a limited life span in the peripheral circulation of approximately 120 days, and these studies were all performed using blood samples collected at times after exposure well beyond the lifetime of erythrocytes, these results demonstrate that exposure to ionizing radiation induces long-lived GPA locus mutations in bone marrow stem cells and that these cells continue to produce circulating erythrocytes expressing these radiation-induced GPA allele-loss mutations years to decades after exposure. The assay therefore provides "biological memory" of past radiation exposures and can thus serve as a retrospective biodosimeter in populations acutely exposed to relatively high radiation doses.

The current study was undertaken to provide new biological information on the effects of relatively low doses of ionizing radiation after protracted whole-body exposures and to provide biodosimetry data useful in assessing the potential importance and practicality of large-scale studies with long-term follow-up of other Chernobyl cleanup workers. Here we report initial biodosimetry data, obtained using the GPA in vivo somatic cell mutation assay, for 453 and 281 cleanup workers and controls from Estonia and Latvia, respectively, together with correlations with reported physical dose estimates for these workers. A preliminary report of the results of the study, including biodosimetry data for 48 Lithuanian cleanup workers, has been published (15).

### MATERIALS AND METHODS

#### Study Populations

The individuals included in this study are male inhabitants of Estonia and Latvia who, primarily as civilian reservists in the army of the former Soviet Union, were conscripted and sent to the Chernobyl area to participate in cleanup activities from 1986 until 1989, primarily in 1986 and 1987. Cleanup workers from Estonia also included individuals who were on active duty in the Soviet Army or who served under contract at Chernobyl. The Estonian cohort of Chernobyl cleanup workers, which numbers 4,833 men, was identified and assembled by the Estonian Institute of Experimental and Clinical Medicine from partially overlapping sets of lists and records maintained by four sources: the former Estonian Chernobyl Radiation Registry, the Estonian Chernobyl Committee (which is a political organization of cleanup workers that maintains lists of individuals sent to Chernobyl), the former Estonian Ministry of Social Welfare (which issues identification cards used for benefits registration of cleanup workers) and the General Staff of the Estonian Defense Forces. The accurate identification and classification of this cohort has been aided by the issuance of Chernobyl military passports to these workers upon their return to Estonia which have been used to document their service at Chernobyl.

The Latvian cohort of Chernobyl cleanup workers, which numbers 6,002 men, was identified from regional Health Ministry lists, the Latvian Chernobyl Registry, military reservists lists made available by the Latvian Chernobyl Association (a political organization formed by cleanup workers) and the Chernobyl Center (a clinic in Riga that provides health care for Chernobyl cleanup workers). The Chernobyl Center has conducted a mass media campaign to identify these workers and, as in Estonia, used Chernobyl military passports to validate their status.

While the main purpose of this study was to identify the population of Chernobyl cleanup workers in Estonia, Latvia and Lithuania, to characterize their work experience and exposures, and to determine health outcomes, the available resources of the study permitted the recruitment of a limited number of subjects for biodosimetric controls. Male control subjects from Estonia, restricted for age, were sampled randomly from the same populations resident in the same geographic locales of Estonia from which the Chernobyl cleanup workers were drawn and were contacted by mail. In Latvia, male control subjects were primarily National Guard reservists and were contacted by telephone. The responding volunteers were screened to exclude service at Chernobyl and were group-matched for age to the cohorts of cleanup workers. Date of birth was determined by personal interview at the time of blood collection. Also reported here are data from historical control subjects recruited from a population of healthy unexposed donors from the U.S. These donors comprised staff and students at the Lawrence Livermore National Laboratory (LLNL), Livermore, CA. GPA data from a subset of these donors, selected to include only male European-Americans and group-matched by 5-year intervals of age to the combined populations of Chernobyl cleanup workers from Estonia and Latvia, were used for comparison.

#### Physical Dosimetry of Radiation Exposures

For both the Estonian and Latvian cohorts, written records as well as personal interviews have documented that approximately one-half of the cleanup workers wore a thermoluminescent dosimeter or other personal dosimeter during their service at Chernobyl. In some instances area radiation monitors were used in place of, or in addition to, personal dosime-
Glycophorin A (GPA) Assay

Since the GPA assay can be performed only on peripheral blood samples from individuals of M/N blood type, who comprise approximately one-half of any study population, upon receipt at Pittsburgh or UCSF the samples were serotyped using microscope slide agglutination tests with commercial anti-M and anti-N typing sera (Ortho Diagnostics, Raritan, NJ). Erythrocyte fixation, immunolabeling and flow cytometric analysis procedures for the GPA assay were performed in a similar manner to that for the BR6 version of the assay described by Langlois et al. (17), except that a modification of the immunolabeling methodology using antibodies labeled directly to fluorescent green (BRIC157-fluorescein, BRIC157-F) and orange (6A7-phycocerythin, 6A7-PE) fluorochrome labels so that the normal erythrocytes in the sample, which express both the GPA<sup>M</sup> and GPA<sup>N</sup> alleles, were doubly labeled. The labeled samples were analyzed using a Becton Dickinson FACScan<sup>TM</sup> flow cytometer and Consort 30<sup>TM</sup> software in dual-fluorescence parameter-correlated mode at a rate of approximately 4,000 cells/s. A dual-parameter low-angle and orthogonal light-scatter live gate was used to limit the analysis to erythrocyte singlets, and the two fluorescence intensities of each of 5 × 10<sup>4</sup> cells per sample were quantified. A small number of erythrocytes which bind the normal level of GPA<sup>M</sup>-specific BRIC157-F antibody, but exhibit no binding of the GPA<sup>N</sup>-specific 6A7-PE antibody, were detected in each analysis, indicating that these cells have lost expression of the GPA<sup>M</sup> allele. These variants are designated allele-loss "hemizygous O/N" cells. A second discrete population of rare variant cells also appear to have lost expression of the GPA<sup>M</sup> allele, but express the GPA<sup>N</sup> allele at twice the normal level. These variants are designated as allele-loss and duplication "homozygous N/N" cells. Variant frequencies (V<sub>P</sub>) for both classes of variants are measured directly by enumerating the number of both variant and normal cells and are expressed as variants per 10<sup>6</sup> cells. Samples of M/N blood type from the U.S. control subjects were analyzed at LLNL using the BR6 version of the assay (17).

A blood sample from a standard control donor was fixed, immunolabeled and analyzed together with each batch of experimental samples for the purposes of quality control and to monitor the long-term baseline stability of the assay. These control samples were drawn from one of several donors in each laboratory. These individuals had been sampled repeatedly over time, yielding stable and comparable GPA V<sub>P</sub> in all samples analyzed in both laboratories. In addition to the shared standardized blood samples, common antibody reagents were also used at Pittsburgh and UCSF to further ensure interlaboratory calibration of the GPA data.

Statistical Analysis

Upon completion of the GPA analyses, the Pittsburgh and UCSF databases were combined, the samples were decoded, and univariate comparisons of the groups of exposed workers and controls were performed. Because of the skewed distributions of the physical dose estimates and GPA V<sub>P</sub> (Figs. 1 and 2), the nonparametric Mann-Whitney U-test, which is based on relative ranks of compared data and therefore does not assume a shape for the underlying distribution of the data, was employed. Accordingly, for the statistical summaries presented in Tables I and II, geometric mean values and the asymmetric dispersion intervals around the geometric mean corresponding to the exponentiated values of the logarithmic mean ± standard deviation values are reported.

For the analysis of covariance (ANCOVA), the GPA V<sub>P</sub> were first log-transformed to stabilize variance and approximate a normal distribution. ANCOVA, using log-transformed GPA V<sub>P</sub> as continuous dependent variables and age and physical radiation dose as continuous independent variables together with the worker/control identifier as a discrete independent variable, was employed to determine the potential confounding effects of these factors on the association between the results from the biodosimetry and the physical dose estimates. All results reported as significant had P values ≤ 0.05.

The non-normal distribution of O/N and N/N V<sub>P</sub> observed in the Estonian, Latvian and U.S. populations, like others studied previously, are driven in part by the presence of a relatively small number of very high "outlier" values previously defined as V<sub>P</sub> ≥ 30 that comprise the approximate highest 5% of the O/N or N/N V<sub>P</sub> observed in the population. The fraction of "outliers" observed in a study population appears to depend, in part, on the age distribution of the subjects as the fraction of individuals within a population displaying "outlier" V<sub>P</sub> increases with age (Bigbee et al., manuscript submitted for publication). In this study, of the total of 785 Estonian and Latvian samples analyzed, 15 (1.9%) had O/N and 22 (2.8%) had N/N V<sub>P</sub> that are classified as "outlier" values by this
TABLE I
Summary Statistics for Estonian and Latvian Chernobyl Cleanup Workers and Control Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Dose (cGy)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Median</td>
</tr>
<tr>
<td>Exposed:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>26-71</td>
<td>39</td>
</tr>
<tr>
<td>Latvia</td>
<td>26-62</td>
<td>40</td>
</tr>
<tr>
<td>Combined</td>
<td>26-71</td>
<td>40</td>
</tr>
<tr>
<td>Controls:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>27</td>
<td>45</td>
</tr>
<tr>
<td>Latvia</td>
<td>24</td>
<td>39</td>
</tr>
<tr>
<td>Combined</td>
<td>51</td>
<td>40</td>
</tr>
<tr>
<td>U.S.</td>
<td>94</td>
<td>39</td>
</tr>
</tbody>
</table>

†For 401 Estonian and 281 Latvian Chernobyl cleanup workers with reported physical doses.

Mean ± SD.

Exponentiated values of the mean and the asymmetric dispersion interval calculated from the logarithmic mean ± SD values (zero doses excluded).

criterion. Among the selected 94 U.S. control samples, 4 (4.3%) and 3 (3.2%) displayed “outlier” O/N and N/N Vf, respectively. To be certain that no potential differences or associations were driven by these observations, or that no significant differences in the majority of the data were obscured by these “outlier” values, all analyses were performed, and are reported, with and without these data.

RESULTS

GPA Vf were measured in blood samples from 453 Estonian and 281 Latvian Chernobyl cleanup workers and from 27 Estonian and 24 Latvian control subjects. The Estonian workers and controls did not differ significantly with respect to age, nor did the Latvian workers and controls (Table I). The two populations also did not differ with respect to age from each other when workers, controls or the combined cohorts were compared (Table I). The age distribution of the 94 matched historical controls from the U.S. also did not differ significantly from any of these groups (Table I). The distribution of recorded physical radiation doses was similar in the two cleanup worker cohorts (Fig. 1), although there were substantially more Latvian workers who were assigned zero doses. For this reason, median as well as arithmetic and geometric mean doses were calculated and are included in Table I. Overall, the doses recorded for the Estonian workers were slightly, and significantly, higher than those of the Latvian cohort (P = 0.022).

Statistical summaries of the GPA O/N and N/N Vf observed in the samples from Estonian and Latvian controls and cleanup workers, together with those of the historical U.S. controls, are presented in Table II. The distributions of O/N and N/N Vf observed in the combined populations of Estonian and Latvian cleanup workers are shown in Fig. 2. As seen from Table II, the distributions of observed GPA Vf were very similar between the Baltic and U.S. control populations and the Vf determined for the cleanup workers were not dramatically elevated over those of the controls.

Univariate analyses were first performed to determine if elevated GPA Vf were demonstrable in either of the Estonian or Latvian cleanup worker cohorts when compared to those of the age-matched control population from each country. As summarized in Table II, no signifi-

FIG. 1. Distribution of the reported physical radiation doses received by 401 Chernobyl cleanup workers from Estonia and 281 workers from Latvia. Median doses were 9.5 Gy for the Estonian workers and 9.4 cGy for the Latvian workers.
### TABLE II

<table>
<thead>
<tr>
<th>Group</th>
<th>GPA Vf in Estonian and Latvian Chernobyl Cleanup Workers and Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GPA variant cell frequencies</td>
</tr>
<tr>
<td></td>
<td>( \Omega/N )</td>
</tr>
<tr>
<td></td>
<td>N/N</td>
</tr>
<tr>
<td></td>
<td>( P )</td>
</tr>
<tr>
<td></td>
<td>( P )</td>
</tr>
<tr>
<td>Exposed:</td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>453^b 0.3–145.6 6.6 6.6 (3.3–13.0) 0.25</td>
</tr>
<tr>
<td></td>
<td>447^e 0.3–26.8 6.6 6.4 (3.4–12.1) 0.33</td>
</tr>
<tr>
<td>Latvia</td>
<td>281^b 0.3–213.8 7.0 6.4 (2.5–16.0) 0.0001</td>
</tr>
<tr>
<td></td>
<td>272^e 0.3–27.2 6.9 5.9 (2.5–13.9) &lt;0.0001</td>
</tr>
<tr>
<td>Combined</td>
<td>734^b 0.3–213.8 6.8 6.5 (3.0–14.2) 0.017</td>
</tr>
<tr>
<td></td>
<td>719^e 0.3–27.2 6.6 6.2 (3.0–12.9) 0.0069</td>
</tr>
<tr>
<td>Controls:</td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>27^b 1.8–38.4 7.4 7.1 (3.8–13.2) —</td>
</tr>
<tr>
<td></td>
<td>26^e 1.8–13.6 7.4 6.7 (3.9–11.3) —</td>
</tr>
<tr>
<td>Latvia</td>
<td>24^b 0.2–11.2 4.0 2.9 (0.9–9.4) —</td>
</tr>
<tr>
<td></td>
<td>23^e 0.8–28.5 6.0 6.1 (2.5–14.7) —</td>
</tr>
<tr>
<td>Combined</td>
<td>51^b 0.2–38.4 6.4 4.6 (1.7–13.0) —</td>
</tr>
<tr>
<td></td>
<td>50^e 0.2–13.6 6.2 4.4 (1.6–12.0) —</td>
</tr>
<tr>
<td>U.S.</td>
<td>94^b 2.2–54.0 5.9 6.1 (3.3–11.5) —</td>
</tr>
<tr>
<td></td>
<td>90^e 2.2–17.0 5.8 5.7 (3.4–9.3) —</td>
</tr>
</tbody>
</table>

\(^a\) Exponentiated values of the mean and the asymmetric dispersion interval calculated from the logarithmic mean ± SD of GPA Vf.

\(^b\) All \( \Omega/N \) data.

\(^c\) Excluding \( \Omega/N \) Vf ≥ 30.

\(^d\) All N/N data.

\(^e\) Excluding N/N Vf ≥ 30.

Significant increases in either \( \Omega/N \) or N/N Vf were observed in the Estonian workers compared to the Estonian controls with or without the inclusion of “outlier” data defined as Vf ≥ 30. For the Latvian workers compared to the Latvian controls, a significant elevation of \( \Omega/N \) Vf, but not N/N Vf, was observed with and without the “outlier” data (\( P = 0.0011 \) and 0.0019, respectively). This observed difference is driven by \( \Omega/N \) Vf in the Latvian control group which are significantly lower than the \( \Omega/N \) Vf in the Estonian controls (\( P = 0.0020 \) and 0.0031 with and without the “outlier” data, respectively). No significant differences in GPA \( \Omega/N \) or N/N Vf were observed between the exposed workers from Estonia and Latvia. When the data from the cohorts were combined for comparison of all control workers to the control populations, the elevation in \( \Omega/N \) Vf in the workers approached significance (\( P = 0.064 \) and 0.058 with and without the inclusion of the “outlier” data, respectively). This apparent elevation is driven by the lower \( \Omega/N \) Vf observed in the Latvian control population; no significant elevation is apparent when the combined \( \Omega/N \) Vf are compared to the Estonian control population alone. No significant differences in N/N Vf were obtained with or without the inclusion of the “outlier” data.

When compared to the GPA Vf observed in the historical group of age-matched U.S. controls, no significant differences were seen in \( \Omega/N \) Vf for the Estonian control population with or without the inclusion of the “outlier” data; \( \Omega/N \) Vf for the Estonian cleanup workers were significantly elevated (\( P = 0.031 \)) with the “outlier” data excluded but not when all of the data were included (Table II). The \( \Omega/N \) Vf for the Latvian control population were significantly lower than the U.S. control population with and without the inclusion of the “outlier” data (\( P = 0.0088 \) and 0.017, respectively); \( \Omega/N \) Vf for the Latvian cleanup workers were not significantly different from those of the U.S. control population (Table II). The N/N Vf in the U.S. control population were not significantly different from the Latvian control population but were significantly elevated over the Estonian control population with and without the inclusion of the “outlier” data (\( P = 0.047 \) and 0.032, respectively), in the Estonian cleanup workers with and without the inclusion of the “outlier” data (\( P ≤ 0.0001 \) for both), in the Latvian cleanup workers with the “outlier” data excluded (\( P = 0.032 \)) and nearly so with all data included (\( P = 0.059 \)). When the data from the U.S. control population are combined with the Estonian and Latvian control populations, the \( \Omega/N \) Vf in the combined groups of Estonian and Latvian cleanup workers becomes significant with and without the inclusion of the “outlier” data (\( P = 0.0184 \) and 0.0064, respectively). Again, the significance is driven by the Latvian control population; there is no significant difference in \( \Omega/N \) Vf between these combined groups with the removal of these data.
The data were next tested for the level of association between the observed GPA \( V_f \) and the reported physical radiation doses. Univariate regressions of both $\theta/N$ and $N/N$ GPA \( V_f \) against subject age suggested significant increases with age in both the Estonian and Latvian cohorts. Therefore, to account for the potentially confounding effect of age in determining if a radiation dose response was present in the data, analyses of covariance were performed treating the GPA $\theta/N$ and $N/N$ \( V_f \) for Estonian and Latvian workers and control populations as single response variables, control compared to exposed groups as a discrete independent variable, and age and reported physical dose as continuous independent variables. As summarized in Tables II and III, these analyses both confirmed and extended the results of the univariate analyses. In the covariance model, no significant differences between the GPA $\theta/N$ or $N/N$ \( V_f \) for Estonian workers and the control population were obtained (Table II), although a marginally significant positive association between $\theta/N$ \( V_f \) and the reported physical doses was observed with and without the inclusion of the “outlier” data ($P = 0.023$ and $0.028$, respectively) (Table III). For the Latvian cohort, $\theta/N$ \( V_f \) were significantly elevated in the workers over the controls with and without the inclusion of the “outlier” data ($P = 0.0001$ and $< 0.0001$, respectively) (Table II), but no association between $\theta/N$ \( V_f \) and the reported physical doses was obtained for the Latvian workers (Table III). When the data for the two countries were combined, there was no significant effect of exposure on $N/N$ \( V_f \) (Tables II and III). The difference between worker and control $\theta/N$ \( V_f \) remained significant with and without the inclusion of the “outlier” data ($P = 0.017$ and $0.0069$, respectively) (Table II), but the association of $\theta/N$ \( V_f \) with the reported physical doses seen for the Estonian workers was lost (Table III).

In addition to these marginally significant effects of the radiation exposures, the model revealed highly significant associations of both $\theta/N$ and $N/N$ \( V_f \) with subject age. These adjusted linear regressions of age-related increases in GPA \( V_f \) were very consistent between the Estonian and Latvian cohorts and were both relatively insensitive to the effect of the “outlier” data (Table III). For both groups, $N/N$ \( V_f \) were seen to increase more steeply with age than $\theta/N$ \( V_f \). Very similar effects of age on the GPA $\theta/N$ and $N/N$ \( V_f \) were also observed in the matched historical U.S. controls (Table III). These age-related increases are quantitatively consistent with our observations in several other European and U.S. populations (19, 20; Bigbee et al., unpublished observations).

The calculated covariance analysis equations including the effects of dose and age on the combined GPA \( V_f \) were $\log \theta/N \ V_f = 0.46 + 0.0030 \text{(dose, cGy)} + 0.0065 \text{(age, years)}$ and $\log N/N \ V_f = 0.38 + 0.0026 \text{(dose, cGy)} + 0.0092 \text{(age, years)}$ with all data included and $\log \theta/N \ V_f = 0.48 + 0.0018 \text{(dose, cGy)} + 0.0057 \text{(age, years)}$ and $\log N/N \ V_f = 0.38 + 0.0010 \text{(dose, cGy)} + 0.0087 \text{(age, years)}$ with the “outlier” data excluded (Table III). The model predicted a geometric mean difference in $\theta/N \ V_f$ between the exposed workers and controls of 1.7 (Table III), which translates to a mean radiation dose estimate for the combined Estonian and Latvian workers of 6.8 cGy using the dose–response relationship of 25 radiation-induced $\theta/N$ erythrocytes/$10^6$ cells/Gy derived previously from studies of populations who sustained acute high-dose exposures to ionizing radiation (7, 8, 14). Linear regression of the nontransformed Estonian GPA $\theta/N \ V_f$ as a function of the reported physical dose estimates yields a slope of 6.0 and 4.7 radiation-induced $\theta/N$ erythrocytes/$10^6$ cells/Gy with and without the inclusion of the “outlier” data, respectively.

**DISCUSSION**

In published studies of Hiroshima atomic bomb survivors and individuals exposed at Chernobyl (7, 8, 14), the GPA locus somatic cell mutation assay has demonstrated
persistent increases in $\theta/NV_f$ that are associated with radiation dose in blood samples obtained years to decades after exposure with consistent dose responses of approximately $25 \times 10^{-6}$/Gy. Replicate blood samples, obtained from several months up to 7 years apart, from selected individuals in these study populations have also demonstrated that these radiation-induced elevations in $GPA \, V_f$ are stable with time (8, 14). Together these data demonstrate that the assay, in these circumstances of exposure, can be used on a population basis as a biodosimeter, providing a measure of past exposure to ionizing radiation.

The current study of Chernobyl cleanup workers from Estonia and Latvia exposed chronically to lower radiation doses did not consistently reveal significant differences in the $GPA \, V_f$ compared to the group-matched concurrent local or historical U.S. controls. Significant increases in $GPA \, \theta/N \, V_f$ for the Estonian and Latvian cleanup workers were observed compared to the Latvian control population, but this result is driven by significantly lower $V_f$ in this control group compared to both the Estonian and historical U.S. control populations. These results suggest the data for the Latvian control population are not typical of the population and/or the selected controls were not well matched for other factors that have an impact on $GPA \, \theta/N \, V_f$. A larger, and presumably more representative, cohort of controls from Latvia and/or Estonia would have been useful in this regard. However, given the logistic difficulties in sampling blood from persons on a large scale in Estonia and Latvia, the recruitment of large numbers of control subjects was not practical as part of the experimental design given the limited resources available for the study in Estonia and Latvia. The consistency of the results for the Estonian and Latvian cleanup workers compared to the larger set of group-matched historical U.S. controls argues that the $GPA \, V_f$ observed for these exposed populations were not substantially elevated over control values.

In many respects, the data from these Chernobyl cleanup worker populations closely resemble those from previous surveys of healthy unexposed groups. The ranges, medians, means and standard deviations of the observed $GPA \, \theta/N$ and $N/N \, V_f$ are very similar (6), as are the quantitative increases of both $GPA \, V_f$ with age (Table III). Finally, these study populations from Estonia and Latvia also contain comparable numbers of individuals with “outlier” $GPA \, V_f$ (Table II) with a similar dependence of occurrence on age as observed previously (Bigbee et al., manuscript submitted for publication).

In addition, there was not a consistent correlation between the $GPA \, V_f$ and the physical radiation dose estimates for the Estonian and Latvian cohorts. For the larger set of Estonian workers and controls, the analysis of covariance revealed a shallow and marginally significant association of $GPA \, \theta/N \, V_f$ with the physical dose estimates. The slope of the regression of the nontransformed data against the estimated doses is approximately one-fourth to one-third of that predicted by the results of prior studies of populations with acute high-dose exposures consistent with a lower biological effectiveness of the protracted exposures to the workers in this study. This association was not demonstrable in the data for the Latvian workers and control population, perhaps due to a greater fraction of inaccurate physical dose estimates as indicated by the larger number of Latvian cleanup workers with assigned zero doses (Fig. 1).

The failure of this study to consistently demonstrate radiation-induced increases in $GPA \, V_f$ in the Estonian and Latvian Chernobyl cleanup workers may be ascribed to several factors. The first involves the apparently low whole-body doses received together with uncertainties regarding the dose estimates. Unlike the previous studies involving much higher radiation doses received by Hiroshima atomic bomb survivors and Chernobyl accident victims, these cleanup worker populations received relatively low doses
that were apparently measured by physical monitoring methods or that were calculated from reconstructed environmental monitoring involving significant uncertainties. In addition, a significant fraction of the cleanup workers from Estonia and Latvia cannot be assigned physical dose estimates with confidence since military records are incomplete. Particularly among early entrants, when conditions were most chaotic and the potential for significant radiation exposures were the highest, not all workers were badgeg. These undocumented exposures are expected to be similar, however, for the Estonian and Latvian cohorts as the Soviet military mixed workers from different republics.

Despite the uncertainties associated with the recorded estimates of dose, the GPA analyses suggest that high doses were unlikely to have been received by many of the cleanup workers since we found no consistent differences between the exposed and control populations. This conclusion is consistent with the current understanding that only about 0.02% of all Chernobyl cleanup workers received doses exceeding 50 cGy (3). Given the sensitivity of the GPA assay, we would be unlikely to detect a biological effect with this biomarker if the mean dose to the population was of the order of 10 cGy. This appears to be the case for the cleanup workers in our study and indicates that the estimates of physical doses for the group, while perhaps incomplete and imprecise, cannot be rejected as inadequately characterizing the exposure experience; however, recorded doses for individual workers may have been reported inaccurately.

Second, the low doses received by these workers were predominantly protracted exposures at low dose rates. Numerous radiobiological studies, involving a number of experimental systems including specific locus mutation studies in mice, have demonstrated attenuated biological effects of equal radiation doses when delivered as fractionated compared to single doses or at low compared to high dose rates (2, 21). A recent study of the GPA response in populations exposed at Chernobyl includes a cohort of cleanup workers from Russia and Ukraine who were at the site the first few months after the accident and received protracted exposures estimated to range from 2 to 93 cGy (14). Analysis of the O/N Vf observed in these subjects yielded a much shallower linear dose response of approximately 4 × 10^{-6}/Gy (14). If the lower value of 4 × 10^{-6}/Gy is used as a more accurate estimate of the GPA dose response under the exposure conditions of this study, then we would have even less ability to detect a biological effect using this assay. This lower value is consistent with the dose regression of the GPA O/N Vf observed for the Estonian cleanup workers.

A main reason for conducting the current biodosimetry study was to learn whether large numbers of cleanup workers who were sent to Chernobyl shortly after the accident might have received substantial radiation exposure that was either undocumented or recorded inaccurately. The use of the GPA assay as a population biodosimeter in this context had the advantage of being relatively inexpensive, requiring only a small volume of peripheral blood, and presented few serious logistic problems associated with shipping and storage of samples. The disadvantages of the assay include the limitation of being applicable to only half the population with the required M/N phenotype, and the inherent biological variation in background Vf which limits the usefulness at low dose levels of approximately 10 cGy. The current analyses were unable to distinguish a consistent significant difference between the GPA Vf distributions of exposed Estonian and Latvian workers and controls. We can conclude that the physical doses were relatively accurate on a population level, that is with a mean dose not exceeding approximately 10 cGy, and/or that the protracted nature of the exposure was such that the increase in Vf induced by the exposure was much lower than anticipated from studies of acute exposures. The limited positive results of the study, consisting of the data from the Estonian population of workers and controls, support the latter hypothesis. Until ongoing biodosimetry studies of chromosomal translocations in peripheral blood lymphocytes are completed, it appears that physical dose estimates and reconstructions should be used in the assessment of possible health hazards and in calculations of statistical power for future epidemiological studies.

These results suggest the need for additional GPA studies of populations exposed to doses greater than 10 cGy that include accurate physical dosimetry to model and calibrate the low-dose, low-dose-rate response of the assay with confidence. Three such studies are currently under way involving nuclear fuel reprocessing personnel at Sellafield, UK, U.S. X-ray personnel and thyroid disease patients treated with \(^{131}\)I. In all three studies, the study subjects will have received protracted whole-body exposures to ionizing radiation generally below 1 Gy. The \(^{131}\)I study may prove to be the most definitive, as the clinical setting for this exposure allows for time series blood sampling of these patients prior to and after therapy, a design which will permit both the assessment of the temporal induction and long-term persistence of radiation-induced mutations as well as the estimation of the practical limit of sensitivity of the assay to detect radiation exposures when pre-exposure background levels of GPA Vf in the exposed subjects have been determined.

Additional studies are also under way which apply automated fluorescence in situ hybridization (FISH)-based stable chromosome translocation analysis in peripheral blood lymphocytes (22) as an independent biodosimetric measure of the exposures experienced by these Estonian and Latvian Chernobyl cleanup workers. A pilot ECP-7 study of stable chromosome aberrations scored by FISH in a sample of 62 Russian cleanup workers revealed no correlation with reported physical doses or with characteristics of work at Chernobyl consistent with low radiation exposures to these workers (3), and Lazutka, using standard cytogenetic methods, reported no significant overall increase in chromosome aberrations over controls in 183 cleanup workers from Lithuania with a mean dose estimate of 14 cGy, although ~20% had elevated frequencies of dicentric and ring chromosomes possibly related to radiation exposure (23).
contrast, in an independent study of Russian cleanup workers with similar physical dose estimates, Tucker et al. (24) have reported a highly significant difference in the frequency of stable aberrations between controls and exposed workers using this methodology, and Thomas et al. (25) have observed significantly elevated mutant frequencies at the HPRT locus in the peripheral blood lymphocytes of these workers. This elevation was driven predominantly by a reported twofold increase in the frequency of total gene deletion mutations in the Russian cleanup workers compared to controls. These studies will provide important additional data for calibration of the radiation doses received and biological effects of these exposures in Chernobyl cleanup workers. Official limits of individual radiation exposures were established for the cleanup workers and were progressively lowered in the years after the accident as work became less hazardous and more routine. These allowed limits were 25 cGy in 1986, 10 cGy in 1987 and 5 cGy in 1988 and following years (2), which is consistent with the mean doses reported for these cohorts. However, given the incomplete physical dosimetry for the cleanup worker populations and the inability to document the quality of the methods used to generate the recorded doses, it is important to assess these exposures accurately and independently using these biodosimetric methods.

Finally, the data obtained using these biodosimetric assays will be included as part of a comprehensive assessment of the potential exposures and health risks attendant to the Chernobyl experience of these cleanup workers from Estonia and Latvia together with a pilot study of workers from Lithuania. A detailed questionnaire has been developed and administered to these workers both to identify potential confounding host factors as well as to provide a detailed assessment of their work at Chernobyl including dates of entry, duration and nature of work, work in special zones or on the roof of the reactors, and construction of the sarcophagus (Tekkel et al., manuscript submitted for publication). These responses will be used to test associations with the biodosimetric data and to identify subpopulations of workers at high risk of exposure independent of estimates of physical dose.

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