Effect of Mustard Gas Exposure on Incidence of Lung Cancer: A Longitudinal Study

Mihoko Doi, Noboru Hattori*, Akihito Yokoyama, Yojiro Onari, Masashi Kanehara, Kenji Masuda, Tetsuji Tonda, Megu Ohtaki, and Nobuoki Kohno

* Correspondence to Dr. Noboru Hattori, Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551, Japan (e-mail: nhattori@hiroshima-u.ac.jp).

Initially submitted April 11, 2010; accepted for publication November 9, 2010.

Sulfur mustard, an agent used in chemical warfare, is an alkylating substance with carcinogenic potential. However, the precise long-term carcinogenic effects of mustard gas are unclear. Since 1952, the authors have conducted health surveys of former workers who were employed from 1929 to 1945 in a poisonous gas factory in Okunoo-jima, Hiroshima, Japan. This prospective study was undertaken from 1952 to 2005 to examine the incidence of lung cancer among the workers who were exposed to mustard gas (n = 480), lewisite (n = 55), and/or diphenylcyanarsine (n = 178), as well as the incidence among unexposed workers (n = 969). The stochastic relation between exposure and lung cancer was explored on the basis of multistage carcinogenesis by using an accelerated hazard model with a transformed age scale. Mustard gas exposure was found to transform the age scale for developing lung cancer. One year of exposure in subjects >18 years old at first exposure shifted the age scale down by 4.9 years and 3.3 years, respectively. On the basis of the long-term follow-up of former workers in the poisonous gas factory, the authors concluded that sulfur mustard decreased the age at which people were at risk of developing lung cancer and that the effect declined with aging.

carcinogens; cohort studies; inhalation exposure; lung neoplasms; mustard gas

Abbreviation: CI, confidence interval.
gas results in a poor substrate for the DNA repair system, and DNA alkylation seems to be primarily responsible for the mutagenic consequences of cellular exposure (6, 13). Tumorigenesis of sulfur mustard has been confirmed by an increasing occurrence of pulmonary tumors via inhalation and injection in laboratory mice (6, 14).

In Okuno-jima, the arsenical gases diphenylcyanarsine and lewisite were also manufactured. Arsenic is categorized as a respiratory carcinogen by the International Agency for Research on Cancer, and inorganic arsenic acid and its derivatives have been implicated as carcinogens of the skin and the respiratory system. In contrast to the evidence about mustard gas, however, the evidence that organic arsenicals, including lewisite, are carcinogenic, mutagenic, or teratogenic is very weak (14, 15).

For over 50 years, we have investigated and provided health care to the former workers employed in the poisonous gas factory in Okuno-jima. In the present study, to clarify the transitional effects of poison gases (mustard gas, lewisite, and diphenylcyanarsine) on the incidence of lung cancer, we conducted a prospective survey in which the risk of lung cancer was analyzed in the former factory workers who were exposed or unexposed to these gases.

MATERIALS AND METHODS

Study population and data collection

Because factory employment records were missing, subjects who worked in the poisonous gas factory were identified through the use of several alternative methods. Since 1966, questionnaires about work history at the factory have been distributed in a house-to-house canvass of the area where most of the former workers were thought to have lived, and spot announcements were repeated on television throughout Hiroshima Prefecture to urge former factory employees to contact the investigators. Workers who came forward were asked whether they had information about other persons who had worked at the factory. In addition, persons who were admitted to hospitals in the area surrounding Okuno-jima were asked whether they had worked at the poisonous gas factory. Since 1952, persons identified as having worked at the factory have been invited to receive a medical examination at least once a year at registered hospitals, and financial support for their medical care and/or living costs has been provided by the government based on the severity of their disorder due to poisonous gas exposure. The identified workers were registered, and complete records of their medical examinations were maintained in our department. Vital status and cause of death were determined by death certificates and notifications from the hospitals or public health authorities. As of March 31, 2005, there were a total of 6,851 subjects registered as former workers at the poisonous gas factory in Okuno-jima.

Study cohorts

Because birth-cohort trends in lung cancer varied considerably depending on gender and geographic area (16), we restricted the study sample to males who had not lived outside of Hiroshima Prefecture since retirement from the poisonous gas factory. Person-years were calculated using the period from the date of entry as a worker at the factory to the earliest of either March 31, 2005, the date of death, or the last day of follow-up. Only individuals who could be followed for >2 years were included in the cohort. Subjects were excluded if a diagnosis of lung cancer preceded the date of entry into the study. Information on smoking status was obtained from a clinical interview or chart review and was assessed repeatedly during the follow-up period. To analyze the effects of poisonous gas exposure and smoking on lung cancer incidence, subjects for whom information about smoking history was missing were excluded. Lung cancer morbidity rates were derived from clinical records, postmortem examinations, or notification from the hospital or public health authority. Moreover, the definition of lung cancer for purposes of our analysis was restricted to cases determined by pathologic confirmation by histologic or cytologic examinations; diagnoses based only on medical certificates or imaging were excluded. Tumor histology was determined using lung biopsy specimens obtained by using bronchoscopy, computed tomography-guided aspiration, or surgical resection.

Exposed and control groups

We had information on the job titles of the subjects who were identified as former workers at the poisonous gas factory in Okuno-jima. On the basis of this information, we first selected the subjects who directly engaged in manufacturing poisonous gases, called “manufacturers.” The manufacturers were categorized into 5 groups on the basis of the gases with which they dealt: 1) mustard gas, 2) lewisite, 3) both mustard gas and lewisite, 4) diphenylcyanarsine, and 5) other gases, such as hydrocyanic acid, phosgene, and chloracetophenone. Because only mustard gas, lewisite, and diphenylcyanarsine are established carcinogens, the exposed group was selected from the subjects categorized in groups 1, 2, and 4 and then classified into subcohorts consisting of subjects who were engaged in producing mustard gas, lewisite, or diphenylcyanarsine. The subjects categorized in group 3 were excluded from the exposed group. As the control group, we selected subjects from the manufacturers categorized in group 5 and former workers whose job titles were classified into activities other than manufacturing poisonous gases, as follows: carriers, construction workers, clerks, cleaning men, and medical staff. The ambient concentration of mustard gas in the factory is estimated to have ranged from 50 mg/m³ to 70 mg/m³ (12, 14). Furthermore, acid corrosion, equipment breakdown, and exhaust-fan troubles occurred frequently. A considerable number of workers seem to have continued working under these conditions, with constant or frequent exposure to small amounts of poisonous gases (12). In the present study, we assumed that the manufacturers had constant exposure to the poisonous gases during their employment, so we utilized duration of employment as a surrogate for level of exposure to poisonous gases.

Statistical analysis

Armitage and Doll (17) established the multistage model of carcinogenesis, which states that the accumulation of
mutations in target cells explains the age-time patterns of excess cancer. They considered cancer to be the end result of the accumulation in a normal cell of a critical number (k) of independent mutations through a series of intermediate states (17). We modified this basic multistage model to formulate an estimate of whether exposure to a specific mutagen shifts the age scale, that is, whether the exposure accelerates the age at risk of carcinogenesis (18, 19). The risk ratio between exposed and unexposed subjects increases in proportion to the intensity of exposure and decreases with time after exposure. This model conforms remarkably well to observations from cohort studies of the atomic-bomb survivors, miners with prolonged exposure to radon, and cigarette smokers who stopped smoking at various ages (20). In the present study, we applied the generalized Armitage-Doll multistage model (18, 19) in which age, duration of exposure, and age at first exposure are specified as effect modifiers and smoking status is treated as a main effect in the baseline rate model. When a subject begins at age a, the hazard of lung cancer at time t is expressed as the function $h(t|a, D) \propto (t - b - w + \beta_w D + \gamma C)^{k-1}$, where $\beta_w$ is the coefficient of the effect of gas exposure, $\gamma$ is the coefficient of smoking status $C$ (1, current or ex-smoker; 0, nonsmoker), and $w$ is the tumor growth time (the time from the final step in carcinogenesis to a clinically detected tumor). As suggested by Collins et al. (21), the number of cancer cells increases exponentially during division. However, as described in a previous report from our group (22), a certain proportion of cancer cells die in the process of tumor growth; therefore, in many deep-growth-type cancers, there is a quite large difference between the generation time (about 7 days) and the doubling time (1–3 months) of cancer cells. Judging from the data in the previous report, we set the doubling time of lung cancer cells as 2 months. We also defined a clinically detectable tumor in the lung as one having a diameter of 10 mm, which is considered to contain $2^{30}$ cancer cells. On the basis of these assumptions, we calculated the amount of time it takes a single cancer cell to proliferate into $2^{30}$ cells and concluded that $w$ was 5 years. The unknown parameters ($k, \beta_w, \gamma$) were estimated by maximizing a partial likelihood (23), and $k$ was optimized with the constraint that it be an integer value. The exposure accelerates the aging process, and the relative risk, the rate ratio between the exposed and control groups, is expressed as relative risk $= \{1 + (\beta_w D + \gamma C)/(t - b - w)\}^{k-1}$. The relative risk was considered to be significantly different from 1.0 when its 95% confidence interval did not include 1.0.

This study was approved by the Hiroshima University Ethics Committee.

**RESULTS**

**Study population and characteristics of the cohorts**

Figure 1 illustrates the subject selection process used in this study. To exclude the effects of gender and
environmental carcinogens such as air pollutants, we restricted the study population to 3,231 men who had never lived outside of Hiroshima Prefecture after retirement from the poisonous gas factory. Among them, 2,841 men met the criteria of having a follow-up time >2 years. Moreover, some subjects were excluded from the analysis because they were considered unclassifiable: 1 whose occupation at the factory was not verifiable, 86 who manufactured both mustard gas and lewisite, and 1,072 who may have had contact with poisonous gases (factory inspection, incineration, and destruction of the factory or disposal of poisonous gases after the war) but did not work in the manufacture of poisonous gases. Consequently, 1,682 subjects were assigned to the exposed and control groups. The exposed group included subjects who were engaged in manufacturing carcinogenic gases and was divided into 3 subcohorts: mustard gas (480 subjects), lewisite (55 subjects), and diphenylcyanarsine (178 subjects). The control group consisted of 969 subjects who were not engaged in the production of carcinogenic gases. Consequently, 1,682 subjects were assigned to the exposed and control groups. The exposed group included subjects who were engaged in manufacturing carcinogenic gases and was divided into 3 subcohorts: mustard gas (480 subjects), lewisite (55 subjects), and diphenylcyanarsine (178 subjects). The control group consisted of 969 subjects who were not engaged in the production of carcinogenic gases. The characteristics of each group are shown in Table 1. Age at employment was younger and duration of employment and length of follow-up were shorter in the exposed and control groups. The exposed group in- cluded subjects who were engaged in manufacturing carcinogenic gases and was divided into 3 subcohorts: mustard gas (480 subjects), lewisite (55 subjects), and diphenylcyanarsine (178 subjects). The control group consisted of 969 subjects who were not engaged in the production of carcinogenic gases. Consequently, 1,682 subjects were assigned to the exposed and control groups. The exposed group included subjects who were engaged in manufacturing carcinogenic gases and was divided into 3 subcohorts: mustard gas (480 subjects), lewisite (55 subjects), and diphenylcyanarsine (178 subjects). The control group consisted of 969 subjects who were not engaged in the production of carcinogenic gases. The characteristics of each group are shown in Table 1. Age at employment was younger and duration of employment and length of follow-up were shorter in the control group than in each of the exposed subcohorts (Table 1).

Smoking history was obtained from about 80% of the subjects. The proportion of subjects with unknown smoking history was higher in the cohort of workers exposed to mustard gas than in the control group (Table 3). Of the 77 incidences of lung cancer occurred in the mustard gas group had survived, but more than half of the subjects in the control group were alive. During follow-up, a total of 77 incidences of lung cancer occurred in the mustard gas (n = 39) and control (n = 38) groups. When the incidence was stratified by age at first employment (10-year strata), the rate of lung cancer was higher in subjects exposed to mustard gas than in the control group (Table 3).

Lung cancer incidence in 10-year intervals

Table 2 shows incidence rates and histologic classifications of lung cancers observed in each group in 10-year intervals from 1955 to 2005. In the control group, the incidence of lung cancer showed a marked increase in recent years; on the other hand, among the workers exposed to mustard gas, the incidence of lung cancer was apparently higher than in the controls in the early years and showed a slow increase in recent years (Table 2). Table 3 shows vital statistics and incidence rates of lung cancer assessed on March 31, 2005, in the subjects exposed to mustard gas and the control group, by age at first employment. As of March 31, 2005, only 17% of subjects in the mustard gas group had survived, but more than half of the subjects in the control group were alive. During follow-up, a total of 77 incidences of lung cancer occurred in the mustard gas (n = 39) and control (n = 38) groups. When the incidence was stratified by age at first employment (10-year strata), the rate of lung cancer was higher in subjects exposed to mustard gas than in the control group (Table 3).

Effects of gas exposure and smoking on the age scale

Under the assumption that malignancy occurs upon the kth mutation, we estimated the relative contributions of carcinogenic-gas exposure and smoking to the carcinogenic process of lung cancer. We analyzed the following 2 groups: the entire gas exposure group, which consisted of subjects exposed to mustard gas, lewisite, or diphenylcyanarsine, and the group of subjects exposed only to mustard gas. Using the multistage model of carcinogenesis modified with an accelerating effect, we assumed that an accumulation of 6 mutations was required in both the entire gas exposure group and the mustard gas only exposure group (Table 4). In the entire gas exposure group, the effect of gas exposure for 1 year was estimated to produce an increase in the age scale by 4.9 years (95% confidence interval (CI): 2.1, 8.2) and 2.9 years (95% CI: 0.7, 5.0) for subjects whose ages at first exposure were ≤18 or >18 years, respectively, after adjustment for attained age and smoking status. In the mustard-gas-only exposure group, the effect of gas exposure for 1 year was estimated to shift the age scale down by 5.2 years (95% confidence interval (CI): 2.1, 8.2) and 2.9 years (95% CI: 0.7, 5.0) for those ≤18 years of age at first exposure and 3.3 years (95% CI: 1.2, 5.5) for those >18 years of age at first exposure. On the other hand, the effect of smoking was estimated to shift the age scale by 26.2 years in the entire gas exposure group and 23.5 years in the mustard-gas-only exposure group after
adjustment for attained age and the effect of gas exposure. An analysis limited to subjects exposed to lewisite or diphenylcyanarsine could not be conducted because the numbers of subjects in those groups were too small. To illustrate the age-dependent effects of exposure to only mustard gas for 1 year on the incidence of lung cancer, we estimated the relation between attained age and relative risk for subjects whose ages at first exposure were \( \leq 18 \) or \( > 18 \) years (Figure 2). The estimated relative risk decreased with increasing attained age and was higher in the subjects whose age at first exposure was \( \leq 18 \) years.

**DISCUSSION**

In the present prospective study, we demonstrated that exposure to mustard gas accelerated the age at risk of developing lung cancer in former poisonous gas factory workers. The relative risk of lung cancer in those who were engaged in the manufacture of mustard gas decreased substantially with increasing age at start of exposure and with attained age, similar to what has been seen in the pattern of excess risk of solid cancer among the atomic-bomb survivors (20, 24).

In the multistage model of carcinogenesis for most cancers, accumulation of 5–7 mutations in a normal stem cell is thought to result in the development of malignant cells (25). In the present study, it was estimated that cancer results after the accumulation of 6 mutations (Table 4, k) in the former poisonous gas factory workers who suffered from lung cancer. This finding is supported by the presence of epithelial hyperplasia and dysplasia, as well as by the fact that lung cancer was frequently observed in the bronchial trees of the workers (5). These pathologic findings suggest the occurrence of multistep carcinogenesis beginning with hyperplasia, moving through metaplasia, dysplasia, and in situ carcinoma, and ultimately becoming invasive cancer (6). In addition, genomic instability in preneoplastic lesions and the presence of p53 mutations in lung cancers have been reported in the former workers (26). On the other hand, Tokuoka et al. (5) reported significant correlations between the incidence of atypical lesions and mustard gas exposure in a multivariate analysis, though the incidence of atypical lesions was also influenced significantly by age, smoking, and chronic bronchitis. In the present study, we explored the relative effects of exposure to the carcinogenic gases and smoking on the age-specific incidence of lung cancer. Exposure to mustard gas was shown to act as a mutagen with a long-term effect on the development of lung cancer that was related to cumulative exposure.

Regarding the association between mustard gas exposure in the former workers of the factory at Okuno-jima and the
Table 3. Vital Status and Incidence Rates of Lung Cancer as of March 31, 2005, by Age at Employment (Age at First Exposure), in Former Workers Employed From 1929 to 1945 in the Poisonous Gas Factory in Okuno-Jima, Hiroshima, Japan

<table>
<thead>
<tr>
<th>Age at First Employment, years</th>
<th>Subjects, No. of Cases</th>
<th>Alive</th>
<th>No. of Cases</th>
<th>Person Years</th>
<th>Lung Cancer</th>
<th>No. of Cases</th>
<th>Incidence Rate Per 1,000 Person Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–9</td>
<td>Mustard gas</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>9</td>
<td>8</td>
<td>454</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10–19</td>
<td>Mustard gas</td>
<td>167</td>
<td>71</td>
<td>7,448</td>
<td>12</td>
<td>1.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>664</td>
<td>459</td>
<td>32,287</td>
<td>30</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>Mustard gas</td>
<td>164</td>
<td>12</td>
<td>6,155</td>
<td>13</td>
<td>2.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>149</td>
<td>28</td>
<td>6,155</td>
<td>4</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>Mustard gas</td>
<td>113</td>
<td>0</td>
<td>3,341</td>
<td>13</td>
<td>3.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>107</td>
<td>5</td>
<td>3,811</td>
<td>4</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>Mustard gas</td>
<td>30</td>
<td>0</td>
<td>644</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>36</td>
<td>0/</td>
<td>1,145</td>
<td>0/</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>50+</td>
<td>Mustard gas</td>
<td>3</td>
<td>0</td>
<td>62</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1</td>
<td>0</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>Mustard gas</td>
<td>3</td>
<td>0</td>
<td>36</td>
<td>1</td>
<td>27.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Mustard gas</td>
<td>480</td>
<td>83</td>
<td>17,686</td>
<td>39</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>969</td>
<td>500</td>
<td>43,943</td>
<td>38</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Estimated Number of Mutations Required for Lung Cancer and the Accelerating Effects of Gas Exposure and Smoking on Aging Scale in a Prospective Study of the Effect of Poison Gas Exposure on Incidence of Lung Cancer, Hiroshima, Japan, 1952–2005

<table>
<thead>
<tr>
<th>Age at Employment,</th>
<th>Entire Gas Exposure</th>
<th>Mustard Gas Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k^a$</td>
<td>$\beta_a^b$ at age $\leq 18$ years</td>
</tr>
<tr>
<td></td>
<td>Year</td>
<td>95% CI</td>
</tr>
<tr>
<td>0–18 years</td>
<td>5.2</td>
<td>2.1, 8.2</td>
</tr>
<tr>
<td>$&gt; 18$ years</td>
<td>2.9</td>
<td>1.2, 5.5</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

*a* $k$ is the estimated number of mutations required for lung cancer.

*b* $\beta_a$ is the aging effect of gas exposure for 1 year in the subjects whose ages at the first gas exposure were $\leq 18$ years and $> 18$ years, and is adjusted for attained age and smoking status.

*c* $\gamma$ is the aging effect of the presence of the smoking history, and is adjusted for attained age and gas exposure.

Figure 2. Relative risk of lung cancer among former workers with mustard gas exposure in Hiroshima, Japan, by age at employment, 1952–2005.
Although ethnic and racial differences have been reported, smoking is the most well-established risk factor for lung cancer (27, 28). The effects of smoking vary depending on the amount and duration of smoking, age at starting or quitting smoking (29), and the product smoked (cigarettes, cigars, or pipes). We sought detailed smoking information from all subjects included in the cohort, but it was fragmentary and of limited accuracy except for presence or absence of smoking. Therefore, we limited our investigation to the effect of this simple variable. Smoking data were more frequently missing for subjects in the group exposed to mustard gas because subjects in the earlier period of the survey comprised many of those with mustard gas exposure, and smoking information from the medical chart was frequently insufficient in the earlier period. Because most subjects smoked, we reanalyzed the data by labeling as a "smoker" all subjects with missing smoking data; the result was essentially the same. In general, long-term smoking elevates the risk of lung cancer 10–30-fold over one’s lifetime compared with not smoking (30), and the occurrence of lung cancer in smokers rises in the middle- to late 40s (31). In the present study, smoking was found to independently shift the age scale of lung cancer occurrence down by 24–26 years in comparison with never smokers. When this result was referred to the accelerated hazard model on the transformed age scale used in the previous study that investigated the effects of smoking cessation on lung cancer mortality in a cohort of 1,000,000 subjects (20), it was estimated that smoking duration was about 30 years in that cohort.

There are several limitations to the present study. First, we failed to include workers who developed lung cancer in the early period after the start of the survey. This is mainly because of inability of the system to enroll former workers and is likely to have resulted in underascertainment of lung cancers. Second, our control group might also have been slightly exposed to poisonous gases because of the lack of industrial hygiene in the factories, which led to contamination by poisonous gases of the air, water, and soil around the factory (12). Thus, the effect of poisonous gases on the incidence of lung cancer in the exposed group might be underestimated. Moreover, we need to consider the unobserved interindividual variability arising from either exposure to carcinogens, such as mustard gas or smoking, and background characteristics, because the stochastic model we applied was based on an assumption of population homogeneity.

To the best of our knowledge, this is the first study to report a long-term effect of poisonous gas exposure on the incidence of lung cancer. In the Iran-Iraq conflict between 1980 and 1988, approximately 45,000 military and civilian casualties were associated with sulfur mustard gas, and not only acute consequences after inhalation but also a series of chronic destructive pulmonary sequelae have been reported (32). We hope that our present investigation will be beneficial for the future health care of all persons exposed to these chemical agents.

**ACKNOWLEDGMENTS**

Author affiliations: Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan (Mihoko Doi, Noboru Hattori, Yojiro Onari, Masashi Kanekura, Kenji Masuda, Nobuoki Kohno); Department of Hematology and Respiratory Medicine, Kochi University, Kochi, Japan (Akihito Yokoyama); and Department of Environmetrics and Biometrics, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan (Tetsuji Tonda, Megu Ohtaki).

This work was partly supported by a grant for the group conducting “Research on the Aftereffects and Prognosis of Poison Gas Injuries” from the Ministry of Health, Labour, and Welfare, Japan.

The authors thank all contributing medical staff who have provided health care to the former workers in the poisonous gas factory, especially Dr. Masato Yukutake.

Conflict of interest: none declared.

**REFERENCES**

13. Ludlum DB, Kent S, Mehta JR. Formation of O6-ethylthioethylguanine in DNA by reaction with the sulfur mustard, chloroethyl sulfide, and its apparent lack of repair by


