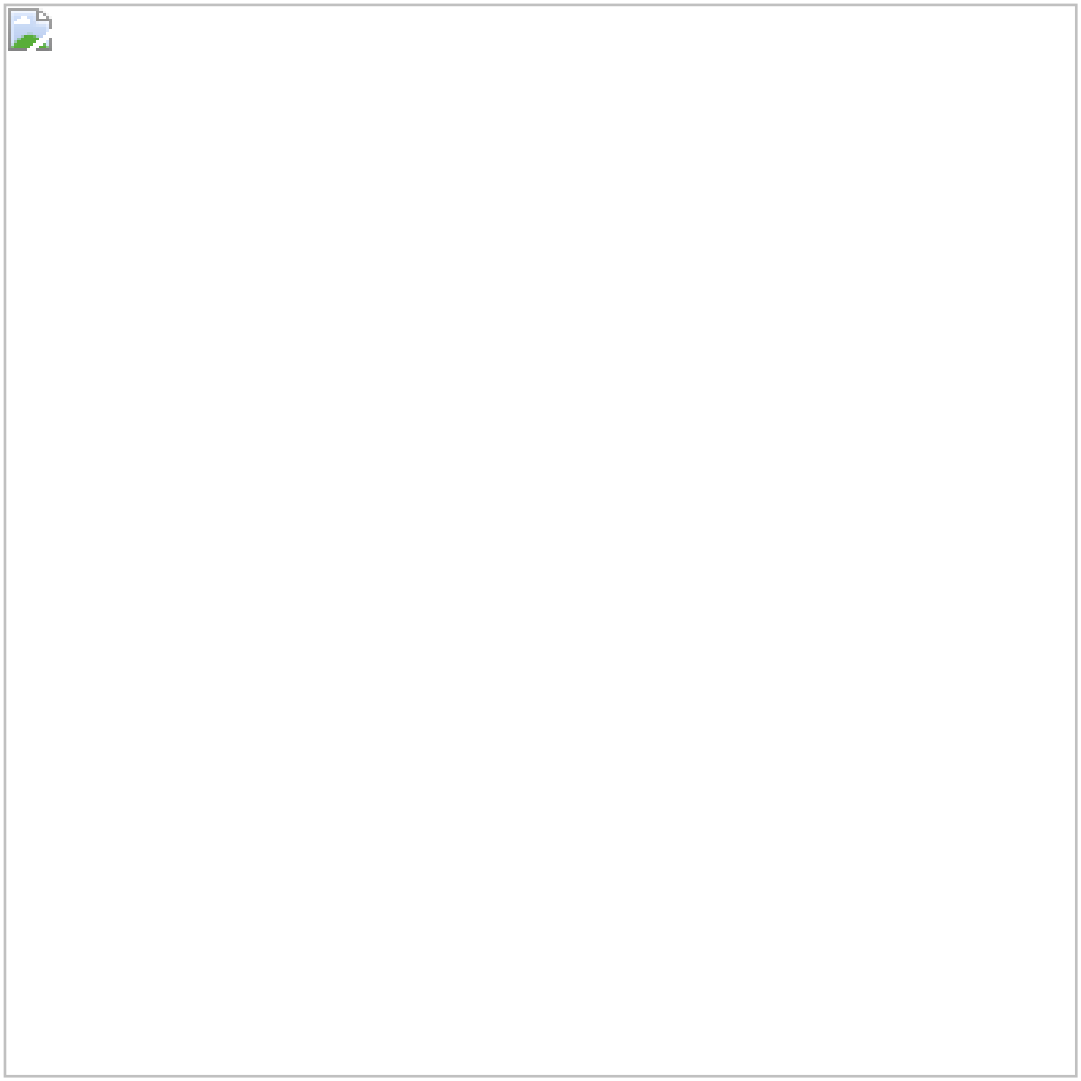

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Authors: Doi, Kazutaka, Kai, Michiaki, Suzuki, Keiji, Imaoka, Tatsuhiko, Sasatani, Megumi, et al.

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Estimation of Dose-Rate Effectiveness Factor for Malignant Tumor Mortality: Joint Analysis of Mouse Data Exposed to Chronic and Acute Radiation

Kazutaka Doi,[a,1] Michiaki Kai,[d] Keiji Suzuki,[e] Tatsuhiko Imaoka,[b] Megumi Sasatani,[f] Satoshi Tanaka,[g] Yutaka Yamada[c] and Shizuko Kakinuma[b]

[a] *Center for Radiation Protection Knowledge,* [b] *Department of Radiation Effects Research,* [c] *Department of Radioecology and Fukushima Project, Center for Advanced Radiation Emergency Medicine, National Institute of Radiological Sciences (NIRS), National Institutes for Quantum and Radiological Science and Technology (QST), Chiba, Japan;* [d] *Environmental Health Science Division, Oita University of Nursing and Health Sciences, Oita, Japan;* [e] *Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan;* [f] *Department of Experimental Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan; and* [g] *Department of Radiobiology, Institute for Environmental Sciences, Aomori, Japan*

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Uncertainties due to confounding factors in epidemiological studies have limited our knowledge of the effects of low-dose-rate chronic exposure on human health. Animal experiments, wherein each subject is considered to be nearly identical, can complement the limitations of epidemiological studies. Therefore, we conducted a joint analysis of previously published cancer mortality data in B6C3F1 female mice chronically and acutely irradiated with ^{137}Cs γ rays to estimate the dose-rate effectiveness factor. In the chronically irradiated animal experiment conducted by the Institute for Environmental Sciences, mice received irradiation at dose rates of 0.05, 1.1 or 21 mGy per day for 400 days from 8 weeks of age. For the acutely irradiated animal experiment conducted by the National Institute of Radiological Sciences, mice received irradiation at 35, 105, 240 or 365 days of age with 1.9, 3.8 or 5.9 Gy at a dose rate of 0.98 Gy per min. Because the preliminary analyses suggested that the risk was dependent on the age at exposure, a model was applied that considered risk differences depending on this factor. The model analysis revealed a three-fold, significantly decreased risk per Gy in mice exposed to 21 mGy per day compared to that in acutely irradiated mice. This resulted in a dose-rate effectiveness factor larger than that reported previously.

INTRODUCTION

The Fukushima Daiichi nuclear power plant accident prompted a crucial need to understand the effects of chronic exposure to low-dose-rate radiation. Although epidemiological studies of atomic bomb survivors have provided valuable information on the effects of radiation on cancer incidence and mortality, they focus on the health effects of a single, high-dose-rate, acute exposure to radiation (*1, 2*). To determine the effects of low-dose-rate radiation, long-term cohort studies, such as those of occupational radiation workers, are useful for obtaining risk estimates.

There are a number of published studies on the effects of low-dose-rate radiation. Compared to studies of atomic bomb survivors, the relative risk estimates for these studies varied widely; while some studies reported similar relative risks (*3*), others have reported risk estimate values several times larger or smaller (*4–6*). One possible explanation for this discordance is that it is difficult to adjust for the confounding factors of a subject population with various background factors. It has been reported that doses of nuclear workers were strongly correlated with smoking due to differences in job category (*7*). It is also possible that meta-analyses of dose-rate effectiveness factors (DREFs), in which the risk estimates from low-dose-rate epidemiological studies were compared with the corresponding risk estimates from atomic bomb survivors, did not completely adjust for differences in genetic factors and lifestyle between countries (*4–6*). Another possible cause is that the risks of low-dose-rate radiation are not considerably increased above the level of baseline risk and that the risks of low-dose-rate radiation can easily be masked by the failure to adjust for confounding factors. Therefore, the current knowledge on the effects of low-dose-rate radiation is still limited.

In contrast to epidemiological studies, genetic factors and lifestyle differences among animals can be considered as

[1] Address for correspondence: National Institutes for Quantum and Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan; email: doi.kazutaka@qst.go.jp.

nearly uniform. Therefore, animal experiments, wherein each subject is considered to be nearly identical, can complement the limitations of epidemiological studies. However, three conditions are important for accurate and precise risk evaluations of low-dose-rate radiation when analyzing animal experimental data. First, the data must include mice that have been exposed to both acute and chronic radiation to allow direct comparisons in the analysis. Second, the mice and conditions must be as similar as possible except for the dose rate of the irradiation. Third, the method of statistical analysis should allow the risk estimate to depend on the age at exposure, since radiation sensitivity is expected to change depending on age during long-term irradiation. In a recently published analysis of the dose and dose-rate effectiveness factor (DDREF), different analytical methods were used (*8*); its analytical methods and the criteria for the selection of studies for DDREF estimation were subsequently questioned (*9*). Thus, analysis of the effectiveness of low-dose-rate effects still requires clarification.

Historically, the low-dose effectiveness factor (LDEF) is the slope ratio of the straight lines at the higher and lower dose ranges by linear extrapolation; this value is typically larger than 1 due to the shape of the linear-quadratic dose response (*10*). The DREF is also the slope ratio of the straight lines from the linear-quadratic dose response from acute exposure and the linear dose response from chronic exposure. The DREF is incorporated into DDREF, assuming that the dose-response curve due to chronic exposure is linear and that the linear term of the acute dose-response curve is equal to that of the chronic dose response. However, these assumptions have been questioned (*11, 12*).

In Japan, the Institute for Environmental Sciences (IES) has conducted animal experiments on the effects of chronic exposure at low dose rates of 0.05, 1.10 and 21.00 mGy per day for 400 days (*13, 14*). However, DREF analysis was not conducted because no data on acutely exposed mice were included. The National Institute of Radiological Sciences (NIRS) has performed mice experiments after acute irradiation (*15*). In this study, the electronic archive data was created from the original experimental records.

For the current study, these two previously published data sets were combined, using B6C3F1 female mice that received chronic and acute ^{137}Cs γ-ray irradiation. Joint analysis was conducted to estimate the DREF, which is defined as the ratio of the risk of chronic exposure to the risk of acute exposure.

MATERIALS AND METHODS

Chronic Irradiation Data

Chronically irradiated animal experiments were performed by the IES to investigate the late effects of low-dose-rate radiation. The details of the experiment are described elsewhere (*13, 14*). Briefly, the study used specific-pathogen-free (SPF) B6C3F1 mice, generated as a hybrid of female C57BL/6JNrs and C3H/HeNrs. The animals were group-housed in plastic cages (5 animals/cage). Sawdust bedding, food pellets, water (chlorinated, 10 ppm), and equipment were sterilized before transfer into the facility. The irradiation and animal rooms were maintained at 21–25°C and 40–60% humidity with a 12:12 h light-dark schedule.

Three irradiation rooms were used and equipped with ^{137}Cs sources. Beginning at 8 weeks of age, mice were irradiated with ^{137}Cs γ rays at dose rates of 0.05, 1.10 or 21.00 mGy per day for 400 days from the beginning of irradiation, corresponding to total cumulative doses for each group of 0.02, 0.40 and 8.00 Gy, respectively. Each group comprised 500 mice, totaling 4,000 mice (eight groups; two categories for sex, and four categories for dose rate including control). Data on male mice were excluded from most of the analyses because the acute irradiation data included only female mice. The 2017 United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) report defines low-dose-rate irradiation as ''0.1 mGy per min, averaged over 1 h or less, for radiations such as external X rays and gamma rays''; all three irradiation conditions described above met this definition (*16*). Mouse irradiations were continued for 22 h per day; the remaining 2 h were used to clean the rooms and care for the animals. After receiving their predetermined total doses, the mice were transferred to the animal rooms. The mice in the control group were kept in the animal rooms with identical husbandry practices as the irradiated groups (the animal facility is environmentally controlled with the same light-dark schedule, ambient temperature, humidity and ventilation). The cages were inspected twice daily (morning and afternoon) on weekdays and once daily on weekends and holidays. All mice were followed up until they died spontaneously, and a complete necropsy was performed as soon as possible after each death based on a standard protocol. Necropsies included gross and microscopic pathological examinations to identify neoplasms and non-neoplastic lesions and to determine the causes of death (CODs). The neoplasms and non-neoplastic lesions were considered fatal if they compromised vital functions. A neoplasm was designated as ''fatal'' when it was considered to be directly or indirectly responsible for the animal's death (COD); alternatively, a neoplasm designated as ''incidental'' was judged not to have caused the death of an animal in which death resulted from an unrelated cause (*13*). The current study defined deaths with the above-mentioned fatal neoplasms as cancer death.

The animal experiments were conducted according to legal regulations in Japan and the guidelines for animal experiments of the IES.

Acute Irradiation Data

Acute irradiation animal experiments were performed by the NIRS to investigate the age dependence of sensitivity to the induction of various types of neoplasms. Details on the procedures are described elsewhere (*15*). Briefly, the study used first-generation hybrid mice between C57BL/6JNrs and C3H/HeNrs strains (B6C3F1). All mice were allowed to live out their entire life spans in the animal rooms at SPF conditions. The animal rooms were maintained at 22–24°C and 45–55% humidity with a 12:12 h light-dark schedule. Three to five mice were housed in aluminum cages with hardwood chip bedding. The mice were provided with a pellet diet and chlorinated water (pH 2.8–3.0) available *ad libitum*.

Female B6C3F1 mice received ^{137}Cs γ-ray irradiation at a dose rate of 0.80–0.98 Gy per min at 2–4 pm. The control group did not undergo sham irradiation. The doses were 1.9, 3.8 and 5.7 Gy at 35 and 105 days, and 3.8 Gy at 240 and 365 days of age. Each irradiated group comprised approximately 80 mice (80, 84 and 84 mice in the 1.9, 3.8 and 5.7 Gy groups at day 35; 81, 80 and 83 mice in the for 1.9, 3.8 and 5.7 Gy groups at day 105; and 85 and 82 mice in the 3.8 Gy groups at days 240 and 365, respectively), with 194 mice in the control group.

The mouse cages were checked for dead animals once daily, six days per week. Upon natural death the mice were necropsied, and gross findings recorded as photographs. Suspicious neoplasms and

non-neoplastic lesions were examined histologically, and the primary cause of death of each mouse was assessed.

The experiment was conducted according to legal regulations in Japan and following the Guidelines for Animal Experiments of the National Institute of Radiological Sciences.

Organization of the Combined Data

Chronic and acute irradiation data were combined and analyzed using the statistical models described below. Although several types of neoplasms were recorded as the main cause of death in the original experiments, the current analyses considered only cancer death.

The combined data included information about the time to event, type of event and radiation dose per day. The time to event was recorded as the postnatal age (days after birth), while the type of event was categorized as cancer death, non-cancer death, or other cause of death such as accidental death. In this analysis cancer deaths were treated as events; all others were treated as censoring. To apply the novel statistical method described below, the dose data were treated separately and doses were recorded for each day. For example, for mice irradiated chronically at a dose rate of 21 mGy per day, the value of 0.02 (21 mGy per day irradiation for 22 h) was continuously recorded from 56 to 455 days; similarly, for mice irradiated acutely with 3.8 Gy at day 105, the value of 3.8 appeared at day 105 while the rest of the dose data were 0.

Statistical Analysis Models

The statistical models used for the analysis of data on times until cancer death include the Poisson regression model. The analyses of data on atomic bomb survivors generally applied Poisson regression models; these model the number of events per person-years (also called a hazard) (*1, 2, 17*). Because the risks from radiation are usually included in the model as relative excess measures compared to the baseline risk in radiation epidemiology (*18*), modeling of the baseline risk is important for the estimation of the risks from radiation. The software implementation is relatively easy (iterative weighted least squares procedure) because the Poisson regression model is classified as a generalized linear model like the logistic regression model (*19*) and has been widely used since the 1970s (*20*).

Another candidate for statistical models is the Cox regression model, also known as survival analysis, in which the hazard is modeled as in Poisson regression models (*21*). One of the advantages of Cox regression models is that it is not necessary to assume any particular form for the time-dependent part of the hazard (*22*). Cox regression models using the attained age as a time scale provide the most flexible control for attained age effects while avoiding the need to include the effect of attained age (*23, 24*). Therefore, to focus on the modeling of DREF without complex adjustments for attained age in the baseline risk, the current study used Cox regression models.

Statistical Analysis Approaches

This study jointly analyzed chronically and acutely irradiated mice data to estimate DREF. By analyzing the combined data with the same statistical model, the DREF and its confidence interval could be directly estimated.

Before analyzing the combined data, a preliminary analysis was performed in which the nonirradiated mice in the two data sets were compared to check the validity of the joint analysis of the combined data. Kaplan-Meier survival curves were plotted for each dose group in the preliminary analyses. In addition to the analyses of the control mice, Cox regression models including only group variables as covariates were applied to estimate the hazard ratio and excess relative risk (ERR) for each group. These ERR estimates can be considered the non-parametric estimates of the ERR for each group compared to the complex modeling described below. The final statistical model was selected based on the information obtained in the preliminary analyses. To examine the possibility of extrapolating the results to male mice, the hazard ratio for each dose group was also obtained separately from chronically irradiated male mice data. Due to the lag time in the dose used in a previously published study (*25*), several candidate lag time values were compared in terms of model fitting.

Statistical Methods to Assess the Effects of Cumulative Dose with Dependence on Age at Exposure

Previously published analyses of data from chronic radiation exposure usually summed radiation doses as the ''cumulative dose'' included in the statistical models as a covariate. The inclusion of the cumulative dose as a covariate implicitly assumes that the risks from exposure in different periods are equal throughout the study period. Therefore, to relax this implicit assumption of cumulative doses, a statistical method for considering the effect of age at exposure was applied to the combined data.

In the joint analyses, Cox regression models with time-dependent covariates were used to handle the effect of cumulative dose depending on age at exposure. We assumed that the subjects were continuously exposed to radiation, and the objective of the analysis was to characterize the relationship between cumulative dose and cancer death. The dose for each subject was assumed to be measured annually at a discrete time point j. Let the dose for subject i ($i = 1 \ldots N$) at time point j ($j = 1 \ldots T$) be d_{ij}. Its measurement time t_j is assumed to be the same for all subjects. Let $\xi(t)$ be the set of time points j where t_j is equal to or smaller than t. Here we consider the baseline covariate vector x_i for each subject.

To model the risk of chronically irradiated mice, one candidate hazard function in the Cox regression models with time-dependent covariates is as follows:

$$\lambda_i(t) = \lambda_0(t)\left(1 + \beta_1 \sum_{j \in \xi(t)} d_{ij}\right), \tag{1}$$

where $\lambda_0(t)$ is the baseline hazard function, and β_1 is the parameter for risk increase due to cumulative dose. The timescale t is days after birth. The radiation risks are usually modeled as relative excess measures compared to the baseline risk in radiation epidemiology (*18*), often referred to as ERR. β_1 is interpreted as the ERR per unit dose, such as Gy in radiation.

To consider the effects of cumulative exposure with dependence on age at irradiation, we introduced a weight function. In this weight function, the hazard function for subject i at time point j can be further modeled as follows:

$$\lambda_i(t) = \lambda_0(t)\left(1 + \beta_1 \sum_{j \in \xi(t)} w(j;\boldsymbol{\alpha}) d_{ij}\right), \tag{2}$$

where $w(j;\boldsymbol{\alpha})$ is a weight function for subject i at time point j with parameter vector $\boldsymbol{\alpha}$. In the current study, an exponential function with linear and quadratic terms of the age at exposure variable was assumed as the weight function for subject i at time point j. $w(j;\boldsymbol{\alpha})$ was then calculated as $w(j;\boldsymbol{\alpha}) = \frac{T \cdot \exp\left(\alpha_1 t_j + \alpha_2 t_j^2\right)}{\sum_{j=1}^{T} \exp\left(\alpha_1 t_j + \alpha_2 t_j^2\right)}$ with the restriction that $\sum_{j=1}^{T} w(j;\boldsymbol{\alpha}) = T$, so that the special case in which $w(j;\boldsymbol{\alpha}) = 1$ for all j can be regarded as conventional cumulative dose analyses. The estimate of β_1 can be interpreted as the ERR per weighted cumulative dose for a subject at the age at which the weight is equal to 1. The age can then be obtained by solving the following equation for t:

$$\frac{T \cdot \exp(\alpha_1 t + \alpha_2 t^2)}{\sum_{j=1}^{T} \exp\left(\alpha_1 t_j + \alpha_2 t_j^2\right)} = 1. \tag{3}$$

Statistical Analysis Models for the Combined Data

For the analysis of the combined data, the model described above [Eq. (2)], was extended, and the hazard function was modeled as follows:

$$\lambda_i(t) = \lambda_s(t)\left\{1 + \rho\left(\sum_{j\in\xi(t)} w(j;\boldsymbol{\alpha})d_{ij};\boldsymbol{\beta}\right)\exp\left(\beta_3 I_{1,i} + \beta_4 I_{2,i} + \beta_5 I_{3,i}\right)\right\}, \tag{4}$$

where $\lambda_s(t)$ is the baseline hazard function, stratified for facility s (IES or NIRS). More flexible adjustments were possible by stratified analysis rather than modeling the facility parametrically. $\rho(\Sigma_{j\in\xi(t)} w(j;\boldsymbol{\alpha})d_{ij};\boldsymbol{\beta})$ describes the shape of the dose-response function with parameter vector $\boldsymbol{\beta}$. The following linear and linear-quadratic dose responses were assumed in the analyses:

$$\rho\left(\sum_{j\in\xi(t)} w(j;\boldsymbol{\alpha})d_{ij};\boldsymbol{\beta}\right) = \beta_1 \sum_{j\in\xi(t)} w(j;\boldsymbol{\alpha})d_{ij}, \tag{5}$$

$$\rho\left(\sum_{j\in\xi(t)} w(j;\boldsymbol{\alpha})d_{ij};\boldsymbol{\beta}\right) = \beta_1 \sum_{j\in\xi(t)} w(j;\boldsymbol{\alpha})d_{ij} + \beta_2\left\{\sum_{j\in\xi(t)} w(j;\boldsymbol{\alpha})d_{ij}\right\}^2, \tag{6}$$

where $I_{1,i}$, $I_{2,i}$, and $I_{3,i}$ are the indicator variables for groups with dose-rate values of 0.05, 1.1 and 21 mGy per day, respectively, and β_1, β_2, β_3, β_4, β_5, α_1 and α_2 are the parameters to be estimated. The applied models included a total of four combinations. The breakdown of dose-response curves revealed two types: a straight line and a linear-quadratic, and two cases with or without consideration of age sensitivity. The ERR term includes the effect modification term by dose rate [$\exp(\beta_3 I_{1,i} + \beta_4 I_{2,i} + \beta_5 I_{3,i})$] and the DREFs for each dose rate were estimated as $1/\exp(\beta_3)$, $1/\exp(\beta_4)$ and $1/\exp(\beta_5)$, respectively. The goodness-of-fit of the models was assessed using the Akaike Information Criterion (AIC) (*26*).

Estimation of Parameters in Cox Regression Models

Estimation of the statistical models described above was difficult using standard statistical software; therefore, a program was written to calculate the partial likelihood of Cox regression models. When maximizing the partial likelihood, the parameter of the weight function was also obtained such that special processing such as separately estimating only the weight function in advance was not required. The maximization of partial likelihood utilized the nlm and optim functions of the R statistical software. The partial likelihood programs were validated by comparing the results of the written program to those of the coxph function, which is the most standard function used to execute Cox regression models in R.

The standard errors of the estimated parameters were obtained using the Hessian matrix calculated during the nonlinear optimization procedure. The Wald confidence interval was obtained from the standard error for each parameter.

RESULTS

Preliminary Analyses

The Kaplan-Meier survival curves for the control groups from the IES and NIRS data are shown in Fig. 1. Although the survival curves from the control groups were similar, the survival curve for IES was slightly lower than that of NIRS in the earlier period, while the opposite trend was observed in the later period. Therefore, the facility was adjusted by stratification, and the baseline hazard function was stratified by the facility in the subsequent analyses.

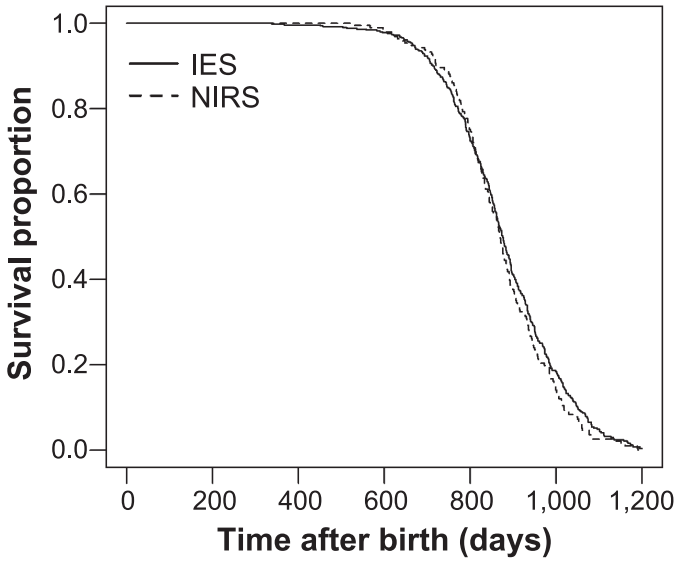

FIG. 1. Kaplan-Meier curve of the control group data from the Institute for Environmental Sciences (IES) and the National Institute of Radiological Sciences (NIRS).

The hazard ratio estimates for each group were obtained from the Cox regression models and are presented in Table 1. Because this model was executable using both the written program and the coxph function in R, the results for both are presented. The results were almost identical; thus, the written program results were validated via this comparison.

The hazard ratio estimates for each group were obtained separately for female and male mice, and are shown in Table 2. The hazard ratios for 0.05 and 1.1 mGy/day in male mice were larger than those in female mice, and vice versa for 21 mGy/day. Although the trends showed slight differences, the estimates did not differ significantly between male and female mice.

Examination of dose lag time showed that the model without lag time fitted best. Therefore, the doses were not lagged in the analyses in this study.

Statistical Analyses of the Combined Data

The parameter estimates of the statistical analyses for the combined data are presented in Table 3. The dose-response curves in the models that included age at exposure effects (AAEE) varied depending on the age at exposure. The parameter estimates (β_1, β_2) in Table 3 represent the dose-response curves for the specific age at exposure where the weight equals 1. The models with linear-quadratic dose response fitted better than those with linear dose response in both models with and without consideration of AAEE. The best-fit model was the linear-quadratic dose response with AAEE consideration in terms of the AIC. The statistical models with AAEE showed an estimate of the dose-rate parameter for 0.05 mGy/day that was larger than 0,

TABLE 1
Hazard Ratio Estimates for Each Group in Cox Regression Models with Group Categorical Variables

	Written program			coxph function in R		
Parameter	Estimate	95% CI		Estimate	95% CI	
0.05 mGy/day	1.004	0.880	1.146	1.004	0.880	1.146
1.1 mGy/day	1.001	0.877	1.143	1.001	0.877	1.143
21 mGy/day	2.315	2.027	2.645	2.316	2.027	2.645
1.9 Gy at 35 days	1.957	1.492	2.567	1.957	1.492	2.567
1.9 Gy at 105 days	1.542	1.184	2.007	1.542	1.184	2.007
3.8 Gy at 35 days	4.587	3.488	6.033	4.587	3.488	6.033
3.8 Gy at 105 days	2.478	1.882	3.264	2.478	1.881	3.265
3.8 Gy at 240 days	1.869	1.419	2.461	1.869	1.419	2.461
3.8 Gy at 365 days	1.431	1.096	1.869	1.431	1.096	1.869
5.7 Gy at 35 days	10.166	7.556	13.678	10.166	7.556	13.678
5.7 Gy at 105 days	4.730	3.594	6.225	4.730	3.594	6.225
AIC	32074.8			32074.8		

suggesting a slight risk increase for this dose compared to that for acute exposure. In contrast, the estimated dose rate for 1.1 mGy/day irradiation was smaller than 0, suggesting a risk decrease for this dose. However, the confidence intervals for both parameters were very wide; thus, no clear conclusion can be suggested from this analysis.

The estimated dose-rate parameter for 21 mGy/day from the linear-quadratic dose response with AAEE was −1.1 (95% confidence intervals (CI): −1.6, −0.60), suggesting that the risk for irradiation with 21 mGy/day was 0.33 (95% CI: 0.20, 0.55) times lower than that for acute irradiation. By taking the reciprocal of these values, the value of DREF was obtained as 3.0 (95% CI: 1.8, 5.1). The model with linear-quadratic dose response without AAEE fitted better than the model with linear dose response. The estimated dose-rate parameter for 21 mGy/day was −1.7 (95% CI: −2.6, −1.4), 0.18 times (95% CI: 0.073, 0.25) that of acute irradiation. The estimated DREF was, therefore, 5.7 (95% CI: 4.0, 8.0), higher than that estimated by the model with AAEE.

The estimated weight functions from the linear and linear-quadratic dose-response functions are shown in Fig. 2. Although the trends of the weight functions from the dose-response models were similar, the change in weight function from the linear dose-response model was larger than that from the linear-quadratic dose-response model.

To clarify the nature of the new statistical method that considered the effect of age at exposure, the fitted dose-response curves from the models with and without AAEE were compared to the non-parametric estimates of the ERR

TABLE 2
Hazard Ratio Estimates for Each Group in Cox Regression Models with Group Categorical Variables

	Female			Male		
Parameter	Estimate	95% CI		Estimate	95% CI	
0.05 mGy/day	1.004	0.880	1.146	1.052	0.922	1.200
1.1 mGy/day	1.001	0.877	1.143	1.124	0.986	1.282
21 mGy/day	2.316	2.027	2.645	1.897	1.661	2.167

obtained from the acutely irradiated data, which were considered to be the least dependent on the model. From the estimate shown in Table 2 and the estimated weight functions, the fitted dose-response curves for acute radiation at days 35, 105, 240 and 365 were calculated and plotted with the non-parametric estimates of the ERR obtained from acutely irradiated mice data (Figs. 3–6). The non-parametric estimates of the ERR obtained from the chronically irradiated mice data were also plotted with a simple cumulative dose and weighted cumulative dose. To make the weighted doses comparable to the acute irradiation dose, they were divided by the weight at days 35, 105, 240 and 365 of irradiation. These divided weighted doses can be interpreted as corresponding doses if they were delivered to mice with constant sensitivity at days 35, 105, 240 and 365 of age. Similarly, the fitted ERR estimate curves at 3.8 Gy were calculated for different days at irradiation and were plotted with the non-parametric estimates of the ERR obtained from acutely irradiated mice data (Fig. 7). In addition to the AIC, the goodness-of-fit of the models was compared visually between the fitted ERR estimate from the models and the non-parametric ERR estimates.

Comparison of the fitted dose-response curves and the non-parametric estimates of the ERR for irradiation at days 35 and 105 (Figs. 3 and 4) suggested that the model with the linear-quadratic dose response with AAEE (solid line) fitted better than the linear dose response including sensitivity (dashed line). The model with linear-quadratic dose response with AAEE (solid line) fitted fairly well to the data for irradiation at days 35, 105, 240 and 365 (Figs. 3–6, respectively). Conversely, the model with linear-quadratic dose response without AAEE (dotted line) fitted poorly to the irradiation data at days 35 (Fig. 3), 240 (Fig. 5) and 365 (Fig. 6) but did fit to the data for irradiation at day 105 (Fig. 4). The model with linear dose response without AAEE (dotted and dashed line) fitted poorly to all irradiation data at days 35, 105, 240 and 365 (Figs. 3–6, respectively). A comparison between the fitted ERR estimate curves at 3.8 Gy and the non-parametric estimates of the ERR (Fig. 7)

TABLE 3
Parameter Estimates of the Statistical Analyses for the Combined Data

	Statistical models with age sensitivity consideration						Statistical models without age sensitivity consideration					
	Linear			Linear quadratic			Linear			Linear quadratic		
Dose response	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI	
Linear term in ERR (β_1)[a]	0.326	0.220	0.432	0.037	–0.112	0.185	0.461	0.339	0.584	–0.033	–0.228	0.162
Quadratic term in ERR (β_2)[a]				0.058	0.020	0.096				0.110	0.065	0.155
0.05 mGy/day (β_3)	0.138	–19.033	19.310	0.132	–34.647	34.912	–0.782	–31.399	29.834	0.134	–17.748	18.016
1.1 mGy/day (β_4)	–5.542	–29.943	18.859	–4.576	–24.102	14.950	–5.535	–29.456	18.386	–4.575	–38.535	29.384
21 mGy/day (β_5)	–0.630	–1.037	–0.222	–1.110	–1.622	–0.597	–1.075	–1.410	–0.740	–1.738	–2.079	–1.396
AIC	32,092.4			32,067.1			32,175.3			32,155.1		

[a] These estimates are calculated at the age of 171.76 and 177.34 for linear and linear-quadratic dose response models with age sensitivity consideration.

showed similar fits for the models with both linear and linear-quadratic dose response.

DISCUSSION

Study Significance

The comparison of risks between acute and chronic radiation exposures is an important topic in terms of radiation protection and has received particular attention in recent years; several studies in the U.S. and Europe, using animal experimental data have been published recently (*25, 27*). Although several studies have assessed DREF and DDREF, most of them assume some dose-response structures based on the knowledge of radiation biology and define DREF as a function of parameters in the dose-response curves. One novelty of the current analyses is that the DREF was modeled directly in the model, assuming common dose-response curves for both acute and chronic irradiation. This study adds new findings to the body of literature on this topic using a different statistical modeling approach and independent data from Japan.

The other novelty of the analyses is that the statistical analysis modeled both the dose rate and AAEE. To our knowledge, the effect of the age at exposure has not been accounted for appropriately in the analyses of animal experimental data to estimate DREF. Without AAEE adjustment, the effects of differences in dose rate and in age at irradiation are mixed as a difference between the risk of chronic and acute irradiation, which will result in a biased DREF estimate. The analyses in the studies on nuclear workers examined the temporal effects on cancer risk and adjusted for the age effects in the risk estimates by calculating the cumulative covariate within a time span separately after defining the number of time spans (*28, 29*).

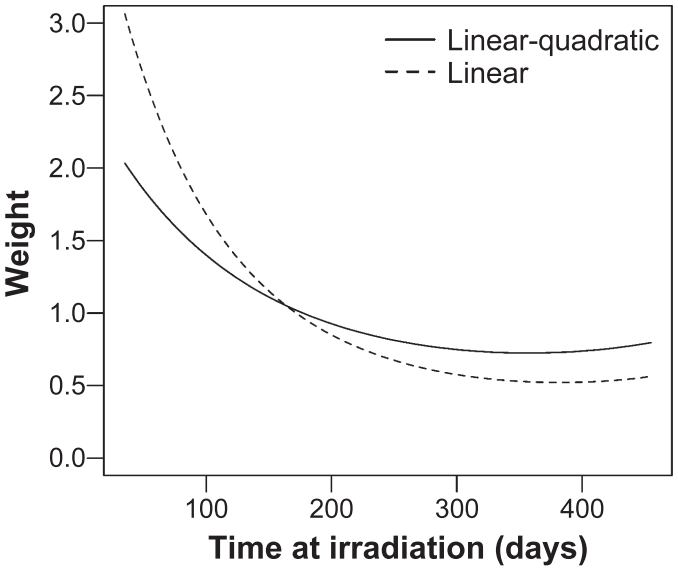

FIG. 2. Estimated weight function from linear and linear-quadratic dose-response models.

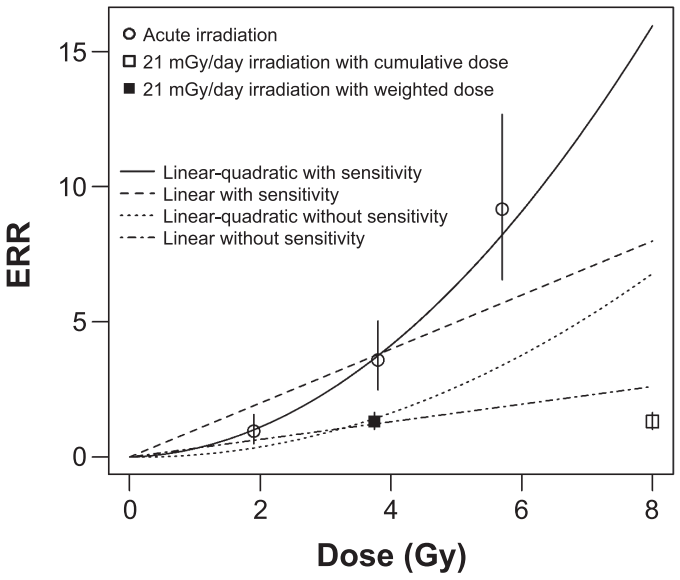

FIG. 3. Fitted dose-response curves for acute irradiation at 35 days with non-parametric excess relative risk (ERR) estimates of acute and chronic irradiation with cumulative and weighted dose. Circles and squares with bars indicate the non-parametric ERR estimates and 95% confidence intervals of data from acutely and chronically irradiated mice.

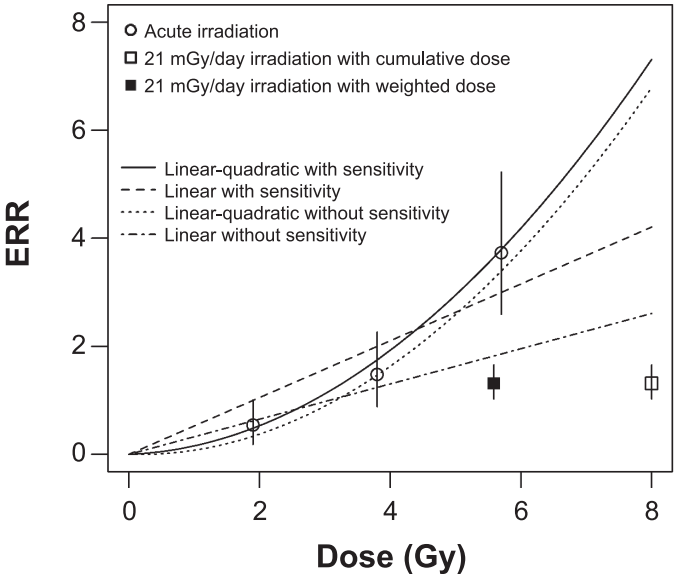

FIG. 4. Fitted dose-response curves for acute irradiation at 105 days with non-parametric excess relative risk (ERR) estimates of acute and chronic irradiation with cumulative and weighted dose. Circles and squares with bars indicate the non-parametric ERR estimates and 95% confidence intervals of data from acutely and chronically irradiated mice.

These approaches increased the complexity of both the analysis and interpretation of the results due to pre-analysis data handling, instability of the estimation due to the increased number of parameters, and multiple estimates of DREF for each time span. Our newly proposed statistical model avoids these complexities and provides results that are easy to interpret.

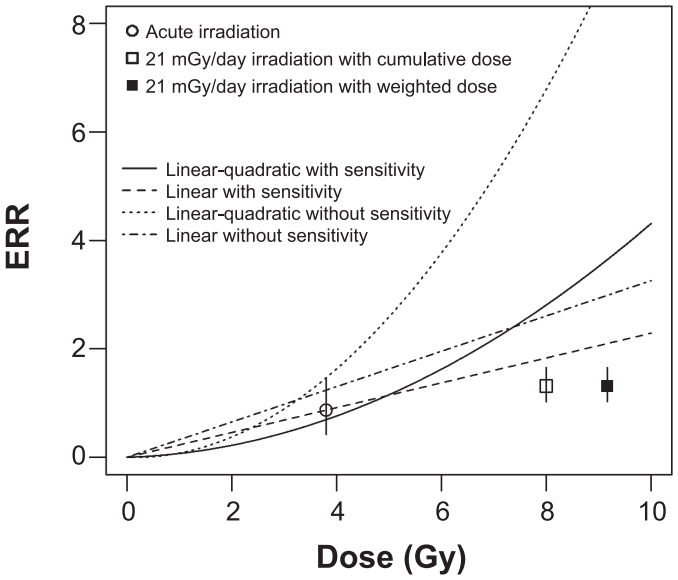

FIG. 5. Fitted dose-response curves for acute irradiation at day 240 with non-parametric excess relative risk (ERR) estimates of acute and chronic irradiation with cumulative and weighted dose. Circles and squares with bars indicate the non-parametric ERR estimates and 95% confidence intervals of data from acutely and chronically irradiated mice.

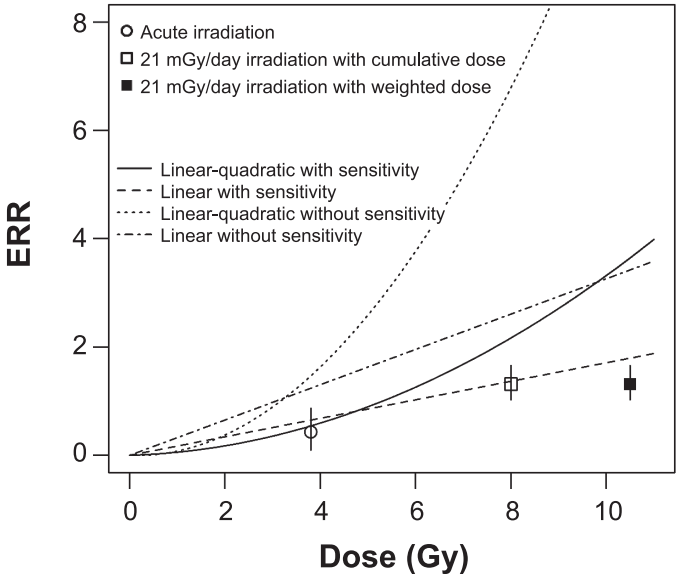

FIG. 6. Fitted dose-response curves for acute irradiation at day 365 with non-parametric excess relative risk (ERR) estimates of acute and chronic irradiation with cumulative and weighted dose. Circles and squares with bars indicate the non-parametric ERR estimates and 95% confidence intervals of data from acutely and chronically irradiated mice.

Interpretation of the Results of the Statistical Analysis Models

The change in the estimated weight function of the linear dose-response model was larger than that of the linear-quadratic model (Fig. 2). This difference in weight functions could be attributed to overfitting of the model with the linear dose response. Because the cumulative doses are correlated with days after birth, the quadratic term that

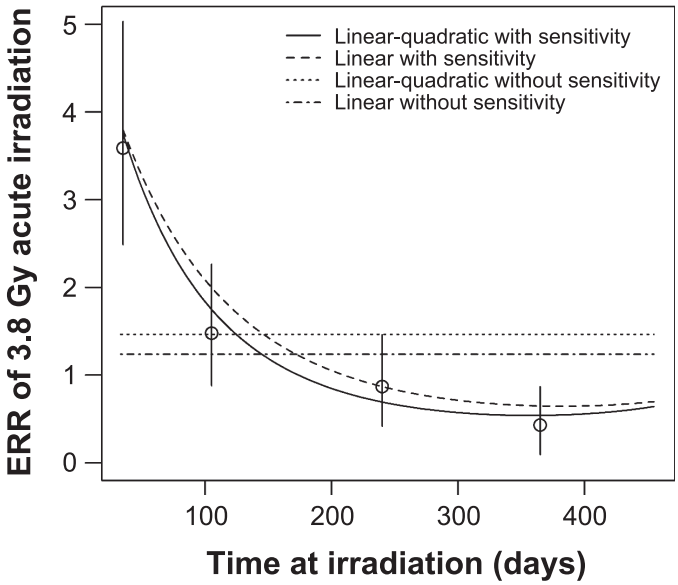

FIG. 7. Predicted excess relative risk (ERR) estimates of 3.8 Gy acute irradiation according to days at irradiation. Circles indicate the non-parametric ERR estimates from data of acutely irradiated mice.

cannot be explained by the linear term in the dose response could be erroneously fitted in the sensitivity function. Due to this overfitting, the predicted ERR estimates from the model with the linear dose response followed the sensitivity change to the same extent as that in the model with the linear-quadratic dose response (Fig. 7), although the dose-response curves did not fit the non-parametric estimates (Figs. 3–6).

In the fitted dose-response curves for acute irradiation at 105 days, the curves from the models with and without AAEE consideration were similar and both were compatible with the non-parametric ERR estimates of acute irradiation. Although the fitted dose-response curves of the models with AAEE fitted in all irradiation days by changing the dose-response curves with age at exposure (days 35, 105, 240 and 365; Figs. 3–6), the dose-response curves of the models without AAEE failed to fit the non-parametric ERR estimate in all irradiation days except for day 105 (Figs. 3, 5 and 6). Unlike the model with AAEE, the models without AAEE fit all data with a single dose-response curve and its dose-response curve was estimated as the average dose-response curve from the data including all irradiation day settings. Therefore, the dose-response curve of the models without AAEE fitted the non-parametric ERR estimate in the irradiation setting of 105 days by chance and did not fit the other settings.

The estimates of DREF differed considerably between linear-quadratic dose-response models with and without AAEE. The estimates can be roughly obtained as the ratio of the non-parametric ERR estimates of chronic irradiation to the ERR value from the fitted dose-response curve at the corresponding dose (Figs. 3–6). The consideration of AAEE in the model corresponds to the change in the dose by weighting. For example, the weighted doses of cumulative irradiation would shrink to zero for acute irradiation at day 35 because the average sensitivity during the chronic irradiation span (from day 56 to 455) was smaller than that at day 35 (Fig. 3). Therefore, the weighted dose of the total 8 Gy chronic irradiation was smaller than 8 Gy at day 35 and 105 (Figs. 3 and 4). The opposite trend was observed for days 240 and 354 of irradiation (Figs. 5 and 6). The estimate of DREF from the model with AAEE corresponded to the ratio of the non-parametric ERR estimate with weighted dose and the corresponding ERR obtained from the fitted dose-response curve at the same dose. For all irradiation days, the ratios were approximately one third (Figs. 3–6). Similarly, the ratio of the non-parametric ERR estimate with simple cumulative dose, and the corresponding ERR obtained from the fitted dose-response curve was less than one third, which corresponded to the estimated DREF from the model without AAEE.

The age sensitivity changed greatly from days 35 to 365 of irradiation (Fig. 7), with a weight ratio greater than five. It is impossible to execute chronic irradiation experiments at the same irradiation age as acute irradiation due to the nature of chronic irradiation. The estimated DREF in this study was approximately three and could be easily masked or overestimated without appropriate consideration of age sensitivity. Therefore, statistical methods that consider the effects of age at exposure are essential for the study of DREF.

Validity of Comparisons between Chronic and Acute Radiation Risk

In general, animal experiments at low dose rates require a large number of animals to be monitored for a long period to detect excess risk increases due to radiation. Because experiments at low dose rates alone require considerable resources, it is difficult to execute experiments that include both chronically and acutely irradiated mice. Therefore, the joint analysis of the chronic and acute irradiation data from the different experiments, as performed in the current study, is especially useful for the estimation of DREF. In such analyses, however, bias could arise due to different baseline risks. In the current study, the survival curve for the nonirradiated group showed a slightly different trend (Fig. 1). Therefore, we conducted a stratified analysis by facility for flexible adjustment of the baseline. By this analysis, the chronically and acutely irradiated data were connected flexibly. Information for the sensitivity due to the age at irradiation in the acutely irradiated mice data was utilized in the chronically irradiated mice data. The comparison of risk between the chronically and acutely irradiated mice data provided an estimated DREF that was adjusted for differences in sensitivity due to the age at irradiation.

Effect of Sham Irradiation

Sham irradiation is often performed in animal experiments in an attempt to reduce systematic differences between nonirradiated and irradiated groups. Sham irradiation was not performed in the IES (*13, 14*) since the environmental conditions were uniform throughout the animal facility and the differences in housing and exposure conditions between the nonirradiated and irradiated groups were minor (rectangular animal rooms vs. square irradiation rooms). Actually, there were no significant differences between the nonirradiated and 0.05 mGy/day irradiated groups in terms of mean life span and neoplasm spectra in both male and female mice (*13*).

Sham irradiation was also not performed in the NIRS, and the risk of the nonirradiated groups between acute and chronic irradiation data was compared to quantify the effect of sham irradiation. The hazard ratio of the acute irradiation to the chronic irradiation was 1.11 (95% CI: 0.94, 1.31). In addition to the hazard ratio, the survival curves of the nonirradiated group (Fig. 1) did not show large differences between the acute and chronic irradiation data. From these results it was also expected that the differences of animals between the nonirradiated and irradiated groups in the acute irradiation experiment were not large. These quantifications

suggested that the absence of sham irradiation had no significant impact on the results of this work.

Merit of Using Animal Experimental Data

There is a tendency for some researchers to assume that there are sufficient data for estimating DDREF from epidemiological studies alone (*8*). However, it may be better to consider the merits of using data from animal experiments. In the context of evidence-based medicine, the order of single-study designs from the most to the least reliable is: randomized controlled trials (RCTs), cohort studies, case-control studies, and others including ecological studies (*30*). In RCTs, subjects are randomly assigned to multiple treatment groups to be compared; this randomization ensures that the background factors of the subjects among groups are almost equal. Because exposure to radiation is known to be harmful, performing an RCT in humans to assess radiation effects is ethically unacceptable; thus, the most reliable research design is an observational cohort study. However, the results of cohort studies have always been limited by the potential for confounding due to a lack of random allocation. Although data analyses in cohort studies usually use relatively complex statistical models to adjust for various confounding factors, they still require caution in interpreting the results due to unmeasured confounding factors (*30*) and the results of epidemiological studies cannot rule out the possibility of biases due to confounding.

In contrast, compared to the variety of background factors of subjects in cohort studies, the subjects in animal experiments can be regarded as uniform in terms of genetic and lifestyle factors; thus, the results of animal experiments are equivalent to those of RCTs in terms of the reliability of study design. The various differences between experimental animals and humans require consideration in extrapolation of the results. The results of experimental animals present a disadvantage in terms of extrapolation, but is also an essential condition in terms of the validity of comparisons. Therefore, comprehensive evaluation of the results from both animal experiments and epidemiological studies is required for further discussion of DREF.

Extrapolation of Animal Results to Humans

Extrapolation of cancer risk from experimental animals to humans is required in the context of radiological protection including estimation of DREF, as discussed elsewhere (*31*). Thus, the animal data sets and statistical methodologies adopted herein may be applied to improving extrapolation. The dose-dependent changes in the predicted cumulative survival curves are concordant among humans, mice and dogs using relative risk models of all-cause or solid cancer mortalities (*32, 33*). Thus, the application of Cox regression and the use of cancer mortality data in the current study support the applicability of our approach to animal-to-human extrapolation. Moreover, in contrast to human populations, wherein the age at exposure is widely distributed, most experimental studies use only young adult animals, impeding the employment of most experimental data in extrapolation to humans (*31*). In this respect, our current approach is again advantageous in that it uses experimental data that include a wide range of ages at exposure (*15*) and a statistical model that considers age-related differences.

The many challenges to interspecies extrapolation also include the tendency of most experimental studies to use only one or a few strains having uniform genetic backgrounds, whereas human populations are more heterogeneous. The current analysis also used data sets obtained from only one hybrid mouse strain (B6C3F1) with uniform genetics combining inbred C57BL/6JNrs and C3H/HeNrs strains (*13, 15*). Another general limitation of extrapolation is the difference in tumor types and subtypes to which experimental animals and humans are susceptible (*31*). Although the current analysis focused on solid cancer as an aggregate of many tumor types to minimize the effect of such interspecies variations, this approach raises another limitation in estimating the cancer risk of individual organs. Despite these limitations, the data set and methodology in the current study demonstrate a novel approach for extrapolating animal data to human cancer risk.

Adjustment for Time-Related Variables in Statistical Models

In addition to the age at exposure, cancer mortality rates also vary greatly with attained age in both humans and mice. In the current study, Cox regression models enabled flexible adjustment for the attained age, as described above in Materials and Methods. Various approaches can be used to adjust for time-related variables and the time since exposure variable is one candidate for them (*28*). However, there is a constraint in the use of three time-related variables (attained age, age at exposure and time since exposure) simultaneously in the model due to collinearity (*34*). Furthermore, the probability of failure in parameter estimation usually increases with model complexity. The current study focused on adjusting for age at exposure in addition to attained age, and we believe that this decision was appropriate because the sensitivity at an irradiation age of 35 days was more than five times that at 365 days.

Study Limitations

This study estimated DREF as the ratio of the risk of cancer death from chronic irradiation to that from acute irradiation using only female mice. Although risk estimates for each group in male mice were not significantly different from those in female mice, the DREF value may vary; thus, it would be desirable to include additional data from acutely irradiated male mice.

There is also an interest in the risk of cancer in individual organs such as the central nervous system (CNS). However, the number of events is reduced by limiting the cancer site

[at most two deaths from CNS in each irradiation group from Table 1 in Tanaka *et al*. (*13*)], and parameters cannot be estimated with a complex model using data with such small numbers of events. When adding new experimental data from other facilities, it is essential to make conditions other than dose rate as equal as possible in chronic and acute irradiation data to obtain unbiased DREF estimates.

Further assessment of DREF from animal experimental data requires estimation of DREF for both male and female animals as well as for various cancer sites. To do so will require collaboration among several organizations so that data from each organization can be treated as single large-scale data.

Comparison of Results with Previous Studies

It may be of interest to compare our results to those of similar studies. Haley *et al*. reported $DDREF_{LSS}$ (the dose and dose-rate effectiveness factor for the life span study of atomic bomb survivors) estimates from 4.8 to infinity by analyzing consolidated animal data from the Janus and ERA databases (*27*). The $DDREF_{LSS}$ was estimated using life span data as a function of the ratio between quadratic and linear coefficients based on the curvature in a linear-quadratic model. The linear-quadratic model poorly fitted the observed data; thus, the $DDREF_{LSS}$ could not be well estimated. Direct comparisons between acute and protracted radiation exposure resulted in a significantly larger estimated $DDREF_{LSS}$ than that based on the curvature of the dose-response model. This curvature theory assumes that the risk from protracted exposure with decreased dose can come close to the linear coefficient of the dose response from acute exposure. However, the linear coefficient could be dose-rate dependent (*11*). Thus, it would be biologically better to estimate the $DDREF_{LSS}$ by direct comparisons between acute and protracted exposure. The same method was used to estimate DREF in the current study.

Tran and Little (*25*) analyzed JANUS data by Cox regression models that were more general than that fitted by Haley *et al*. (*27*). The model considered adjustments for the effects of both dose and dose rate. The result indicated *high*-dose-rate to low-dose-rate (<5 mGy/h) ratios of 1.2–2.3 for many tumor sites. This analysis differs from our analysis in two ways. First, Tran and Little used a threshold dose rate of 120 mGy/day (5 mGy/h) to define protracted exposure at low dose rates. This treatment would result in limitations in the analysis of the dose-rate effect. In contrast, our analysis used data from longer, protracted exposure at lower dose rates of 0.05, 1.1 and 21 mGy per day for 400 days. Secondly, our analysis considered age-dependent modification for protracted exposure. The effect of radiation on cancer incidence or mortality decreases with increasing age at exposure for most cancer sites in the atomic bomb survivors. The difference in risk between acute and protracted exposure may include not only dose-rate but also age-at-exposure effects. However, previous analyses have never modeled age-at-exposure effects because it would not be easy to be statistically treated without using our approach.

The dose and dose-rate response of lymphocyte chromosome aberrations in our mouse data were specifically analyzed to clarify the dose-rate effects. In unstable-type chromosome aberrations, the DREF was 4.5 for dicentrics by fluorescent *in situ* hybridization (FISH) and 5.2 for dicentrics and centric rings (*35*). While these DREF values may not be directly comparable to those in tumors, they suggest the presence of dose-rate effects at lower dose rates.

The results of epidemiological studies are more useful in estimating human DREF but require caution when comparing the risk of protracted exposure to that of acute exposure. The meta-analysis by Shore *et al*. of low-dose-rate radiation epidemiological studies showed a DREF of approximately 3, which was also statistically compatible with 2 (*6*). However, the study observed a lower DREF after excluding the cohort of Mayak workers. Similar meta-analyses of nuclear worker cohorts concluded that the best estimate of DREF was approximately 2 (*4*), although two large worker studies, INWORKS and Mayak, had various limitations that might have affected the DREF. In contrast, another meta-analysis using a similar method reported a DREF of approximately 1 (*5*). To reach convincing conclusions, however, current meta-analyses of epidemiological studies of low-dose-rate radiation require further consideration of the limitations of each study.

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REFERENCES

1. Grant EJ, Brenner A, Sugiyama H, Sakata R, Sadakane A, Utada M, et al. Solid cancer incidence among the life span study of atomic bomb survivors: 1958–2009. Radiat Res 2017; 187:513–37.
2. Ozasa K, Shimizu Y, Suyama A, Kasagi F, Soda M, Grant EJ, et al. Studies of the mortality of atomic bomb survivors, Report 14, 1950–2003: an overview of cancer and noncancer diseases. Radiat Res 2012; 177:229–43.
3. Richardson DB, Cardis E, Daniels RD, Gillies M, O'Hagan JA, Hamra GB, et al. Risk of cancer from occupational exposure to ionising radiation: retrospective cohort study of workers in France, the United Kingdom, and the United States (INWORKS). BMJ 2015; 351:h5359.
4. Hoel DG. Nuclear epidemiologic studies and the estimation of DREF. Int J Radiat Biol 2018; 94:307–14.
5. Jacob P, Ruhm W, Walsh L, Blettner M, Hammer G, Zeeb H. Is

cancer risk of radiation workers larger than expected? Occup Environ Med 2009; 66:789–96.

6. Shore R, Walsh L, Azizova T, Ruhm W. Risk of solid cancer in low dose-rate radiation epidemiological studies and the dose-rate effectiveness factor. Int J Radiat Biol 2017; 93:1064–78.
7. Kudo S, Ishida J, Yoshimoto K, Mizuno S, Ohshima S, Furuta H, et al. Direct adjustment for confounding by smoking reduces radiation-related cancer risk estimates of mortality among male nuclear workers in Japan, 1999–2010. J Radiol Prot 2018; 38:357–71.
8. Kocher DC, Apostoaei AI, Hoffman FO, Trabalka JR. Probability distribution of dose and dose-rate effectiveness factor for use in estimating risks of solid cancers from exposure to low-let radiation. Health Phys 2018; 114:602–22.
9. Wakeford R, Azizova T, Dorr W, Garnier-Laplace J, Hauptmann M, Ozasa K, et al. The dose and dose-rate effectiveness factor (Ddref). Health Phys 2019; 116:96–9.
10. Ruhm W, Azizova TV, Bouffler SD, Little MP, Shore RE, Walsh L, et al. Dose-rate effects in radiation biology and radiation protection. Ann ICRP 2016; 45:262–79.
11. Niwa O. Roles of stem cells in tissue turnover and radiation carcinogenesis. Radiat Res 2010; 174:833–9.
12. Ruhm W, Woloschak GE, Shore RE, Azizova TV, Grosche B, Niwa O, et al. Dose and dose-rate effects of ionizing radiation: a discussion in the light of radiological protection. Radiat Environ Biophys 2015; 54:379–401.
13. Tanaka IB 3rd, Tanaka S, Ichinohe K, Matsushita S, Matsumoto T, Otsu H, et al. Cause of death and neoplasia in mice continuously exposed to very low dose rates of gamma rays. Radiat Res 2007; 167:417–37.
14. Tanaka S, Tanaka IB, 3rd, Sasagawa S, Ichinohe K, Takabatake T, Matsushita S, et al. No lengthening of life span in mice continuously exposed to gamma rays at very low dose rates. Radiat Res 2003; 160:376–9.
15. Sasaki S, Influence of the age of mice at exposure to radiation on life-shortening and carcinogenesis. J Radiat Res 1991; 32:S73–85.
16. United Nations Scientific Committee on the Effects of Atomic Radiation. Sources, effects and risks of ionizing radiation. UNSCEAR 2017 report to the general assembly, with scientific annexes A and B. New York: United Nations; 2018.
17. Fleiss JL, Levin B, Paik MC. Statistical methods for rates and proportions. 3rd ed. Hoboken: John Wiley & Sons; 2003.
18. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
19. Dobson AJ, Barnett AG. An introduction to generalized linear models. 4th ed. Boca Raton, FL: Taylor & Francis Group; 2018.
20. Stokes ME, Davis CS, Kock GG. Categorical data analysis using the SAS system. 2nd ed. Cary, NC: SAS Institute Inc.; 2000.
21. Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. 2nd ed. Hoboken, NJ: John Wiley & Sons; 2002.
22. Amitage P, Berry G, Matthews JNS. Statistical methods in medical research. 4th ed. London: Blackwell Science; 2002.
23. Griffin BA, Anderson GL, Shih RA, Whitsel EA. Use of alternative time scales in Cox proportional hazard models: implications for time-varying environmental exposures. Stat Med 2012; 31:3320–7.
24. Thiebaut AC, Benichou J. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: a simulation study. Stat Med 2004; 23:3803–20.
25. Tran V, Little MP. Dose and dose rate extrapolation factors for malignant and non-malignant health endpoints after exposure to gamma and neutron radiation. Radiat Environ Biophys 2017; 56:299–328.
26. Akaike H. Likelihood of a model and information criteria. J Econom 1981; 16:3–14.
27. Haley BM, Paunesku T, Grdina DJ, Woloschak GE. The increase in animal mortality risk following exposure to sparsely ionizing radiation is not linear quadratic with dose. PLoS One 2015; 10:e0140989.
28. Daniels RD, Bertke SJ, Richardson DB, Cardis E, Gillies M, O'Hagan JA, et al. Examining temporal effects on cancer risk in the international nuclear workers' study. Int J Cancer 2017; 140:1260–69.
29. Ritz B, Morgenstern H, Moncau J. Age at exposure modifies the effects of low-level ionizing radiation on cancer mortality in an occupational cohort. Epidemiology 1999; 10:135–40.
30. Sackett DL, Richardson WS, Rosenberg W, Haynes RB. Evidence-based medicine. New York: Churchill Livingstone; 1997.
31. Extrapolation of radiation-induced cancer risks from nonhuman experimental systems to humans. Report No. 150. Bethesda, MD: National Council on Radiation Protection and Measurements; 2005.
32. Carnes BA, Grahn D, Hoel D. Mortality of atomic bomb survivors predicted from laboratory animals. Radiat Res 2003; 160:159–67.
33. Carnes BA, Olshansky SJ, Grahn D. An interspecies prediction of the risk of radiation-induced mortality. Radiat Res 1998; 149:487–92.
34. Lagarde F. Understanding estimation of time and age effect-modification of radiation-induced cancer risks among atomic-bomb survivors. Health Phys 2006; 91:608–18.
35. Tanaka K, Kohda A, Satoh K, Toyokawa T, Ichinohe K, Ohtaki M, et al. Dose-rate effectiveness for unstable-type chromosome aberrations detected in mice after continuous irradiation with low-dose-rate gamma rays. Radiat Res 2009; 171:290–301.