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Statistical Analysis of Survival Data From Radiation Countermeasure Experiments

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Abstract

We present an introduction to, and examples of, Cox proportional hazards regression in the context of animal lethality studies of potential radioprotective agents. This established method is seldom used to analyze survival data collected in such studies, but is appropriate in many instances. Presenting a hypothetical radiation study that examines the efficacy of a potential radioprotectant both in the absence and presence of a potential modifier, we detail how to implement and interpret results from a Cox proportional hazards regression analysis used to analyze the survival data, and we provide relevant SAS[®] code. Cox proportional hazards regression analysis of survival data from lethal radiation experiments (1) considers the whole distribution of survival times rather than simply the commonly used proportions of animals that survived, (2) provides a unified analysis when multiple factors are present, and (3) can increase statistical power by combining information across different levels of a factor. Cox proportional hazards regression should be considered as a potential statistical method in the toolbox of radiation researchers.

INTRODUCTION

Development of radioprotective agents to reduce the mortality and morbidity resulting from the acute toxicity of radiation exposure involves extensive animal testing. The U.S. Food and Drug Administration (FDA) requires rigorous animal lethality studies for approval of medical countermeasures against radiation because efficacy testing cannot be performed in humans (1). Balancing the critical need to improve the public's survival after a radiological or nuclear assault with animal welfare considerations necessitates that these experiments are designed and analyzed so that the maximal amount of information can be derived from the smallest possible number of animal subjects (2–7). Reductions in animal numbers can be achieved by using efficient study designs, with consideration of a number of factors which may compete for importance (8). To the extent possible, animal numbers should also be limited by the anticipated use of the most informative analytical methods, which is the focus of this paper.

In radiation lethality experiments, a variety of study designs are utilized to investigate the impact of different radioprotective treatments on survival after radiation exposure. Treatment groups may represent one or more investigative agents [in addition to a vehicle control (9–12)], different timings of treatment administration [e.g., before or after radiation

exposure (9–13)], different combinations of treatments [$\pm$ treatment A and $\pm$ treatment B (9)], different doses of treatment (13–17), as well as different age groups or genders of animals. Additionally, radiation may be investigated at one dose level or multiple dose levels (9, 11, 12, 14, 16, 17). Consequently, experiments often have more than two study groups defined by combinations of treatment and radiation levels to which animals are randomly assigned.

Depending on the end point of interest, survival studies in rodents typically last between 10 and 30 days. Although total body irradiation causes injury in all organ systems, 10-day lethality is generally considered indicative of gastrointestinal injury, while 30-day lethality generally indicates hematopoietic system damage (3). For each animal, the day of death after radiation exposure is typically recorded for those dying prior to the predetermined study end date. Occasionally, studies will make full use of the survival data by comparing survival time distributions between two groups with, for example, a log-rank test [e.g., ref. (16)]. These comparisons are limited, though, since they can only control for one factor at a time, and must be repeated for each radiation dose used. More often, the level of detail in characterizing the survival experience is disregarded, and study problems are simplified into comparing two groups on the proportions of animals surviving to a particular point in time; these comparisons are made with a χ^2 test or Fisher's exact test. Alternatively, for studies that use multiple radiation doses, investigators sometimes estimate a dose reduction factor (DRF) with probit analyses; for example, see refs. (11, 17). A DRF is a meaningful parameter to estimate, but few studies go on to statistically compare the DRF to 1 (the usual null value) with either a confidence interval or formal test [e.g., refs. (10, 16)]. When more than two groups are compared, these procedures are often applied multiple times for each pair, thereby increasing the overall Type I error rate when not controlled with a multiple comparison method (e.g., Bonferroni's method).

Although intuitively appealing in its simplicity, analyzing the proportion surviving at a particular time point, as with the commonly used probit analysis, is not an efficient approach. It does not incorporate the full information about the animals' survival experience. In an experiment where one group of animals has many more early deaths than the other, the proportions surviving at the end could still be similar. Fisher's exact test (for comparing two groups at a single radiation dose level), or probit analysis (for comparing groups across multiple radiation dose levels), would be insensitive to such a difference in survival distributions, although this difference could have important implications for the window of opportunity for treatment. Even when the survival time distributions are compared by applying more sensitive log-rank tests, gains in power over multiple pair-wise comparisons can be achieved when the treatment effect can be estimated across multiple groups of animals in an experiment. For example, if the relative effect of a radioprotective agent on survival is similar across groups of animals exposed to different radiation doses, then it is more efficient to examine the average treatment effect over radiation doses as compared to examining the treatment effect for each dose separately.

Cox proportional hazards (CPH) regression modeling (18) is an ideal tool for addressing the above deficiencies in the current popular analytic approaches. This method models the time-to-death of each animal and has been shown to be more powerful than the common approach of analyzing the proportion surviving at a fixed point in time, especially when the death rate is high (19, 20). Also, inferences about interactions between treatment and other factors can be objectively made; when interactions may be reasonably assumed to not exist, the treatment effect can be more precisely estimated using information from more than one group of animals. Furthermore, a regression approach allows continuous explanatory variables, such as radiation dose, radioprotectant dose, age, or weight, to be modeled as they

are – continuous. Other factors like sex, genotype, or interfering interventions may also be simultaneously modeled, thus generating mechanistic information.

Cox regression models have a long history of use in the analysis and reporting of clinical trials, and the original seminal paper (18) is one of the top cited papers in research (21). Although Cox models are extensively used in human studies, they do not appear to have made many inroads into assessments of survival in animal studies conducted to evaluate radiation countermeasures. In this paper, we advocate the use of CPH regression analyses for radiation lethality studies. We aim to show how CPH analyses can

- directly address usual survival questions of interest, and;
- effectively combine multiple factors into an overall analysis.

After describing the CPH model, we use a hypothetical radiation survival experiment to illustrate CPH regression analysis. Specifically, we discuss statistical power considerations for CPH analysis versus more elementary analytic methods, provide results in written and graphical form, and interpret the results.

COX PROPORTIONAL HAZARDS MODEL

For radiation experiments in which death is an endpoint, it is common for some animals to live until the predetermined end of the experiment, at which time they are often euthanized. These animals are thus known to have survived *at least* a certain amount of time; their survival times are called “right censored.” Cox (18) first proposed a semi-parametric regression model for survival times of which some are right censored. The regression actually models the hazard of dying at time t (i.e., the probability of dying within a narrow window about time t). Since the hazard of dying may depend on some known factors (collectively, a vector of independent variables denoted as $\mathbf{x}$), and also depends on time, the hazard function is typically written as $\lambda(t, \mathbf{x})$, showing its relationship to both time and $\mathbf{x}$. The CPH model is the product of an unspecified baseline hazard, $\lambda_0(t)$, and an exponentiated regression model, $\mathbf{x}'\boldsymbol{\beta}$:

$$\lambda(t, \mathbf{x}) = \lambda_0(t) e^{\mathbf{x}'\boldsymbol{\beta}}. \quad (1)$$

The “semi” of “semi-parametric” comes from the unspecified nature of $\lambda_0(t)$, and the “parametric” portion from the regression parameters, $\boldsymbol{\beta}$. Common statistical shorthand for a (linear) regression function is $\mathbf{x}'\boldsymbol{\beta} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \dots + \beta_q x_q$ for q independent variables defined in the context of a specific problem. Lacking a defined function for $\lambda_0(t)$, the CPH model focuses on the relationship of the hazards of two observations at the same time point, say t^* , but differing in their values of $\mathbf{x}'\boldsymbol{\beta}$. The nomenclature “proportional hazards” speaks to this relationship, and derives its name from the multiplier, $\exp(\mathbf{x}'\boldsymbol{\beta})$, of the baseline hazard.

We note that if all of the animals in the survival experiment died during the study, then using analysis of variance (ANOVA) or covariance (ANCOVA) on the observed survival times *could* be reasonable. These two methods cannot, however, be used appropriately when some of the data are right censored. CPH regression allows us to use the concepts in ANOVA and ANCOVA while appropriately handling the censored nature of the data. Indeed, we may simply insert the $\mathbf{x}'\boldsymbol{\beta}$ that makes up the ANOVA or ANCOVA into the CPH model framework.

HYPOTHETICAL EXPERIMENT

As described in the Introduction, a study to evaluate a potential radioprotectant often includes two or more factors that may influence the performance of the radioprotectant. Determining under what conditions the agent may be useful as a radioprotectant with the factors under consideration is like putting together a puzzle. Including all the data from a multi-factorial experiment provides a complete picture of the radioprotectant's performance, albeit hazy from inherent variability. The common historical practice of performing a series of analyses on such data is akin to examining a few pieces of the puzzle at a time; whereas analyzing the data in its entirety is to consider all of the pieces of the puzzle simultaneously. We now describe a hypothetical experiment that examines a potential radioprotectant alone (Part A) and in the presence of a potential modifying factor (Part B), and ultimately describe how to examine all the pieces of its puzzle at once (combining Parts A and B).

Hypothetical Experiment: Part A

A potential radioprotectant, R , is being investigated in mice initially at a single dose, compared to a vehicle control. In the strain of mice to be used in the experiment (say C57), a total body irradiation dose, D , of about 10.6 Gy kills 50% of control mice by day 10; that is, $LD_{50/10} = 10.6$ Gy. The researchers want to capture the $LD_{50/10}$ in the control mice, but also want to consider higher irradiation doses in hopes the $LD_{50/10}$ for radioprotectant-treated mice is higher than 10.6 Gy. They decide upon irradiation doses of 10, 11 and 12 Gy. Having about 50 mice to partition among the 6 combinations of radioprotectant [R = on (1) and off (0)] and irradiation dose (10, 11 and 12 Gy), they allot 8 mice to each group, for a total of 48 mice. They intend to monitor mouse survival for 30 days to capture information about deaths due to both gastrointestinal and hematopoietic injuries; the latter of which usually takes 2–4 weeks when using the planned radiation doses.

The question of interest is whether treatment with the radioprotectant reduces lethality (increases life expectancy) after radiation exposure. With this question in mind, and before conducting the experiment, the researchers determine the treatment effects they can detect with at least 0.80 power using two-tailed tests with significance level $\alpha = 0.05$. One way to evaluate the radioprotectant's effect on survival is to compare radioprotectant group to control group at each irradiation dose using a log-rank test. Since three tests will be made, one for each irradiation dose, the researchers use Bonferroni's method, adjusting their significance level to $0.05/3 \approx 0.017$ for each comparison. They also compute the treatment effect they can detect when combining all of the irradiation doses with a CPH regression analysis. Since only one test is made on the effect of radioprotectant in the CPH context, no adjustment of α is needed. It is important to note that, when comparing survival between exactly two treatments without adjusting for other covariates, CPH regression and log-rank tests are asymptotically the same; that is, as the sample size gets larger, the statistical inferences (confidence intervals and/or P values) approach equality². However, even when using small samples, the results from the two tests will not be appreciably different.

In log-rank tests comparing two groups, the treatment effect is expressed in terms of hazard ratios (HRs). A formula that provides the effect size for this simple two-tailed comparison at each dose is:

²The two analyses return exactly the same results no matter what the sample size when the CPH analysis (1) assumes survival times are discrete and (2) uses the score χ^2 statistic to make inference. Assumptions about the nature of survival times (e.g., discrete vs. continuous) and types of χ^2 statistic (e.g., score vs. Wald) can often be changed in the statistical software used.

$$HR = \exp \left[\frac{2(z_{1-\alpha/2} + z_{1-\beta})}{\sqrt{N \times ODR}} \right], \quad (2)$$

where $z_{1-\alpha/2}$ and $z_{1-\beta}$ are quantiles from the standard normal distribution, N is the total sample size assumed to be split equally between the two groups, and ODR is the overall death rate among the N animals. For the present example, $N = 16$, $z_{1-\alpha/2} = 1.960$ where $1 - \alpha/2 = 0.975$, appropriate for a two-tailed 0.05 level test, and $z_{1-\beta} = 0.842$ where $1 - \beta = 0.80$, the desired power. The investigators realize that the death rate (ODR , expressed as a proportion) depends on irradiation dose and the length of observation. Based on previous data, they conservatively estimate that 0.65, 0.80 and 0.90 of the control mice will die by 30 days after undergoing total-body irradiation of 10, 11 and 12 Gy, respectively. The detectable HRs for both unadjusted and adjusted significance levels are provided in Table 1.

When using a log-rank to compare the radioprotectant and control survival curves, survival experience cannot be combined across the three-irradiation doses. However, using a CPH regression, the investigators *can* combine data across irradiation doses and evaluate the effect of radio-protectant while accounting for irradiation dose. With $N = 48$, $ODR = (0.65 + 0.80 + 0.90)/3 = 0.783$, the HR comparing control to radioprotectant detectable with 0.80 power on a two-sided 0.05 level test is about half the size of that detectable using the individual log-rank tests.

Part A Results and Discussion

The results are based on simulated data from the above hypothetical experiment (see Appendix). In the three panels of Fig. 1A, we provide the Kaplan-Meier survival curves for radioprotectant and control animals for each of the irradiation doses: 10, 11 and 12 Gy. From these plots, radioprotectant-treated animals appear to survive longer than the control animals; indeed, the HRs – all less than 1 – confirm in a number what the eyes see. However, none of three comparisons reach the usual 0.05 significance level, let alone the 0.017 Bonferroni-adjusted significance level (Table 2). To fit our proposed CPH model,

$$\lambda(t, x) = \lambda_0(t) \exp(\beta_0 + \beta_1 R + \beta_2 D), \quad (3)$$

most statistical software will need a data set that identifies for each observed survival time T , the particular values of the radioprotectant, R , and total body irradiation, D . Additionally, the data set must include a censoring variable that indicates whether the survival time was observed or not; that is, whether the animal died or lived to the end of the experiment (see example data set in the Appendix). Combining the information across irradiation doses in the CPH model that has $x' \beta = \beta_0 + \beta_1 R + \beta_2 D$, we find that radioprotectant-treated animals survive significantly longer than those *not* receiving radioprotectant (Table 2, $P = 0.023$). The CPH-estimated survival curves at 10.6 Gy, the control's $LD_{50/10}$, are plotted in Fig. 2A.

Hypothetical Experiment: Part B

In addition to considering the effect of radioprotectant on survival (Part A of our example), the investigators are also interested in how the radioprotectant effect might change for a potential modifying factor, M . The modifying factor the investigators are considering here has two levels: whether “off/on” or “now/then” or “young/old” or, more generally, 0/1. Part A covered the case when the modifying factor was at the 0-level. For the second part, the investigators had another 48 mice, which all were $M = 1$ mice. These modified mice were randomly and equally assigned to the six combinations of the radioprotectant (on/off) and total body irradiation dose (10, 11 and 12 Gy). Survival was monitored for 30 days.

Part B Results and Discussion

The investigators' interest in whether the modifying factor actually modifies the radioprotectant effect means that they will need to jointly consider the data from Parts A and B. Repeating the same steps in Part A with data from the modified mice, and comparing both graphical and numerical results to Part A results provide some insight into the effects of the modifying factor. See Table 3 and Fig. 1B. Unlike in Part A, the comparisons between radioprotectant and no-radioprotectant mice are not consistent across the three irradiation doses; HRs are on both sides of 1. Additionally, all three HR values are greater than those observed for the unmodified mice. Combining the data from modified mice across the three irradiation doses in a CPH model (Eq. 3), the estimated HR is close to unity (1.107), implying equality of the hazards for radioprotectant and no-radioprotectant mice (Table 3, $P = 0.746$). These three observations all indicate that the modifying factor tends to suppress the beneficial effects of the radioprotectant observed in Part A (see also Fig. 2B). But, based solely on the results presented thus far, we cannot determine whether these indications of an $R \times M$ interaction are within the realm of chance occurrence.

Combining Parts A and B

We may combine data from animals in Part A with those in Part B with a CPH model. This model will not only allow us to estimate the effects of the radioprotectant and modifying factor, but will provide a formal test for the interaction of the two factors, all the while accounting for irradiation dose. The $x'\beta$ portion of the model is

$$x'\beta = \beta_0 + \beta_1 R + \beta_2 M + \beta_3 R \times M + \beta_4 D. \quad (4)$$

Fitting this model to all 96 animals, the interaction of the radioprotectant and modifying factor ($R \times M$) is verified ($\chi^2[1] = 3.866$, $P = 0.049$). The presence of an interaction means we should not directly interpret the slopes (β s) of the radioprotectant (R) and modifying factor (M), whether the slope is significant (β_1 for R : $\chi^2[1] = 5.179$, $P = 0.023$) or not (β_2 for M : $\chi^2[1] = 0.418$, $P = 0.518$). Indeed, the presence of an interaction means that the slopes for R and M in Eq. (4) become

$$\beta_{R|M} R = \beta_1 R + \beta_3 R \times M = (\beta_1 + \beta_3 M) R$$

and

$$\beta_{M|R} M = \beta_2 M + \beta_3 R \times M = (\beta_2 + \beta_3 R) M,$$

respectively. Interpretation for one factor depends on the value of the other factor. Since the radioprotectant is the primary factor of interest, it makes sense to compare radioprotectant to no-radioprotectant at each level of the modifying factor. Our estimated slope for R when $M = 0$ is

$$\beta_{R|M=0} = -0.697$$

and when $M = 1$ is

$$\beta_{R|M=1} = -0.697 + 0.840 = 0.143.$$

To get the M -dependent HRs of radioprotectant-treated to no-radioprotectant animals, we raise e to the $\beta_{R|M=0}$ and $\beta_{R|M=1}$ powers to get 0.498 [95% CI (0.273, 0.908)] and 1.153 [95% CI (0.640, 2.077)], respectively. These estimates of the radioprotectant effect are consistent with those estimated in Parts A and B, respectively (see “CPH regression” in Tables 2 and 3). That is, in the absence of M , R provides significant radioprotection ($P = 0.023$, 95% CI for HR excludes 1), but, in the presence of M , the radioprotection provided by R is not significant ($P = 0.635$, 95% CI for HR includes 1). If the interaction between M and R had not been statistically significant, it would have been permissible for the researchers to fit a CPH model with no interaction term in order to bolster statistical inferences on the main effects of M and R .

For each of the analyses presented above (Parts A, B and combined Parts A and B), we have assumed the hazards of the various effects (whether the effects of the radio-protectant, modifier, or radiation dose) are proportional to each other over time. We may get an idea of whether the proportional hazard (PH) assumption is valid by looking at the estimated survival curves in Fig. 1A and B for Parts A and B, respectively, and considering both figures simultaneously for the combined analysis. If groups differ in their survival, PH implies that their corresponding survival curves should gradually drift apart over time. Two violations that may be seen easily are when two curves depart early on, then (1) come back together toward the end, or (2) intersect and depart again. If the survival experiences for two groups are the same, we would likely see the curves intersect several times. However, since each curve in Figs. 1A and 2B is estimated with eight animals, we can only look for gross violations of the PH assumption. Rather than relying on our eyes alone, statistical tests also exist for detecting PH assumption violations. Employing those tests for Parts A, B, and combined analysis above, we found no evidence that the PH assumption was violated for any of the factors in the models (Part A both $P > 0.24$; Part B both $P > 0.26$; Combined Parts A and B four $P > 0.07$).

DISCUSSION

More finely characterizing the time-to-death (in days) in radiation lethality studies may lead to detection of subtler differences than those detectable using simple proportions of animals surviving at the end of a study, or from estimating LD50s and dose reduction factors with probit or logit analyses. Thus, statistical tests like the log-rank test for comparing survival distributions are preferable to tests which compare proportions surviving at study end. However, as we have shown, CPH models can provide a more complete picture of the outcome of interest than can multiple pairwise log-rank tests in a multifactor radiation lethality study. CPH allows one to model the time-to-death and simultaneously account for multiple experimental factors. Because of the many advantages of CPH models, CPH has become an established method in clinical research. More animal researchers should utilize this powerful approach.

When there are no differences among levels of one factor in the CPH model, that factor can be removed from the model, so that the treatment effect is essentially estimated across levels of that factor and, consequently, with greater precision (i.e., narrower confidence intervals). Intuitively, this makes sense as more animals are contributing information to the outcome of interest. Combining animals across groups which clearly do not differ could potentially allow fewer animals to be studied, and thus allow investigators to better satisfy ethical constraints regarding animal welfare while maximizing the information gained from

experiments. Moreover, various confounding factors that may (or may not) influence survival and/or the effect of a radiation response modifier may be taken into account. Such factors may, for example, be the animal's age, sex or strain. In the example analyzed here, there was a significant interaction between the radioprotectant and the modifying factor, so that this potential gain in efficiency was not realized. However, the gain in efficiency from analyzing the three radiation doses simultaneously was clear (Tables 2 and 3).

We have demonstrated that a detectable hazard ratio can be calculated for a given sample size, significance level and power (Table 1). Similarly, if the power and the hazard ratio to be detected are specified, then the required sample size can be determined. Depending on the stage of research, different approaches can be taken. In the earliest stage, an investigator may want to conservatively design an experiment based on two-sample comparisons since the information on relationships between different factors and the outcome may not be known. Still, the final analysis could be based on a CPH model. If an investigator is further along in the research and has a fairly good idea about the expected relationships, then the sample size could be based on the CPH model. Even in the earliest stage of research, the CPH approach could be used to evaluate multiple design scenarios, which could be evaluated as a range of possibilities for the final study design.

Underlying these analyses is the assumption of proportional hazards. When this assumption is mildly violated, it still may be reasonable to apply a CPH model, especially when the goal is to show one treatment is superior to the other, and explicitly quantifying survival is secondary. Practically, only gross departures from the assumption of proportional hazards can be detected in this setting where often times there are less than 20 animals per group. If gross violations are found, alternative methods based on extensions to the traditional CPH model can be used to remedy the problem. The lack of proportional hazards implies that the hazard depends on time, so time-dependent covariates in the CPH model can be used to resolve this problem. For categorical covariates, stratified CPH models can be used where different baseline hazards are used for different strata (22). Additionally, there are alternative approaches outside of the CPH model framework, such as accelerated failure time models (23) and accelerated hazards models (24). Although these models can address an important problem, the price to be paid is that interpretation becomes more complicated.

As a final note, if investigators insist on analyzing the proportion surviving beyond a specified time point (a binary outcome) instead of comparing survival distributions, the benefits of jointly assessing factors using a multivariable approach can still be had by using the analogous multivariable approach of logistic regression modeling. This would be preferable to making multiple comparisons of proportions between two groups.

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APPENDIX

Statistical software packages that perform CPH regression analyses can differ in the types of data they allow. For example, one package may allow the radioprotectant variable, R , to take values “present” and “absent”; whereas another may only allow numeric values. All of the packages will be able to handle strictly numeric values. For this reason, we recommend creating indicator variables (a.k.a. dummy variables) for those variables that are not inherently numeric, like gender or our variable R . Indicator variables take values 0 and 1.

For both Parts A and B, the regression portion of the CPH model is $\beta_0 + \beta_1 R + \beta_2 D$. The modifying factor, M , is simply “off” in Part A and “on” in Part B, so it is not included in the model. Letting 1 indicate R 's presence and 0 its absence, we see that survival times from animals without R are only providing information for $\beta_0 + \beta_1(0) + \beta_2 D = \beta_0 + \beta_2 D$. Animals with R provide the same information as animals without R , plus information for β_1 . So β_1 is the difference between those treated with R and those not. Confidence intervals for β_1 that do not include 0 indicate a statistical difference. In terms of the hazard of dying at any given point in time, those treated with R have a hazard that is e^{β_1} times the hazard for animals without R . An $(1 - \alpha)$ 100% confidence interval for e^{β_1} that does not include 1 indicates a statistical difference at level α . The difference in the analyses between Part A and Part B is the data that are being analyzed. The modifier (M) is absent ($M = 0$) in Part A and present ($M = 1$) in Part B. We include a portion of the data in Table A1 to show how the data may be formatted for analysis. SAS code for the analysis of Parts A and B is provided below.

When combining the data from Parts A and B, the regression portion of the model is $\beta_0 + \beta_1 R + \beta_2 M + \beta_3 R \times M + \beta_4 D$. Because M can be either “on” or “off” in the combined analysis, it is explicitly included in the model. We illustrate the meaning of the different parameters (the β s) with the help of Table A2, which contains the remaining portion of the regression model at the four combinations of the radioprotectant R and modifier M at a given radiation dose, D . Subtracting Row 1 from Row 2, we are left with β_1 which is the effect of the radioprotectant in the absence of the modifier. Similarly β_2 – the difference between Rows 3 and 1 – is the effect of the modifier in the absence of the radioprotectant. The change in the hazard of the control case ($M = 0$, $R = 0$) when adding the radioprotectant is e^{β_1} or the modifier is e^{β_2} . When both the radioprotectant and modifier are present, β_3 is the amount added to (or subtracted from) the individual effects of the two factors. Finally, we may interpret e^{β_4} as the expected change in the hazard for a 1-unit increase in the radiation dose, D . Hypotheses about the parameters can be tested with $(1 - \alpha)$ 100% confidence intervals for either the β s or e^{β} s. Most statistical software packages also supply the χ^2 tests and P values. The SAS code for this model analysis is given below.

SAS code for Parts A and B, and the Combined analysis

```
/*=====
In the code below, removing the “*” before the “ods listing select ...”
statements will limit output only to the results presented in this paper;
otherwise default output appears.
=====*/
proc sort data=SIMDAT1 ;
by D R M ID ;
run ;
```

```

/*----- Part A analyses -----*/
title "Unmodified animals only: by Radiation Dose (D)" ;
title2 "Log-rank Analyses" ;
proc lifetest data=SIMDAT1 plots=s ;
by D ; /* Performs analysis for each value of D */
where M=0 ; /* Allows only the data with M=0 */
strata R ;
time T*DIED(0) ;
* ods listing select homtests;
run ;
title2 "CPH Analysis" ;
title3 "Table 2: HRS, their CIs, and 'Score' p-values" ;
proc phreg data=SIMDAT1 ;
by D ; where M=0 ;
model T*DIED(0) = R / ties=discrete ;
hazardratio R ;
* ods listing select globaltests hazardratios;
run ;
title "Unmodified animals only: across Radiation Dose (D)" ;
title2 "CPH Analysis" ;
title3 "Table 2: HRS, their CIs, and 'Score' p-values" ;
proc phreg data=SIMDAT1 ;
where M=0 ;
model T*DIED(0) = R D / ties=discrete ;
hazardratio R ;
assess ph var=(D) / resample=1000 crpanel seed=8172011 ;
* ods listing select parameterestimates hazardratios
ProportionalHazards SupTest;
run ;
/*----- Part B analyses -----*/
title "Modified animals only: by Radiation Dose (D)" ;
title2 "Log-rank Analyses" ;
proc lifetest data=SIMDAT1 plots=s ;
by D ; where M=1 ;
trata R ;
time T*DIED(0) ;
* ods listing select homtests;
run ;
title2 "CPH Analysis" ;
title3 "Table 3: HRS, their CIs, and 'Score' p-values" ;
proc phreg data=SIMDAT1 ;
by D ; where M=1 ;
model T*DIED(0) = R / ties=discrete ;
hazardratio R ;
* ods listing select globaltests hazardratios;
run ;
title "Modified animals only: across Radiation Dose (D)" ;
title2 "CPH Analysis" ;
title2 "Table 3: HRS, their CIs, and 'Score' p-values" ;
proc phreg data=SIMDAT1 ;

```

```
where M=1 ;  
model T*DIED(0) = R D / ties=discrete ;  
hazardratio R ;  
assess ph var=(D) / resample=1000 crpanel seed=8172011 ;  
* ods listing select parameterestimates hazardratios  
ProportionalHazardsSupTest ;  
run ;
```

FIG. 1. Survival of hypothetical animals treated with radioprotectant (RP) or not (no RP) at 10, 11, and 12 Gy of radiation for unmodified (panel A) and modified (panel B) groups.

FIG. 2.

Estimated survival derived from Cox PH models for hypothetical animals treated with radioprotectant (RP) or not (no RP); panels A and B for the unmodified and modified groups, respectively. Estimates shown for 10.6 Gy of radiation – the LD_{50/10} for control animals.

TABLE 1Detectable Hazard Ratios (HRs) with 0.80 Power Using Tests Conducted at the $\alpha = 0.05$ Level

<i>N</i>	Irradiation dose	Analysis method	Detectable HR ^a	
			(unadjusted α)	(adjusted α)
16	10	Log-rank	0.176	0.134
16	11	Log-rank	0.209	0.164
16	12	Log-rank	0.228	0.182
48	10, 11, 12	CPH regression	0.401	0.401 ^b

^aHR = radioprotectant to control hazard. HR < 1 indicates radioprotectant-treated animals have a decreased hazard of death, compared to the hazard of control animals. HRs closer to (either side of) 1 indicate smaller effect sizes.

^bSince there is only one test, no adjustment for multiple comparisons is needed.

TABLE 2

Estimated Hazard Ratios of Radioprotectant to Control in the Absence of the Modifying Factor

Analysis method	Irradiation dose	<i>N</i>	Estimated HR and 95% CI	Unadjusted <i>P</i>
Log-rank	10	16	0.614 (0.200, 1.885)	0.390
Log-rank	11	16	0.593 (0.198, 1.773)	0.346
Log-rank	12	16	0.340 (0.093, 1.251)	0.094
CPH regression	10, 11, 12	48	0.473 (0.249, 0.900)	0.023

TABLE 3

Estimated Hazard Ratios of Radioprotectant to Control in the Presence of the Modifying Factor

Analysis method	Irradiation dose	N	Estimated HR and 95% CI	Unadjusted <i>P</i>
Log-rank	10	16	2.131 (0.684, 6.644)	0.183
Log-rank	11	16	0.697 (0.241, 2.016)	0.504
Log-rank	12	16	1.098 (0.361, 3.340)	0.869
CPH regression	10, 11, 12	48	1.107 (0.598, 2.050)	0.746

TABLE A1

Hypothetical Data

ID	R	M	D	T	DIED
1	0	0	10	13.0	1
4	0	0	10	10.0	1
6	0	0	10	25.0	1
8	0	0	10	26.5	1
18	0	0	10	10.5	1
20	0	0	10	30.0	0
22	0	0	10	14.5	1
24	0	0	10	11.0	1
1	0	1	10	20.5	1
3	0	1	10	20.0	1
5	0	1	10	30.0	0
7	0	1	10	10.5	1
17	0	1	10	28.0	1
19	0	1	10	15.0	1
21	0	1	10	30.0	0
23	0	1	10	14.5	1
10	1	0	10	10.0	1
12	1	0	10	26.0	1
14	1	0	10	30.0	0
16	1	0	10	30.0	0
26	1	0	10	29.0	1
28	1	0	10	10.0	1
30	1	0	10	24.0	1
32	1	0	10	19.0	1
9	1	1	10	16.5	1
11	1	1	10	15.0	1
13	1	1	10	28.0	1
15	1	1	10	13.0	1
25	1	1	10	24.0	1

ID	R	M	D	T	DIED
27	1	1	10	11.5	1
29	1	1	10	17.0	1
31	1	1	10	12.0	1
34	0	0	11	24.5	1
36	0	0	11	11.0	1
38	0	0	11	9.0	1
40	0	0	11	13.5	1
50	0	0	11	18.0	1
52	0	0	11	8.0	1
54	0	0	11	13.5	1
56	0	0	11	5.0	1
33	0	1	11	11.5	1
35	0	1	11	18.5	1
37	0	1	11	8	1
39	0	1	11	7.5	1
49	0	1	11	23	1
51	0	1	11	10	1
53	0	1	11	7.5	1
55	0	1	11	9	1
42	1	0	11	14	1
44	1	0	11	24.5	1
46	1	0	11	13	1
48	1	0	11	20.5	1
58	1	0	11	12.5	1
60	1	0	11	19.5	1
62	1	0	11	11	1
64	1	0	11	18	1
41	1	1	11	13.5	1
43	1	1	11	6.5	1
45	1	1	11	13.5	1
47	1	1	11	17	1

ID	R	M	D	T	DIED
57	1	1	11	5.5	1
59	1	1	11	23.5	1
61	1	1	11	15.5	1
63	1	1	11	15	1
66	0	0	12	10	1
68	0	0	12	9	1
70	0	0	12	8.5	1
72	0	0	12	5	1
82	0	0	12	8.5	1
84	0	0	12	8.5	1
86	0	0	12	7.5	1
88	0	0	12	9.5	1
65	0	1	12	8.5	1
67	0	1	12	13.5	1
69	0	1	12	5.5	1
71	0	1	12	10.5	1
81	0	1	12	11.5	1
83	0	1	12	8.5	1
85	0	1	12	5.5	1
87	0	1	12	7	1
74	1	0	12	6.5	1
76	1	0	12	9.5	1
78	1	0	12	6.5	1
80	1	0	12	12.5	1
90	1	0	12	5.5	1
92	1	0	12	13.5	1
94	1	0	12	18	1
96	1	0	12	13.5	1
73	1	1	12	6	1
75	1	1	12	7	1
77	1	1	12	8.5	1

ID	R	M	D	T	DIED
79	1	1	12	5	1
89	1	1	12	13.5	1
91	1	1	12	5.5	1
93	1	1	12	9	1
95	1	1	12	13	1

Notes. ID uniquely identifies the hypothetical animal; R indicates the absence (0) or presence (1) of the radioprotectant; M indicates the absence (0) or presence (1) of the modifier; D is the total body irradiation dose in Gy; T is the survival time in days; DIED indicates whether the animal died (1) before the end of the study or survived (0).

TABLE A2
CPH Model for the Analysis Combining Both M and R , Evaluated at the Indicated Values of M and R

M	R	Remaining portion of the regression model			Row
0	0	$\beta_0 +$	$\beta_4 D$		1
0	1	$\beta_0 +$	$\beta_1 +$	$\beta_4 D$	2
1	0	$\beta_0 +$	$\beta_2 +$	$\beta_4 D$	3
1	1	$\beta_0 +$	$\beta_1 +$	$\beta_2 +$	4