A Biological Approach to the Interspecies Prediction of Radiation-Induced Mortality Risk

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Abstract

Background: Evolutionary explanations for why sexually reproducing organisms grow old suggest that the forces of natural selection affect the ages when diseases occur that are subject to a genetic influence (referred to here as intrinsic diseases). When extended to the population level for a species, this logic leads to the general prediction that age-specific death rates from intrinsic causes should begin to rise as the force of selection wanes once the characteristic age of sexual maturity is attained. Results consistent with these predictions have been found for laboratory mice, beagles, and humans where, after adjusting for differences in life span, it was demonstrated that these species share a common age pattern of mortality for intrinsic causes of death. In quantitative models used to predict radiation-induced mortality, risks are often expressed as multiples of those observed in a control population. A control population, however, is an aging population. As such, mortality risks related to exposure must be interpreted relative to the age-specific risk of death associated with aging. *Purpose:* Given the previous success in making interspecies predictions of age-related mortality, the purpose of this study was to determine whether radiation-induced mortality observed in one species could also be predicted quantitatively from a model used to describe the mortality consequences of exposure to radiation in a different species. *Methods:* Mortality data for $B6CF_1$ mice and beagles exposed to ^{60}Co γ-rays for the duration of life were used for analysis. The only statistical manipulation of the mortality data for the beagle was the generation of Kaplan-Meier survival curves and their 95% confidence intervals for each of the observed dose rate groups. A proportional hazard model was used to describe the dose-response of the $B6CF_1$ mouse. This model was then used to predict survivorship curves at dose rates that would give mice the same total

exposure burdens that the longer lived beagles received. A Gompertz distribution was fit to the control data for each species in order to estimate the median age at death from intrinsic causes. The ratio of the Gompertz medians was used as a scaling device to adjust for differences in the life span of the mouse and beagle. Finally, the scaled survivorship curves predicted from the mouse dose-response model and the survivorship curves observed for the beagle were plotted together to see whether the mouse predictions fell within the confidence intervals observed for the beagle. *Results:* After adjusting for differences in exposure burden and life span, it was demonstrated that age-specific mortality in irradiated beagles could be predicted from the mortality experience of comparably exposed mice. *Implications:* The successful interspecies extrapolation of mortality risks for species as different as the mouse and dog enhances both the value of studies involving laboratory animals and the potential relevance of these studies to the prediction of human health effects.

Introduction

Laboratory animals are and have been used to study both the positive and the detrimental health effects of foods, vitamins, chemicals (natural and man-made), and radiation exposure. An explicit assumption underlying these studies is that the biological effects observed apply to more than one species, thus justifying the use of animal models for the prediction of human health effects. The prediction may be no more than determining whether exposure to a particular agent is harmful, but more often there are regulatory pressures to produce quantitative estimates of human health and mortality risks for specific biological effects (e.g., cancer)(1). When human data are available, results derived from laboratory animal studies may be judged unnecessary. When human data are

unavailable, the relevance of animal data to the prediction of human health effects is sometimes questioned (2, 3).

One fundamental problem making the interspecies extrapolation of health and mortality risks difficult is that every species has a unique genome that affects virtually every aspect of its biology (e.g., growth and development, reproductive biology, physiology, aging, life span). Inherent differences between species in these basic biological characteristics (host factors) create particular problems for the interspecies study of toxic and carcinogenic agents that must be metabolized in order to manifest their harmful effects. Host factor complexities are reduced in the case of exposure to radiation because harmful effects occur through routes that involve either direct hits to DNA or DNA damage caused by the free radicals formed when radioactive particles interact with water.

Humans exposed to radiation from a variety of sources (e.g., sunlight, drinking water, building materials, medical and dental treatments and diagnostics, Chernobyl, and the bombing of Hiroshima and Nagasaki) have created a public health need to understand the biological consequences of exposure to radiation. Experimental studies involving laboratory animals have been the only way to systematically acquire this knowledge. In order to utilize the knowledge gained, theoretical justifications (4-7) and methods (6, 8-13) had to be developed that would allow comparisons and predictions of radiation effects to be made between species. The biologically motivated approach described in this report contributes to this body of prior work by illustrating that age-specific risks for radiation-induced mortality can be extrapolated across species — extending the generality of an approach that has already been used successfully to make interspecies predictions of age-specific mortality associated with aging (14).

Motivation

In quantitative models used for the prediction of radiation-induced mortality, risks are often expressed as multiples of those observed in a control population. A control population, however, is an aging population. As such, risks related to exposure must be interpreted relative to an age-specific risk of death associated with growing old — the biological basis of which has been explored at length by evolution biologists (16-18).

A basic tenet of modern evolution biology is that the force of natural selection alters the genetic composition of a population through the differential reproductive success of individuals. Evolution theory predicts that, starting with the age of sexual maturity, the ability of selection to affect the frequency of competing alleles in a population begins a progressive decline that continues throughout the reproductive period of a species (15-18). Thus, diseases that are subject to a genetic influence (referred to as intrinsic diseases), whether inherited or acquired, should also be subject to the forces of natural selection (17) — leading to the prediction that age-specific death rates from intrinsic diseases should rise for populations as the force of natural selection wanes (14).

This logic forms the basis for the evolutionary explanations for the existence of senescence or aging (see refs. 19, 20 for a review) and has been extended to populations in order to study age-related patterns of mortality within and between species (14). For example, intrinsic mortality signatures (age-specific death rates arising from intrinsic causes) have been shown to remain unchanged for populations of laboratory mice even though changes in animal husbandry over time caused significant shifts in total mortality (14). It was also shown that age-specific mortality for laboratory mice, beagles, and humans could be described by a single intrinsic mortality signature

when death times for these organisms were normalized (scaled) to a common median age at death from intrinsic causes (14).

Since unexposed populations of different species appear to share a common intrinsic mortality signature (14), it was of interest to determine whether exposure to radiation modifies the intrinsic mortality signatures of different species in the same way. A successful interspecies prediction of radiation-induced mortality has important implications for the establishment of exposure standards for radiation and would enhance the perceived relevance to humans of studies involving laboratory animals.

Data

This study would not be possible if it were not for a unique assemblage of mortality data for laboratory animals meticulously compiled in an extensive database located at the Argonne National Laboratory. The data come from experiments that were conducted over a 50-year period in the Division of Biological and Medical Research in order to investigate the biological effects of exposure to radiation (21). The laboratory animals selected for this comparative mortality analysis are the $B6CF_1$ (C57BL/6 ♀ x BALB/c ♂) mouse and the beagle (Table 1).

Mice entered the study at approximately 100 days of age. They were then exposed to ^{60}Co γ rays for the duration of life at 1.17, 2.34, 5.4, or 10.8 cGy/d (22). Beagles entered the study at approximately 400 days of age and were exposed to ^{60}Co γ-rays for the duration of life at 0.3, 0.75, 1.88, or 3.75 cGy per day (23). Exposure rates for mice and beagles are expressed as average absorbed dose at the midline of the body.

Exact birth and death dates were recorded for both mice and beagles. Detailed necropsy and/or histopathological information for the $B6CF_1$ mouse utilized 9 codes describing the most frequently observed neoplastic diseases and 12 codes identifying the most prevalent acute or chronic infectious and degenerative diseases. For the beagle, specimens from all major organs were routinely fixed for histochemical and histologic classification. Based on an evaluation of all clinical, hematological, necropsy, and histopathological findings, a cause of death was assigned for each dog. Deaths caused by infectious and communicable diseases, inflammatory diseases, amyloidosis, environmental stress, accidents, wounds, and iatrogenic events were declared extrinsic causes of death. All other causes of death were defined as intrinsic (see ref. 14, 24 for more detail).

Analysis

All analyses used the individual death times of the animals. Using the method of maximum likelihood, a Gompertz distribution was fit to the control population of the $B6CF_1$ mouse and the beagle, with extrinsic deaths treated as censored observations. The two parameters estimated for the Gompertz distribution of each species were then used in the cumulative survivorship function, S(t), in order to estimate a median age at death from intrinsic causes (i.e., set S(t) to 0.5 and solve for t; mouse = 898 days, beagle = 4948 days). The resulting ratio of control medians (5.51 dog days/mouse day) was used for all scaling.

The $B6CF_1$ mouse was selected as the predictor species. A proportional relationship between the hazard function for the control population of mice, $\lambda_0(t)$, and the hazard function for each of the irradiated groups of mice, $\lambda(t;z)$, was assumed (25):

$$\lambda(t;z)=\lambda_0(t)\,e^{[f(z)]} \qquad (1)$$

where t is age at death (in days), f(z) is a linear function of two covariates (age at entry and dose rate), and $e^{[f(z)]}$ is the relative risk term of the model. Once coefficients for the two covariates were determined, the dose-response model for the $B6CF_1$ mouse was then used to generate predicted survival curves (26) for an average age at entry (101 days) and for dose rates that would result in the same accumulated dose at the same relative points of the life span as observed in the beagle (i.e., 5.51 times the daily dose rate to which the beagle was exposed).

The beagle was selected as the species whose mortality risks associated with exposure to radiation were being predicted. The only statistical manipulation of the mortality data for the beagle was the generation of Kaplan-Meier survival curves and their 95% confidence interval for each of the observed dose rate groups (27). Next, for each survival curve predicted from the dose-response model for the mouse, the failure times of the mice were multiplied by the scaling factor in order to convert them from mouse days to dog days. Finally, for each dose rate group in the beagle, the observed Kaplan-Meier survival curve (with 95% confidence intervals) for the beagle and the scaled survival curve predicted from the dose-response model for the mouse were plotted together.

Results

Three different estimates of the cumulative survivorship function for the mouse control group (Gompertz, Kaplan-Meier, and dose-response model) were nearly indistinguishable. Similarly, the Gompertz and Kaplan-Meier estimators of the survivorship function of the control population for

beagles were congruent. These results suggest that the median age at death from intrinsic causes used in the scaling factor for both the mouse and dog was reasonably estimated from the Gompertz survivorship function.

When scaled to dog days, the survivorship function predicted for the control population of $B6CF_1$ mice from the dose-response model fell within the 95% confidence interval for the observed survivorship function of the beagle controls (fig. 1). Survivorship functions were predicted for the irradiated mouse populations from the dose-response model evaluated at dose rates scaled to equal the accumulating dose burdens observed in the beagle. After scaling to dog days, these predicted survival curves for the mouse also fell within the 95% confidence intervals for the observed survivorship functions of the irradiated beagle populations (fig. 2).

Discussion

An approach used successfully for an interspecies comparison of age-specific mortality associated with aging (14) was generalized in this paper to make quantitative predictions of radiation-induced mortality from the $B6CF_1$ mouse to the beagle. Although a scaling factor was used to adjust for differences in life span and accumulated dose, no attempts were made to adjust for such host factor effects as differences in radiation sensitivity between the species.

At least two situations might be expected to give rise to host factor differences that would affect interspecies extrapolations. The analyses presented here were based on daily exposure levels of radiation where the biological effect was expected to depend only upon the accumulated dose received rather than upon the rate at which the dose was delivered (28). At higher daily dose rates (e.g., 10-20 cGy/d or higher) where acute radiation syndromes arise and the repair capacities of

species are more severely challenged, differences in species' response would be expected.

Species' differences in mortality risks would also be expected at finer levels of pathology resolution (e.g., a specific type of neoplasm) (9). The specific diseases that constitute the intrinsic mortality signature of a species reflect its unique genetic constitution. Because intrinsic causes of death each compete for the lives of individuals along the time axis of the life span, a specific disease observed in more than one species need not occur at the same relative position in the life span even if an identical etiology and pathogenesis was responsible for all occurrences of that disease. As such, the extrapolation of mortality risks across species for specific pathologic events may fail even though these events, when considered collectively (i.e., intrinsic mortality), can be extrapolated from one species to another.

The predictions made between mouse and beagle in this paper were relatively uncomplicated by differences in environment, diet, exposure conditions, and medical care. These confounding factors as well as others can, however, quickly become serious issues when predicting human health effects from those observed in laboratory animals. For example, if age at exposure is important, the animal cohort data may only be useful for predicting mortality risks for humans exposed within a restricted age range (e.g., young adults). In addition, comparable pathology for comparative analysis can be difficult to acquire because cause of death for humans is most often determined by the attending physician and only rarely by autopsy, whereas these determinations for laboratory animals are generally made only after histological examination and then only by a limited number of pathologists. These issues are currently being investigated by the authors as mortality patterns

among laboratory mice receiving single exposures of ^{60}Co γ rays are being used to predict patterns of mortality among the Japanese survivors of the atomic bombs in Hiroshima and Nagasaki.

In this report, a scaling factor for the comparison of intrinsic mortality between species was presented based on previous research suggesting that the median age of intrinsic mortality occurs at a comparable time point within the life span of species (14). Using this scaling factor, it was demonstrated that age-specific intrinsic mortality in beagles exposed to ^{60}Co γ-rays for the duration of life could be predicted quantitatively from the relative risk model used to describe the dose-response of comparably exposed $B6CF_1$ mice. These findings demonstrate that mortality risks for radiation exposure can be extrapolated across species that are as different as mouse and dog. The modeling approach used here will provide a basis for making quantitative predictions of age-specific radiation-induced mortality in humans from those observed in comparably exposed laboratory animals.

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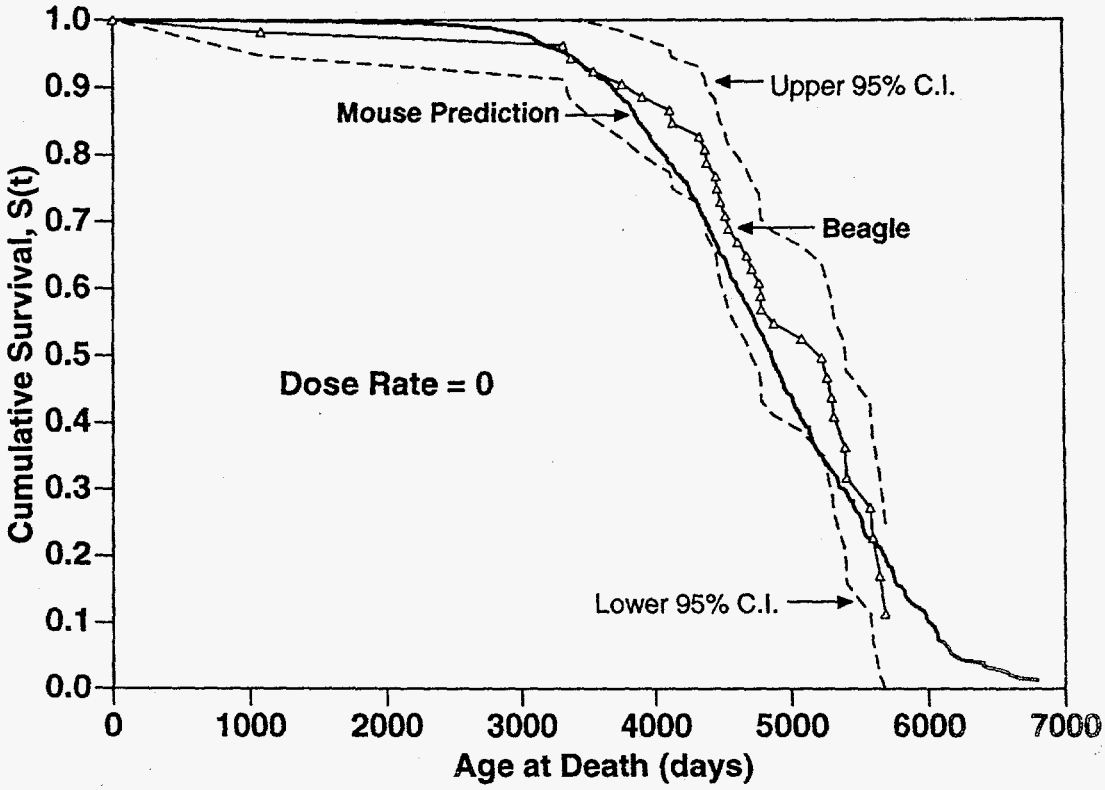
Table 1. Summary information for the $B6CF_1$ mouse and beagle populations used in the prediction from mouse to dog of age-specific radiation-induced mortality following daily exposure to ^{60}Co gamma rays for the duration of life.

Animal	Daily Dose Rate (cGy/d)	N	Intrinsic Deaths	Average Age at Entry (days)	Gompertz Median (days)
$B6CF_1$	0.00	280	229	97	898
	1.17	287	260	99	847
	2.34	170	148	106	861
	5.40	176	150	102	736
	10.80	81	68	104	612
Beagle	0.00	56	34	449	4948
	0.30	92	64	405	4602
	0.75	46	39	403	4072
	1.88	47	44	396	3311
	3.75	24	23	513	2656

Figure Captions

Figure 1. Cumulative survivorship curve (scaled to dog days) for $B6CF_1$ control mice predicted from the dose-response model (equation 1), and the empirical survival curve (with 95% confidence intervals) observed for the beagle control population.

Figure 2. Cumulative survivorship curves (scaled to dog days) for $B6CF_1$ irradiated mice predicted from the dose-response model (equation 1) evaluated at dose rates that give the same accumulated doses as the beagle, and the empirical survival curve (with 95% confidence intervals) observed for each of the irradiated beagle populations.


1.0
0.9
0.8
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0.5
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0.3
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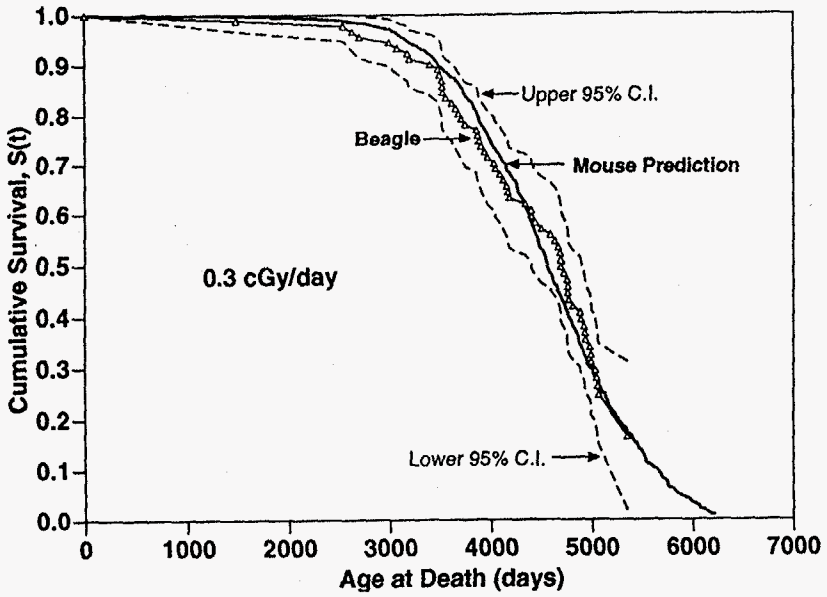
0.0
Cumulative Survival, S(t)
Upper 95% C.I.
Mouse Prediction
Beagle
Dose Rate = 0
Lower 95% C.I.
0
1000
2000
3000
4000
5000
6000
7000
Age at Death (days)


Cumulative Survival, S(t)
1.0
0.9
0.8
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6000
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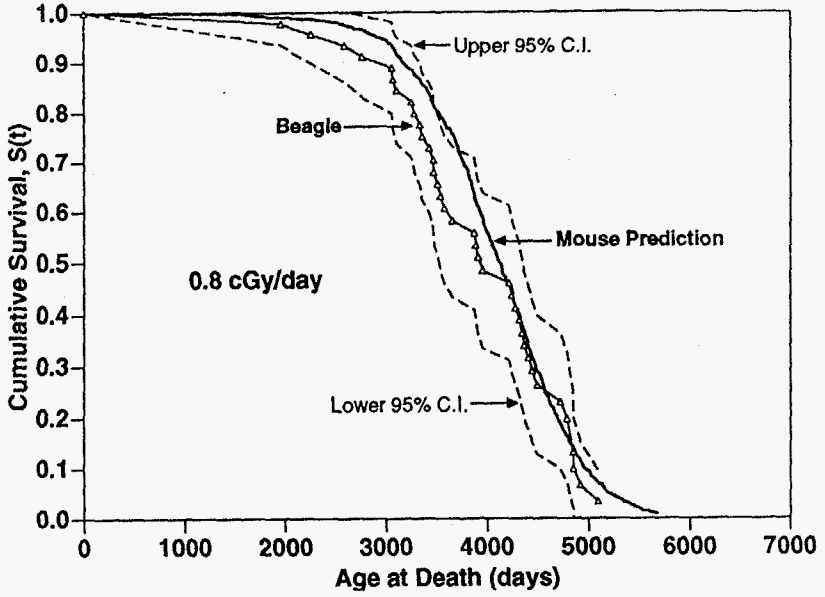
Age at Death (days)
Upper 95% C.I.
Beagle
Mouse Prediction
0.3 cGy/day
Lower 95% C.I.


Cumulative Survival, S(t)
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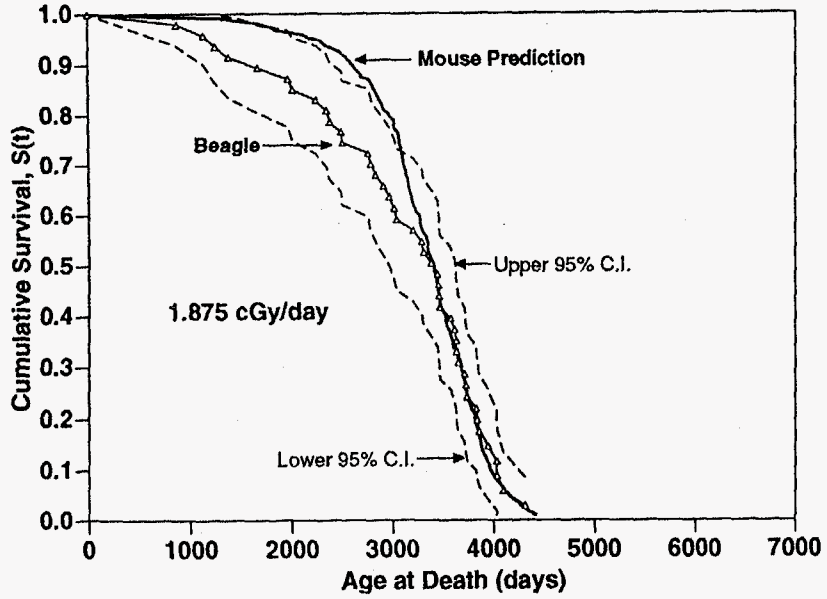
Age at Death (days)
Upper 95% C.I.
Beagle
Mouse Prediction
0.8 cGy/day
Lower 95% C.I.


Cumulative Survival, S(t)
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1000
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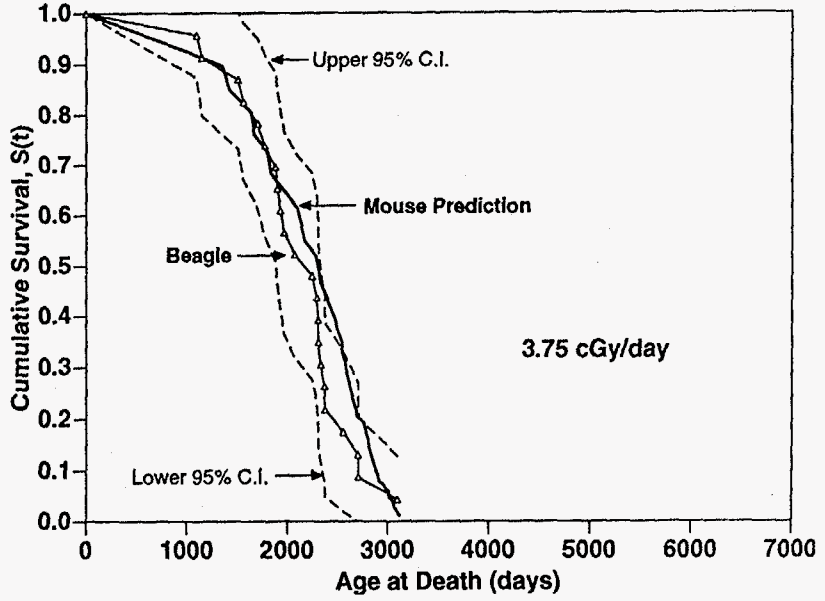
7000
Age at Death (days)
Mouse Prediction
Beagle
Upper 95% C.I.
1.875 cGy/day
Lower 95% C.I.


Cumulative Survival, S(t)
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5000
6000
7000
Age at Death (days)
Upper 95% C.I.
Mouse Prediction
Beagle
3.75 cGy/day
Lower 95% C.I.