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How Protraction Moderates Radiation Risk in Animal Mortality Studies

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Abstract

Radiation is a ubiquitous health risk. Contemporary populations are exposed to several hundred milliSieverts per person over their lifetimes from both natural and human made sources such as radon, cosmic rays, CT-scans, etc. Risk estimates based on studies of atomic bomb survivors suggest that these exposures induce excess cancer mortality at a rate of several percent per Sievert.

To develop accurate risk estimates, it is important to recognize that contemporary exposures are different than atomic bomb survivor exposures. Instead of a single acute high dose rate exposure from an atomic explosion, populations today experience many small, protracted exposures accumulating to moderate total doses over their lifetimes. Therefore, in order to estimate the risk of contemporary exposures using atomic bomb survivor data, it is important to determine the differences in radiation dose response following acute vs. protracted exposures.

The committee to estimate the biological effects of ionizing radiation exposure in humans (BEIR) is one of the central authorities in the United States tasked with estimating radiation risk. Their seventh and most recent report (BEIR VII) written in 2006 estimated that contemporary protracted exposures induce 1.5 fold less risk than atomic bomb survivor exposures.

The work presented in this dissertation leverages a large body of historical animal mortality data to argue that BEIR VII overestimates the risk of protracted exposures.

Concretely, evidence is presented from animal exposures that support the concept that contemporary protracted exposures induce about 2 fold less risk than atomic bomb survivor exposures.

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Gayle and Tanja are my mentors. They taught me how to research – from radiation biology to wine party statistics. Anna is my best friend. We eat tater tots sometimes. Mom and Dad raised me. They taught our family cards. Dave, Fred, Warren, and Wayne advised this work. They've listened to my talk four times and still pay attention.

Steve organizes our graduate program. He keeps everything running. U.S. taxpayers support us all. We try to do them right. Three billion years of ancestors connect us back to pools of pre-biotic soup. The sun powers it all.

I'm grateful.

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Chapter 1: Introduction

Why radiation matters

Ionizing radiation exposure is a ubiquitous health risk. The National Council on Radiation Protection (NCRP) estimates that Americans were cumulatively exposed to 2.7 million Sieverts (Sv) of non-therapeutic ionizing radiation in 2006 alone [1]. Note that a Sievert is equal to 1 Gray (Gy) for X-ray and γ-ray exposures, low-linear energy (low-LET) transfer types of radiation, which are the subject of this study.

In 2006, the US National Research Council organized a committee to estimate the Biological Effects of Ionizing Radiation (BEIR). This committee's 7th report, BEIR VII, stated that the risk of fatal cancer development in a population increases 3-12% per Sievert of low dose or protracted ionizing radiation that the population is exposed to [2]. A four fold difference between the high and low ends of this risk estimate makes it difficult to judge how much effort should be expended to protect society from radiation exposures [3–5]. Moreover, some recent epidemiological evidence has suggested that risks may be underestimated [6] while a recent critique of the BEIR VII methodology suggested that risks may be overestimated [7]. Details of these contradicting claims are discussed below.

While many of the epidemiologic studies focused on protracted occupational exposures, a significant increase in contemporary annual (non-therapeutic) exposures per person is due to medical imaging technologies like computed tomography (CT), nuclear medicine, and fluoroscopy. These medical imaging exposures now constitute roughly

50% of the total dose to the population in America. By comparison, in 1980 diagnostic radiation exposures contributed less than 10% of the cumulative US population dose [1,3,8,9]. While accrual of a total yearly exposure dose by protracted occupational exposure vs. medical diagnostic exposures may correlate with different biological/medical endpoints, in the BEIR VII report, and most radiation protection recommendations, all such protracted and low dose acute exposures are presumed to induce equal risk per dose.

It is critical to understand the risks of radiation in order to guide efforts to reduce exposure – especially in light of rising medical exposures.

Estimating low dose and protracted risk from acute high dose data

Most national and international radiation protection agencies use data from the lifespan study (LSS) of Japanese atomic bomb survivors as their primary source to estimate cancer risk following radiation exposure. Survivors experienced excess risks of cancer development and mortality that increased with the dose received (Figure 1).

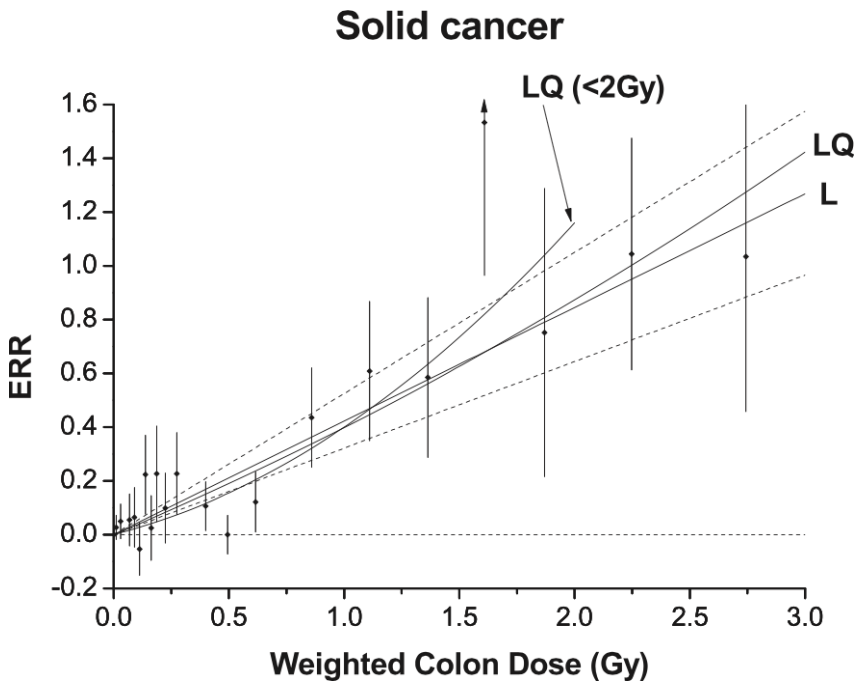


Figure 1: Excess relative risk of solid cancers as a function of dose in atomic bomb survivors

Reprinted with permission from Fig 3 of "Studies of the mortality of atomic bomb survivors, Report 14, 1950-2003: an overview of cancer and noncancer diseases." [10].

Estimated excess relative risk (ERR - equal to relative risk minus one) of solid cancer development vs. mean total colon dose for atomic bomb survivors. These estimates represent the risk of solid cancer development by age 70 to a person exposed at 30 years of age after controlling for the influence of gender and city (Hiroshima vs. Nagasaki) using models specified by Ozasa and others [10]. Black points represent central estimates for each exposure group. Vertical bars represent 95% confidence intervals. Linear (L) and

linear-quadratic (LQ) dose response models were both fit to the data and appear as labeled. A linear-quadratic model fit to doses below 2 Gy is shown as well (LQ (<2Gy)). The apparent quadratic component of ERR increase with dose is most pronounced for LQ model limited to data from exposures less than 2 Gy.

Unlike the high-dose acute A-bomb exposures that inform radiation risk models, most contemporary exposures are low-dose exposures. Acute exposures greater than 20 mSv are rare [11]. Instead, people receive many small low dose rate exposures that result in cumulative lifetime exposures at moderate total doses of a few hundred mSv. Notably most of these exposures derive from medical imaging and natural sources. European and American radiation protection groups limit work exposures to less than 100 mSv over a lifetime.

The health effects of these acute low dose and protracted moderate dose exposures are challenging to estimate. Analysis of atomic bomb survivor data does not provide sufficient information to formulate firm guidelines because the risk per individual at doses lower than 20 mSv is too small to be detected with statistical significance [12] and because all atomic bomb survivors were acutely exposed. Other epidemiological datasets developed to explore the risk of protracted exposures do not have sufficient statistical power to resolve these questions definitively [6]. Therefore, the health risks of low dose and protracted exposures are currently estimated based on the health consequences observed following acute, high dose exposures and a model of the relationship between dose, risk, and protraction. In the BEIR VII report, and most contemporary radiation protection guidelines, this model is the linear-quadratic model that was originally developed to describe effects of high does rate therapeutic radiation exposures.

Most radiation protection groups estimate the risk of low dose and protracted exposures by applying linear-quadratic dose response models to data from atomic bomb survivors and animal studies as described below. The work reported here argues that it is possible (and necessary) to estimate the risk of protracted exposures more accurately by using additional exposure data and a linear, not linear-quadratic, dose response model with separate slopes for acute and protracted exposures – a model this work refers to as a linear-linear model.

Protraction often reduces risk, though not always.

While it is challenging to directly measure the long-term effects of low-dose and protracted radiation exposures in humans, there are multiple reasons to suspect that protracted exposures might induce less risk than dose matched acute exposures. In this work, mouse and rat mortality studies were analyzed in order to estimate the relative risk of protracted exposures, but other lines of evidence are also informative. The fact that fractionation reduces tissue toxicity (used daily in therapeutic clinical practice) and reduces induction of lethal radiation syndromes provides evidence that protraction reduces risk in many circumstances; this suggests, by analogy, that protracted delivery of moderate and low doses may reduce the risk of other long term health consequences such as carcinogenesis and mortality. The mechanisms responsible for this difference in the effects of protracted vs acute exposure are most likely due to cellular and DNA

repair/recovery processes that are known to occur between radiation doses in a fractionated exposure.

At the most general level, it is well known that dangerous things often become less dangerous when exposure to them is spread out over time. Acute exposures to alcohol, carbon dioxide, or even water can all be lethal [13–15]. However, the same agents are non-lethal when the exposure consists of many small doses delivered over time. While the body has mechanisms to cope with frequent low-dose exposures to many substances or stresses, these defenses can be overwhelmed by acute high-dose exposures outside of the range to which an organism has adapted.

This general principle applies to acute radiation toxicity in particular. Whole body exposures to gamma rays or X-rays at doses higher than 3-4 Gy are usually lethal to humans and many other animals in the absence of medical intervention [16]. These exposures are deadly because critical cell populations are eradicated, most notably rapidly dividing bone marrow and intestinal mucosal cells. When stem cell populations in these tissues become small enough, they lose the ability to repopulate depleted cell populations in the organs they support. Within days or weeks functional differentiated cell populations become depleted by natural processes and, because they are not restored from stem cell stocks, the organs fail. Therefore, acutely exposed individuals may die from a failure of the hematopoietic and gastrointestinal systems, organs that depend the most on cell replenishment. If identical doses are delivered as fractions or low dose rate exposures protracted over time, these critical cell populations remain sufficiently

numerous and healthy to carry out their life-maintaining roles. Therefore, for animals, multi-cellularity is a critical defense mechanism that resists radiation toxicity.

Protraction is relevant at a cellular level too. Specifically, protraction reduces the risk of reproductive cell death as well as the accumulation of chromosomal aberrations in individual cells, predominantly through the induction of cellular repair processes [17–19]. However, no level of protraction can completely eliminate these risks to a cell. To understand why this is, it is important to consider the mechanism by which radiation causes cell damage.

The most critical form of radiation damage to cells are double stranded DNA breaks [20]. Two or more such breaks, close together in time and space, often lead to misrepair and chromosomal damage that can result either in reproductive cell death (which can cause tissue and organ dysfunction) or continued propagation of the damage which is presumed to be a precursor to cancer development [21].

Any exposure poses a risk for cells because even a single photon or particle of ionizing radiation may induce two neighboring double stranded DNA breaks. The probability of this kind of DNA damaging event increases linearly with dose regardless of the rate of protraction.

During acute high dose rate exposures, the DNA of a given cell may be damaged by multiple different ionization events. Therefore, there is a chance that two double stranded DNA breaks will occur where each break comes from a separate ionization event. The risk of this kind of DNA damage rises quadratically with total dose, the square

of the number of photons or particles that could potentially interact with the DNA. Unlike the risk from isolated tracks of radiation, this quadratic element of risk can be completely attenuated by protraction because isolated double stranded breaks are more easily correctly repaired than concurrent neighboring double stranded breaks.

Notably, some forms of radiation are just as dangerous to cells when protracted as when delivered acutely. So called high linear-energy-transfer radiation (such as neutron radiation, n°) leads to a dense track of ionization events so that even a single neutron is likely to cause two sets of double stranded breaks all on its own [21]. The damage to DNA is most often so extensive locally that no complete cell repair is possible regardless of the time that the cell is allowed to attempt to repair itself. The cell loses the ability to divide following damage from a single high energy particle. In this case, protracted exposures are just as damaging as acute exposures. While atomic bomb survivors mostly received low linear energy transfer gamma ray exposures, where protraction would reduce risk to cells, this cannot be extrapolated to high LET exposures.

In summary, there is a range of possible responses to an equal total dose of radiation and the effect of protraction on acute radiation toxicity, cellular DNA integrity, and overall cell health status is profound. Protraction reduces risk when cells or tissues are capable of repairing the damage radiation causes, either through DNA repair in a single cell or repopulation within a tissue. But when a single track of radiation causes critical damage on its own, or if repair is impossible, then protraction does not reduce risk.

It is challenging to estimate from the known mechanisms of radiation damage whether the long-term health consequences of radiation exposure, like carcinogenesis and life-shortening, should be reduced by protraction. Are the relevant risk factors associated with structures (or functions) that are repairable? Does repair simply depend on time? What is the relevance of multiple sites of damage within a same cell? These questions are hard to answer because the mechanisms that bridge cellular damage, carcinogenesis, and aging are not fully understood. Even if these systems were understood better, it would still be challenging to *quantitatively* estimate how much protraction moderates risk of ionizing radiation exposure. Therefore, estimating the effect of protraction requires empirical evidence. This thesis focuses on lifespan mouse and rat studies where all the risks are evaluated summarily (as life shortening) in controlled experiments that were designed to study effects of radiation protraction. While these studies have been extensively analyzed to estimate the risk of protraction, no work to date has combined so many sources into a coherent quantitative model of risk.

BEIR VII's dose response model

Several national and international agencies estimate the risk of low dose exposures. This thesis, especially its first aim, is focused closely on the work of one of these committees, the BEIR VII committee in the US. The radiation effects model, used by BEIR VII, described in this section and the next section, does not fit the observed

animal data. Moreover, this work argues that protracted exposures induce less risk than BEIR VII estimates indicate.

To evaluate the risk of low dose and protracted exposures, the BEIR VII report committee employed a linear-quadratic dose response model to estimate that the dose and dose rate effectiveness factor for the lifespan study of atomic bomb survivors ($DDREF_{LSS}$) is between 1.1 and 2.3 with a most probable value of 1.5 [2]. Acute dose-responses are divided by this $DDREF_{LSS}$ value in order to estimate the risk of contemporary low dose and protracted exposures. Therefore, the BEIR VII report estimates that contemporary exposures carry 1.5 fold less risk per Sv than acute exposures to atomic bomb survivors.

The BEIR VII dose response model, illustrated in Figure 2.a, predicts that the risk of carcinogenesis and mortality following exposures to X-rays or γ-rays in the moderate dose range (below 1.5 or 2 Gy) is the sum of two components, one that increases linearly with dose and another that increases quadratically with dose, where the linear and quadratic coefficients, α and β, are estimated based on observed data.

As argued in the introduction the linear-quadratic model dose-response model cannot be justified from biological principals because the full details of mechanisms of carcinogenesis and aging are unknown. This work further challenges the linear-quadratic model on empirical grounds. It argues that the data are better modeled by two separate linear dose response models one for acute high dose rate exposures and another for protracted low dose rate exposures (Figure 2.b).

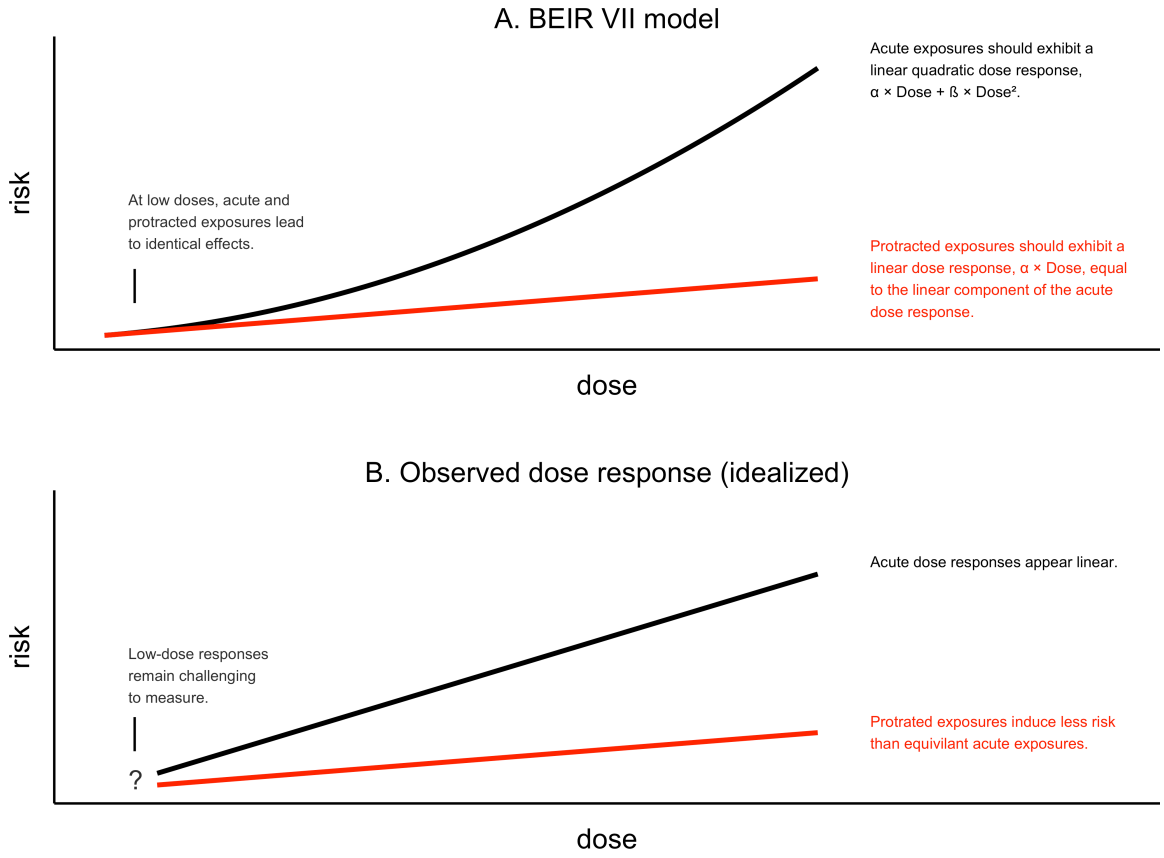


Figure 2: Two possible dose response models based on linear-quadratic model (a) and linear-linear model (b)

a. A schematic representation of a linear-quadratic dose response model, like the one used in the BEIR VII report, is shown above **b.** an idealized representation of the results of the analysis in aim 1. Each panel shows dose (x-axis) vs. risk (y-axis) in which risk represents the excess risk of carcinogenesis or organism mortality. Black lines represent the response to acute exposures. Red lines represent the response to protracted exposures. Both are applicable to exposures of less than 1.5 Sv or 2.0 Sv, the maximum doses considered in the BEIR VII report and in this chapter. While the linear-quadratic model

predicts that protracted dose-response can be estimated based on the curvature of acute dose response, the results presented here show that this is not the case. Also, while the linear-quadratic model predicts that responses to low dose exposure are collinear with responses to protracted exposures, the linear-linear model is inconclusive with regard to low dose exposures.

The linear-quadratic model assumes that the linear component of risk is unavoidable regardless of the pattern of exposure, while the quadratic component is attenuated at low doses or when an exposure is delivered over sufficient time to allow repair of initial damage before additional damage occurs. Therefore, the risk following a low dose, low dose rate, or fractionated exposure is described by only the linear risk component, α, of a corresponding acute exposure.

Concretely, if an exposure is fractionated into distinct equally sized acute exposures separated by enough time for maximum repair, then risk is:

$$risk \sim \alpha \cdot dose + \frac{\beta \cdot dose^2}{fractions}$$

where "fractions" is the number of distinct fractions that a dose has been divided into.

Using the linear-quadratic model, DDREF can be calculated by dividing risk from acute irradiation with the risk of protracted dose exposures.

$$\begin{aligned} DDREF &= \frac{acute\ risk}{protracted\ risk} \\ &= \frac{\alpha \cdot dose + \beta \cdot dose^2}{\alpha \cdot dose} \\ &= 1 + \frac{\beta}{\alpha} \cdot dose \end{aligned}$$

By this definition, DDREF is a function of the ratio between quadratic and linear coefficients, β/α, and dose. As formulated, it can be derived from direct comparisons of protracted and acute exposures. However, because acute exposure risk depends on linear and quadratic terms both, DDREF can also be derived using the linear quadratic model from acute exposure data alone. For example, given the linear-quadratic model, the risk of contemporary protracted exposure could be extrapolated from acutely exposed atomic bomb survivors. This is done by estimating α and β terms based on a quadratic fit to the data and then by extrapolating the risk of protracted exposures from the α term. The more curved the graph of risk from an acute dose is, the higher the DDREF estimate will be. Notably most of the data that BEIR VII used to estimate DDREF came only from acute exposures.

According to the linear-quadratic model, DDREF depends on dose. The DDREF applicable to atomic bomb survivors, $DDREF_{LSS}$, must be calculated across the range of exposure doses that survivors received, though excluding exposures greater than 3 or 4 Gy - exposures which are excluded from estimates of long term radiation risk because they are sufficient to induce acute radiation toxicity. The BEIR VII report showed that $DDREF_{LSS}$ is nearly equivalent to DDREF at 1.08 Sv [2]. Therefore, $DDREF_{LSS}$ is approximately $1 + 1.08 * \beta / \alpha$. The same approximation is employed throughout this work.

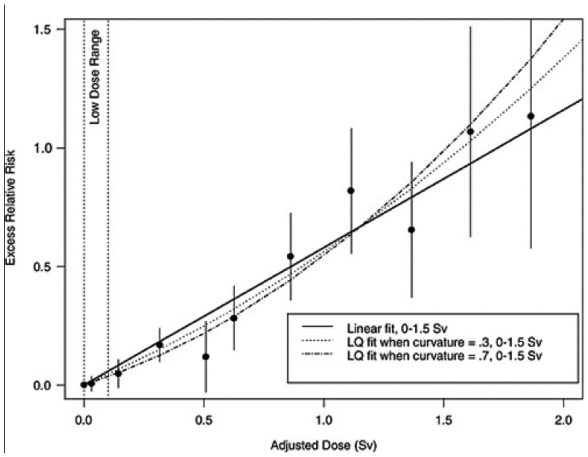
Finally, the BEIR VII model includes the notion that there is interference between cancer induction and reproductive cell death (which includes both cell killing and terminal senescence) following acute exposures to doses greater than 1.5 Sv or 2 Sv. According to this assumption, response to acute exposures deviates from the linear quadratic model at doses above these thresholds. Specifically, actual risk is lower than projected by linear quadratic fits due to reproductive cell death that terminates cells that might otherwise become neoplastic. Therefore, BEIR VII estimated $DDREF_{LSS}$ based only on data for exposures less than 1.5 Sv or 2.0 Sv; these two different cutoffs were used for different datasets, however a rationale for these particular cutoffs was not provided.

BEIR VII's data sources and $DDREF_{LSS}$ estimates

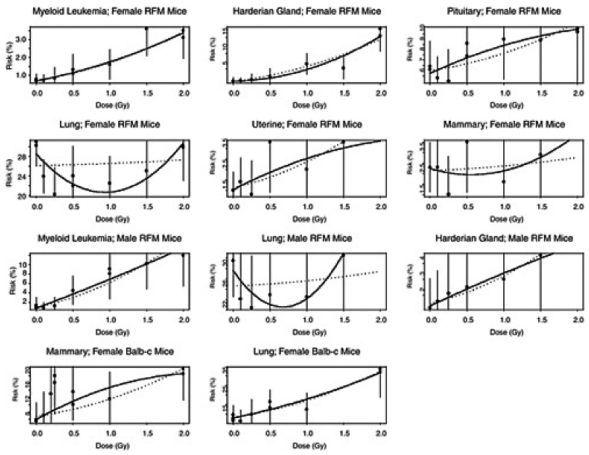
The BEIR VII report fit linear-quadratic dose response models to three distinct data sets: excess cancer incidence rates in atomic bomb survivors exposed to doses less than 1.5 Sv, cancer incidence rates in 11 animal studies with exposures up to 2 Sv, and mortality rates in 2 animal studies with total exposure doses less than 1.5 Sv. Figure 3, reproduced from the BEIR VII report, shows linear-quadratic fit and $DDREF_{LSS}$ estimates for each of these data sets. The profile-likelihood method was used to estimate the relative likelihood of different $DDREF_{LSS}$ values from each data source and Bayesian update was used to combine these separate estimates into one central estimate: $DDREF_{LSS}$

equal to 1.5 with a 95% credible interval from 1.1 to 2.3. Full details of the techniques employed by BEIR VII committee are provided in Chapter 6. An effort to reproduce the same analysis is detailed in Chapter 2.

A. LSS Carcinogenesis: $DDREF_{LSS}$ ~ 1.3 (0.8, 2.4)



B. Animal Carcinogenesis: $DDREF_{LSS}$ ~ 1.4 (1.1, 2.6)



C. Animal Mortality: $DDREF_{LSS}$ ~ 2.0 (1.3, 7.7)

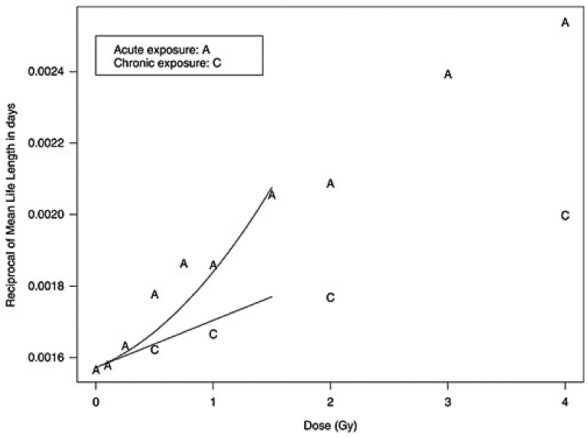


Figure 3: BEIR VII $DDREF_{LSS}$ estimates from 3 data sources

Reproduced with permission from Figs 10-2, 10B-2, and 10B-3 of "Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2" [2]. Linear-quadratic models used for $DDREF_{LSS}$ evaluation fit to three data sources, (A) excess risk of carcinogenesis in atomic bomb survivors, (B) risk of tumor development in various animal studies, and (C) inverse mean lifespan in two animal studies. All panels show dose (x-axis) vs. various measurements of risk (y-axis). Best-fit linear-quadratic models are shown for each model. The LSS carcinogenesis data show best-fit models with various curvatures. Animal carcinogenesis data show best-fit models for each panel individually (solid black lines) and the consensus curvature across all panels (dashed lines). The animal mortality data show the single best-fit model with both acute (linear-quadratic) and protracted (linear) projections. Only animal mortality data included both acute, "A", and protracted, "C", exposures. Above each panel, $DDREF_{LSS}$ estimates derived from the corresponding data source are shown with 95% credible intervals in parenthesis. These estimates were combined (using Bayesian update) to form BEIR VII's central estimate, $DDREF_{LSS} \sim 1.5$ (1.1, 2.3).

Different radiation protection agencies have used a mixture of dose response models

Other national and international agencies, in addition to the BEIR VII committee, have made their own efforts to estimate the effects of low dose and protracted radiation exposures. Most of their estimates have employed linear-quadratic models to some degree.

The 2006 report from the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) used both linear-quadratic and linear-quadratic-exponential (an "S shaped" dose-response) fits to atomic bomb survivor data in order to estimate the risk of a range of exposure doses [22]. These models predict that low dose exposures carry less risk, corresponding to $DDREF_{LSS}$ values of 1.2 to 2.85 (depending on the model and the outcome).

This thesis concludes that a linear-quadratic model, like the one UNSCEAR used in some cases, is not appropriate for estimating the relative risk of protracted exposures. However, the linear-quadratic-exponential model that UNSCEAR also employed that predicted less risk following low-dose exposures than the linear-quadratic model predicted, might be appropriate. If it was determined that protracted exposures and low-dose exposures induce the same risk per Gy, then such a model could explain the observations presented in this work. Chapter 5 discusses this possibility in detail.

The National Council on Radiation Protection 1980 report estimated that $DDREF_{LSS}$ was between 2 and 10 based on an analysis of animal studies [23]. This report directly compared linear fits to acute exposure data with linear fits to protracted exposure

data to estimate $DDREF_{LSS}$. While this technique is the one recommended in this thesis, it is important to note that NCRP's analysis had two significant flaws. It failed to account for age at exposure and was not systematic in combining results from multiple studies.

NCRP's failure to account for age at exposure is the more problematic, because radiation-induced cancer risk decreases with age of exposure. Animals given protracted exposures tended to be older (for any except the first exposure) than comparison groups given acute exposures. This is so because, in most studies, acute radiation was delivered to animals of the same age as the age of the first exposure for protracted exposure animals. Therefore, subsequent (protracted) exposures were delivered to older animals.

NCRP's $DDREF_{LSS}$ value range, 2-10 is also problematic. It simply describes the range of $DDREF_{LSS}$ values calculated from different animal mortality and carcinogenesis studies (see Table 9.3 in the report). No attempt was made to estimate confidence intervals for each of these studies. Moreover, no attempt was made to combine the results of the different studies using meta-analysis techniques that would weight these individual estimates by their confidence. Therefore, the $DDREF_{LSS}$ range estimated by the NCRP is biased upwards by a failure to account for age at exposure and biased in unknown directions by outlier studies that were implicitly given equal weight to high confidence studies.

Finally, the International Commission on Radiological Protection uses a $DDREF_{LSS}$ value of 2. This choice was informed by the work of the NCRP from 1980

detailed above, combined with a linear-quadratic model fitted to atomic bomb survivor data [24].

Ultimately, all of these dose response estimates have flaws either because they rely too heavily on linear-quadratic dose-response models or because of other problems with their analyses. Notably the UNSCEAR linear-quadratic-exponential model, if generalized to both low dose and protracted exposures, is compatible with results described here. Nevertheless, there is not enough evidence available to show clearly that dose response fits some particular model. Therefore, this thesis takes the cautious approach of comparing linear fits to acute and protracted exposures as in NCRP's 1980 report, rather than attempting to extrapolate protracted risk based on acute observations and a model of dose response.

Estimates of contemporary risk should be improved

Several factors make it challenging to measure the risk of contemporary radiation exposures. There is uncertainty in the estimates of the dose that atomic bomb survivors received, in radiation sensitivities of Japanese populations vs. world populations, the value of $DDREF_{LSS}$, the relative effectiveness of partial vs. whole body irradiation as well as other possible issues. Of these, BEIR VII estimates that uncertainty in the risk of low-dose and protracted exposures is the dominant source of uncertainty in the estimate of the

risk of contemporary exposures [2]. A more recent report from UNSCEAR agrees with this conclusion [25].

Recent literature on $DDREF_{LSS}$ highlights these uncertainties. Several studies in the last 6 years have suggested that BEIR VII's $DDREF_{LSS}$ estimate may underestimate risk (the $DDREF_{LSS}$ value is too high), which would imply that low dose and protracted radiation exposures pose more of a health risk than the current estimates indicate. Jacob and others performed a meta-analysis in 2009 that found that workers exposed to protracted radiation and atomic bomb survivors exposed to acute radiation showed comparable increases in cancer risk for the same total exposure dose [6]. This result, albeit with substantial uncertainty, implies that acute and protracted exposures are equally dangerous, that $DDREF_{LSS}$ is close to 1, and that existing radiation protection standards underestimate the risk of radiation exposure.

A more recent follow up study corroborates Jacob's findings. The International Nuclear Workers Study (INWORKS) updated cancer mortality estimates for more than 300 thousand workers from France, the United Kingdom, and the United States [26]. They estimated that the risk of solid cancer mortality increases by 0.48 (0.2, 0.79) per Sv. This is on the high end of risk estimates from atomic bomb survivors, 0.42 (0.32, 0.53) ERR/Sv for a 70 year old exposed at age 30 [10]. If true, they would imply that these protracted exposures were more dangerous than the acute atomic bomb survivor exposures and $DDREF_{LSS}$ is less than 1!

Based on these studies and other arguments, the German Commission on Radiological Protection (SSK) has recommended that $DDREF_{LSS}$ corrections should not be used to estimate the risks of low dose and protracted exposures [27,28].

On the other hand, two other studies suggest that BEIR VII's estimate of $DDREF_{LSS}$ may be too low. For one, a pooled study of US nuclear weapons facilities workers, published after Jacob's analysis, estimated that the lifetime excess relative risk of fatal solid cancer development is 0.14 (-0.17, 0.48) per Sievert [29]. This is substantially lower than the risk estimates from atomic bomb survivors, 0.42 (0.32, 0.53) ERR/Sv for a 70 year old exposed at age 30 [10]. Roughly, this study of US workers suggests that the most likely value of $DDREF_{LSS}$ is 3, though again with substantial uncertainty.

Hoel (2015) also argued that the $DDREF_{LSS}$ estimate made by the BEIR VII report is too low, and that plausible alterations to the BEIR VII assumptions result in $DDREF_{LSS}$ estimates at or above 2, the number adopted by the International Commission on Radiological Protection (ICRP) [7,24]. Part of Hoel's argument is based on the observation by Little in 2008 that a linear-quadratic-exponential ("S" shaped) dose response describes the atomic bomb survivor data better than a linear-quadratic dose response [30].

Hoel's observation is an example of a general point. If protracted dose response is extrapolated from acute dose response, then the shape of the dose-response function has a substantial influence over estimates of low total dose and protracted exposure risk.

Unfortunately, the true shape of the dose-response function for carcinogenesis and mortality is still subject to substantial debate. This risk model represents only one of the possible approaches to estimate low dose and protracted exposure risks [9]. A variety of alternative models could have been used for the same range of doses, each with distinct implications for the risk of low total dose and protracted exposures [31].

Ultimately, the true dose response function is difficult to derive. In part, this is caused by the fact that cellular responses and whole organism responses to radiation exposure are complex. In addition to chromosomal re-arrangements, irradiation of cells, tissues, and whole organisms leads to other mutations [32], epigenetic changes [33], genomic instability [34], adaptive effects [35], hypersensitivity [36], and off-target effects [37,38]. Even if the cellular and tissue level radiation response was completely understood, estimating the carcinogenesis and mortality dose response curves would be difficult because the molecular, cellular, and tissue level mechanisms that lead to cancer and mortality are also not completely understood [39,40]. Even if the most relevant forms of cellular level radiation damage follow a linear-quadratic dose response it is not certain that the dose-response of different whole organism endpoints such as cancer induction and life shortening could be described by the same formula.

Notably, even the proponents of the linear-quadratic dose response model, like BEIR VII, limit its use to describing dose-responses below some total dose limit (e.g. 2 Sv), above which it is assumed that cell reproductive death mitigates dose response.

Finally, it should be mentioned that the linear-quadratic model as applied to cellular systems describes cell reproductive death in response to therapeutically relevant doses of radiation. In radiation oncology it is often necessary to convert one therapeutic radiation regimen with another and linear quadratic models served as a basis for such conversions [41]. Therefore, the primary application of the linear quadratic model is on data obtained for high dose rate/high dose per fraction exposures. It is therefore not surprising that applicability of the linear quadratic model to low dose or protracted exposures lower than 1.5 Sv total dose is not guaranteed.

Specific aims

This thesis represents an effort to fulfill the need for better estimates of the risk of protracted exposures and to provide evidence for the hypothesis that "**protracted exposures induce less risk than BEIR VII's estimate.**" To test this hypothesis this thesis developed two aims:

Aim 1. Re-estimate the relative risk of protracted exposures using BEIR VII's linear-quadratic methodology applied to archived animal data not previously used by BEIR VII. The European Radiobiology Archives (ERA) and Janus tissues archives contain 16 rat and mouse mortality studies that fit BEIR VII's inclusion criteria, 15 of which were not included in the original BEIR VII analysis. This data was curated

and developed into a dataset suitable for analysis that was then done following the approach used by BEIR VII committee. The goal of this effort was to improve the precision of BEIR VII's estimate and also test the validity of their dose-response model.

Aim 2. Estimate the relative risk of protracted exposures using studies that included both acute and protracted exposures to doses up to 4 Sv while accounting for age at exposure [2,10]**.** This aim is designed to closely mirror the analysis of atomic bomb survivor data by the radiation effects research foundation (RERF) so that the risk of protracted exposures might be extrapolated from that data. To accomplish it, a second dataset was curated, again from the ERA and Janus archives with data from 14 studies. The data included exposures up to 4 Sv, the maximum doses sometimes analyzed in atomic bomb survivor data (as opposed to Aim 1 which only includes data up to 1.5 Sv). Data was limited to studies that directly compared acute and protracted exposures or studies that exposed animals at different ages. The effects of protraction and age at exposure were estimated as linear and exponential multipliers to observed dose responses; this approach too was adopted from RERF approach and will be justified in more detail later. However, unique to this work, data was limited to studies that directly compared acute and protracted exposures or studies that exposed animals at different ages, an approach to analysis that is not applicable to data used by RERF which consists solely of acute exposures.

Aim 1 Summary: Additional data discredit use of the linear-quadratic model (and BEIR VII's $DDREF_{LSS}$ estimates)

The original goal of the first aim was to improve the estimate of $DDREF_{LSS}$ by increasing the pool of data used to estimate it. The animal mortality dataset used for BEIR VII report consisted of 17,322 mice from two studies conducted at Oak Ridge National Laboratory. During the same period there have been dozens of other large, lifespan animal studies that have been conducted to estimate the effects of dose and dose protraction. Efforts by the International Radiobiology Archives [42], The European Radiobiology Archives [43], and the Janus Tissue Archives [44,45] have made many of these datasets readily available on the internet. All of the available archived information was used to establish an expanded animal dataset of 28,289 mice from 16 studies in order to revisit BEIR VII's $DDREF_{LSS}$ estimate with more information. Details of this dataset are described in Chapter 3, Tables 3.1 and 3.2. However, rather than establishing a better estimate of $DDREF_{LSS}$, the results showed that BEIR VII's dose response model did not fit the observed data (Figure 2). Specifically, estimates of $DDREF_{LSS}$ based on the curvature of acute exposure data were low, never significantly greater than 1, implying that protracted and low-total-dose exposures have a similar risk per Sievert as acute exposures. By contrast, estimates of $DDREF_{LSS}$ based on data that directly compared acute and protracted exposures were infinitely high, implying that low dose exposures are neutral with respect to carcinogenesis or life shortening.

The linear-quadratic dose response model does not allow this contradiction. Both ways of determining $DDREF_{LSS}$ should lead to the same estimate, not two significantly different estimates. Therefore, this work questions the validity of the linear-quadratic model and the $DDREF_{LSS}$ estimate that was derived from it.

Attempts to estimate the risk of *protracted* exposures should not assume that these risks could be derived from the apparent curvature of acute responses. Therefore, the component of $DDREF_{LSS}$ that pertains to protracted (but not low-total-dose) exposures, the so-called dose rate effectiveness factor ($DREF_{LSS}$) should be estimated based on direct comparisons of acute and protracted exposures. Data should be selected based on the exposures to atomic bomb survivors, low-LET exposures up to 4 Sv. Models should be based on those used to estimate risk from atomic bomb survivor data including a correction for age at exposure, because animals given protracted exposures were regularly older at the conclusion of irradiation than the acute exposure groups they are compared to.

Ideally, the low-total-dose effectiveness factor (DEF_{LSS}) should also be estimated based on direct comparisons, in this case of high-dose and low dose exposures. Unfortunately, statistical considerations make it challenging to conduct this comparison with meaningful precision. The risk of low dose exposures will probably continue to be extrapolated from the risks observed following high dose exposures. The question of whether these risks are collinear with the risks from protracted exposures (as implicit in the term $DDREF_{LSS}$) should continue to be debated.

Aim 2 Summary: Linear-linear models confirm that protracted exposures pose less risk than BEIR VII estimates.

Aim 2 proceeds from the premise that that attempts to estimate the moderating effects of protraction should be based on analyses that closely mirror atomic bomb survivor analyses, because such estimates are used to extrapolate protracted risk from atomic bomb survivor data. Further, the results of Aim 1 made it clear that the moderating effects of protraction should be based on direct comparison of acute and protracted exposures with a correction for age at exposure. Aim 2 is focused on conducting such an analysis, described in detail in Chapter 4. As in aim 1, results showed that protracted exposures induce significantly less risk than BEIR VII estimated that they do, probably 2.1 (from 1.7 to 2.7 with 95% confidence) fold less risk than the dose response observed in acutely exposed atomic bomb survivors.

The dose response model used for this work is based closely on the model developed by the radiation effects research foundation (RERF). As the agency that conducts the lifespan study of atomic bomb survivors, data analyses conducted by RERF inform the work of most other radiation protection groups. Particularly, the analysis in this dissertation is based on the 2012 analysis by Ozasa et al. [10] but in this case applied to acute and protracted animal exposure data.

The model used to complete this aim, based on the one developed by Ozasa, differs from the model used by the BEIR VII, used for the first aim, in three important

ways. The work in Aim 2 fits a linear dose response model, rather than a linear quadratic model, in order to estimate dose response. Secondly, the data used for Aim 2 are also more inclusive than those used in Aim 1. Ozasa's RERF model uses data from survivors who were exposed to colon doses as high as 3 Sv (~ 4 Sv surface dose), while BEIR VII limited their data to doses below a 1.5 or 2.0 Sv cutoff. Finally, the Ozasa based model used in this work explicitly accounts for the effects of age at exposure. Age at exposure is particularly important because the risk of exposure decreases with age [10]. In most animal studies protracted exposures began at the same age at which acute exposed groups received their first and only exposure. Thus, experimental animal groups exposed to protracted exposures were older than the acute exposure groups at the conclusion of radiation exposure – most of the total dose they received, all but the first exposure, occurred to older animals than the ones from the acute exposure series. Therefore, some of the reduction in dose response that appears in protracted exposure groups can be accounted for as a consequence of differences in age at exposure between acute and protracted treatment groups. Failure to account for this bias inflates the value of $DREF_{LSS}$ as it presumably did in the NCRP analysis discussed earlier.

The critical differences between this model (based on Ozasa's RERF model) and BEIR VII's dose response model are illustrated in Figure 4.

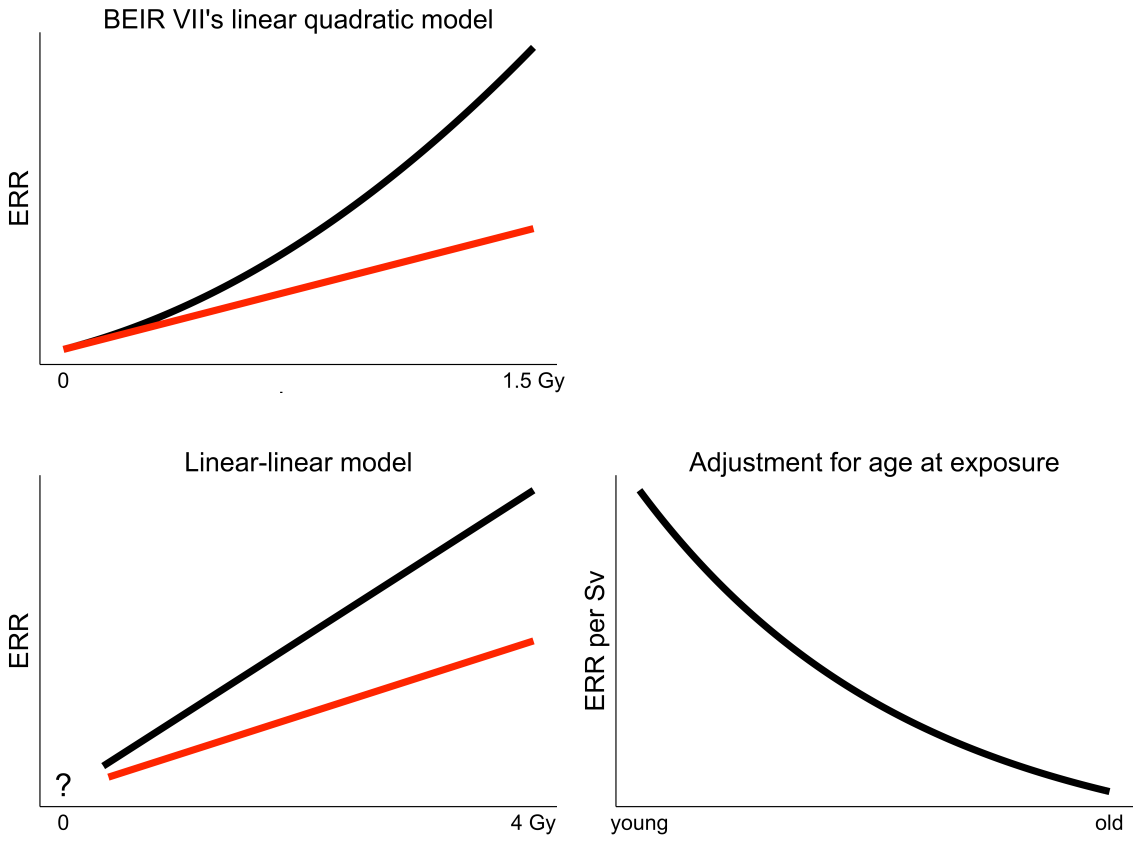


Figure 4: Differences between BEIR VII's dose response model and the RERF based linear-linear model used in this work

Black lines represent acute exposures and red lines represent protracted exposures. The BEIR VII model, represented in panel a, is a linear quadratic model applied to exposures less than 1.5 Sv (or 2.0 Sv) such that protracted risk is proposed to be equal to the linear component of acute risk, and low dose exposures are co-linear with protracted risk. The model proposed in Aim 2, based on atomic bomb survivor analyses, is represented in panels b and c. Like the atomic bomb survivor analysis, it fits a linear model to acute

exposures up to 4 Sv. Protracted exposures are modeled also as linear dose responses and their slopes are compared to those of acute exposures in order to estimate a dose rate effectiveness factor ($DREF_{LSS}$). This thesis does not try to estimate the risk of low-dose exposures that cannot be measured with statistical precision. Finally, as in the atomic bomb survivor analyses, this model explicitly accounts for age at exposure (c).

As in aim 1, archival data suitable for analysis was selected in this case, from 8 distinct studies (Table 6). The Ozasa-RERF based model was applied to these data and $DREF_{LSS}$ was calculated (Table 9). $DREF_{LSS}$ was estimated to be 2.1 with a 95% credible interval from (1.7, 2.7). The analysis was also applied to several different subsets of the data to perform a sensitivity analysis, ensuring that the results obtained were consistent across several possible ways of defining the data set. These results were in agreement with the central estimate and no evidence was found that total dose (with the prior limit of 4 Sv) or acute radiation toxicity influence $DREF_{LSS}$. These findings support the general hypothesis that $DREF_{LSS}$ is significantly greater than the 1.5 central value suggested by the BEIR VII committee. In general, the results agree with the $DREF_{LSS}$ estimate of 2, currently recommended by the NCRP and ICRP.

Chapter 2: Reproducing the original BEIR VII analysis using the same data

Before the BEIR VII's linear-quadratic dose response model could be applied to additional animal data it was important to be sure that all the nuances of the model the committee used were understood. Therefore, this chapter discusses the effort to reproduce the original BEIR VII analysis based on its description in Chapter 10 (pages 246 to 250 and 254 to 258) of the BEIR VII report [2].

Reproducing BEIR VII's analysis was cumbersome for several reasons:

1. Certain methodology details were not clearly specified in the report. Most notably BEIR VII did not make it clear that regressions were weighted by the inverse variance observed in each treatment group. This approach, while justifiable, is not the only approach that could be used. Results from individual treatment groups could have been equally weighted for example, producing different $DDREF_{LSS}$ estimates for reasons unrelated to the data used to generate these estimates.
2. The author of the BEIR VII analysis, Dr. Ethel S. Gilbert, has passed away and it became impossible to clarify methodological details that were omitted from the reports.
3. Only some of the original data used to generate BEIR VII's estimates was available for this analysis (e.g. the formulation of atomic bomb survivor data used in the BEIR VII analysis is not publically available).

4. The BEIR VII report gives limited intermediate results to validate attempts to reproduce the original analysis.

The methods described in the BEIR VII report and the limited data available were used to create a close reproduction of the original BEIR VII analysis. Nearly identical replications of significant tables, figures, and $DDREF_{LSS}$ estimates, from the BEIR VII report were reproduced. Those small discrepancies that remain can be attributed to errors in extracting data from figures or trivial methodological differences like rounding. Regardless of this, the model developed in this chapter produces very similar results to the original BEIR VII analysis. Therefore, the results of Chapter 3, which uses this reproduced BEIR VII model, are faithful to the original BEIR VII analysis.

These efforts are presented in this dissertation in order to subject them to public scrutiny and with the hopes to save future researchers from needing to conduct a similar effort.

Reproducing BEIR VII's animal mortality analysis

As discussed in the introduction, BEIR VII committee used three data sets to form their estimate of $DDREF_{LSS}$. Of these three collections of data only animal mortality data from experiments conducted at Oak Ridge National Laboratory are directly accessible at

this time. These data can be found in Storer et al. 1979 [46]. Therefore, efforts to reproduce BEIR VII's analyses began with this data set.

Two figures that are relevant to BEIR VII's animal mortality analysis are shown in Figures 5 and 6. Top panel of each corresponds to a Figure from BEIR VII report. Figure 5.a (BEIR VII Figure 10B-3) shows the mortality of each treatment group, while Figure 6.a (BEIR VII Figure 10B-4) shows the likelihood of a range of $DDREF_{LSS}$ values necessary to find the most likely value of $DDREF_{LSS}$ and a 95% credible interval for this estimate. The original figures are shown alongside attempts to reproduce (or partially reproduce) this work in b panels of Figures 5 and 6. In the course of this reproduction effort, several unstated steps of the BEIR VII analysis were uncovered. Most notably, mortality regressions were weighted by the inverse variance of lifespan observed in the treatment groups. With this understanding, the other analyses of animal carcinogenesis data and atomic bomb survivor data could also be reproduced as discussed below.

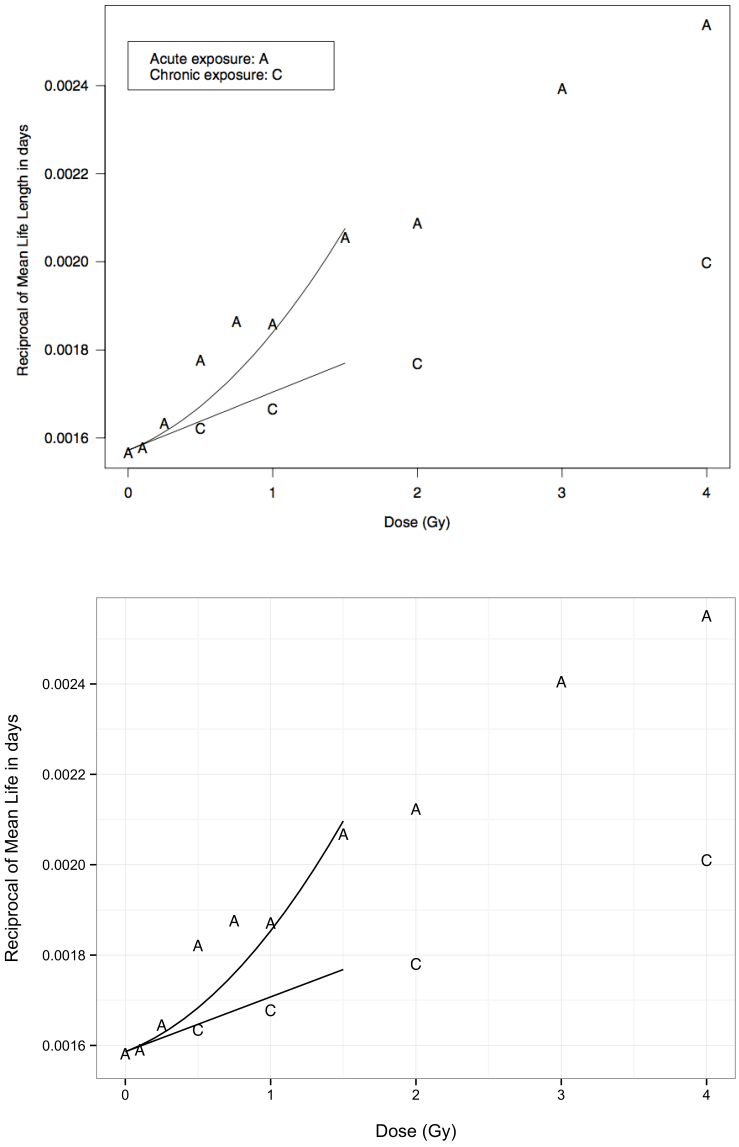


Figure 5: Reproduction of animal mortality dose response, Figure 10B-3 from the BEIR VII report

Reproduced with permission from Figure 10B-3 of "Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2" **a.** The original 10B-3 figure from the

BEIR VII report reproduced with permission, **b.** attempt to reproduce this figure based on the same data and models. Each plot shows exposure dose in Gy on the x-axis vs. the reciprocal of mean lifespan in days, a proxy for mortality, on the y-axis for female RFM mice irradiated at Oak Ridge National Laboratory as reported in Storer et al. 1979 [46]. Acute treatment groups (denoted with the letter "A") from Table 1 of Storer et al. 1979 were exposed at a dose rate of 0.45 Gy/min to total doses indicated on the x-axis. Protracted treatment groups (denoted by the letter "C") from Table 2 of Storer et al. 1979 [46] were exposed to total doses indicated in the figure, but at a dose rate of 0.000069 Gy/min over several days. Notably, acutely exposed female RFM mice from Table 2 of Storer's report do not appear in this figure (a fact omitted from the BEIR VII report). A linear quadratic model fitted to the exposures equal to or less than 1.5 Gy is also shown. This model was fitted so that acute and protracted exposures share the same linear term, and only acute exposures have a quadratic term. The regression was weighted by the inverse variance of the mean lifespan reported in Storer et al. 1979 [46] (a second detail omitted from the BEIR VII report). The two figures, data points and regression fits are nearly identical indicating that this part of BEIR VII's analysis has been successfully reproduced.

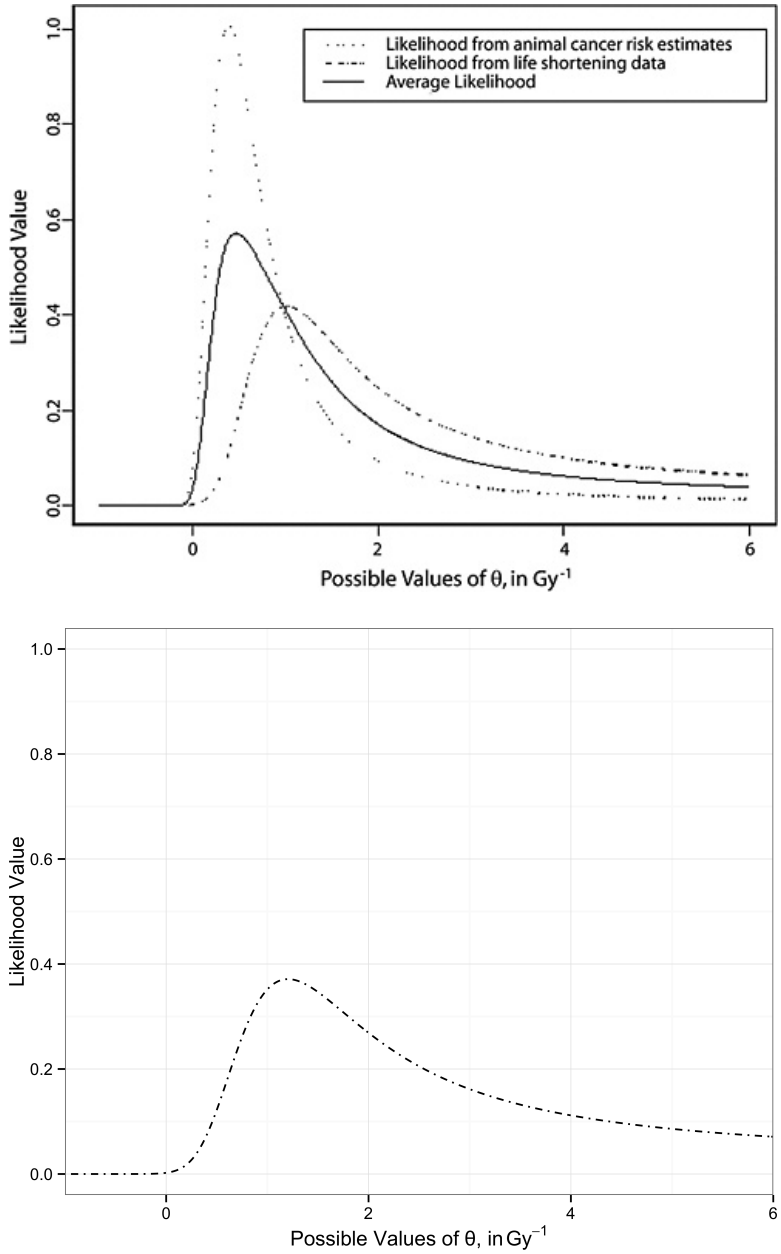


Figure 6: Reproduction of the likelihood profile based on animal mortality data from the BEIR VII's report Figure 10B-4

Reproduced with permission from Figure 10B-4 of "Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2" **a.** Original 10B-4 figure from the BEIR VII report reproduced with permission, **b.** attempt to reproduce the animal mortality portion of the graph in a. using the same method and data as the original BEIR VII report. Each panel shows the relative likelihood (y-axis) of various curvatures (x-axis) where curvatures correspond to the ratio between quadratic (β) and linear (α) coefficients of regression models fitted to dose response data. These curvatures are approximately equal to $DDREF_{LSS} - 1$ according to the BEIR VII report. From these figures credible intervals were estimated (Table 1), from the range of $DDREF_{LSS}$ likelihoods within 14.6% of the peak likelihood. Likelihoods were estimated based on the goodness of fit of treatment groups to the linear quadratic model assuming normal error and weighted by the measured variance in each treatment group. Likelihoods were normalized, so that the area under the curve was equal to 1. In the BEIR VII report models were fit to animal carcinogenesis data (dotted line) and animal mortality data (dot-dashed line). The two profile likelihood curves were also averaged (solid line). The attempt to reproduce the profile curve from animal mortality data, as in Figure 5.b, is shown. The regression models used to generate the likelihood are the same as those described in 2.1.b, the data is very similar. It is worth noting that acute exposure groups from Table 2 of Storer et al. 1979 [46] were used to establish this likelihood profile even though they did not appear in Figure 5, because they fit BEIR VII's inclusion criteria and result in a closer reproduction of the original curve. The resulting reproduction is very similar to the

original, with small remaining discrepancies, for example the peak relative likelihood in the reproduction is slightly less than 0.4 whereas it appears to be slightly higher than 0.4 in the BEIR VII analysis. These remaining discrepancies have not been accounted for, but are considered by the author to be trivial because the figures are otherwise nearly identical.

As mentioned before, the first step in reproducing the animal mortality portion of the BEIR VII analysis is to identify exactly what data were used to produce it. The BEIR VII report describes this data as "Life-shortening data from Tables 1, 2, and 3 of Storer and others (1979)" (pg. 257 [2]) within the "0–1.5 Gy dose range" (pg. 256 [2]). This description is accurate, but incomplete. This work finds that BEIR VII analyzed only female RFM mice from Tables 1 and 2. Male RFM mice and female BALB/c mice were apparently excluded from the analysis even though they could have contributed to a $DDREF_{LSS}$ estimate. It is not clear why these data would be excluded from the analysis. But results from these treatment groups are not depicted in figures nor were these strains explicitly mentioned in the text. Appropriately, none of the mice from Table 3 Storer et al. (1979) [46] were included from the analysis, probably because they were exposed to neutron radiation, not low-linear energy transfer photons (γ -ray or X-rays) that are the subject of the BEIR VII analysis. It appears that Table 3 was listed among the data sources by accident.

The suspicion that only female RFM mice were used was confirmed when reproductions of the curves depicted in Figure 6.a (corresponding to BEIR VII report Figure 10B-4) were produced (Figure 6.b). This reproduction was successful, only when data was limited to female RFM mice. Notably, however, Figure 5.a (BEIR VII Figure 10B-3) did not include acutely exposed female RFM mice from Table 2 of Storer's report [46]. It is not clear why these animals would be excluded from BEIR VII Figure 10B-3,

as they were female RFM mice from the studies under analysis. Perhaps their omission from this figure was a simple oversight by the BEIR VII authors.

Only small errors and omissions were found, in the effort to identify the exact animal mortality data that BEIR VII analyzed. However, it was critical to discover these errors and omissions because the estimate of $DDREF_{LSS}$ depends on the data set under analysis. Once this data set was identified, an attempt to reproduce the $DDREF_{LSS}$ estimate began.

BEIR VII report [2] describes:

> *"…the curvature of interest can be ascertained by fitting an LQ model to the reciprocal of the mean survival.*
>
> *Figure 10B-3 shows the reciprocal mean survival times plotted versus dose, with different plotting characters for means based on acutely and chronically exposed mice. Also shown on the plot are the fits to the model that has the age-specific death rate equal to a constant plus αDose for chronically exposed mice and the same constant plus α(Dose + θDose2) for acutely exposed mice, following the reasoning in the first section of this Annex. (The estimates are maximum likelihood estimates based on normality of the reciprocal means, which are estimates from a large number of mice.) …*
>
> *The (profile) likelihood function for θ is shown in Figure 10B-4…"*
>
> - BEIR VII pg. 255-256 [2]

This description is accurate, but it ignores one crucial detail: these models were fit weighted by the inverse variance in lifespan observed in each treatment group. Thus, groups with more variance in mortality (usually smaller studies) were given less weight in the regression.

Also unstated, chronically exposed animals were apparently treated as if their life shortening had no quadratic component. This would match a situation where fractionation was infinite and is a reasonable approximation in cases where fractionation is high. For example, given the LQ model, the quadratic component of a dose separated into 10 distinct fractions caries only 1% ($1/10^2$) the risk of an acute exposure at the same dose. The exposures analyzed by BEIR were delivered continuously over several days. And so BEIR VII's analysis assumes that this time was sufficient for repair of damage that might contribute to the quadratic component of risk. Essentially, that several days of exposure is equivalent to many fractions (i.e. >10) and therefore that repair takes place over intervals of hours, not days or weeks. This is a reasonable assumption, though not intrinsically obvious.

Only when weighted linear quadratic models assuming infinite fractionation were applied to the Storer et al. [46] data as identified above, were reproductions of the BEIR VII results achieved. A best-fit curve and profile likelihood curves made in this way are very similar to the BEIR VII analysis reproduced (see Figure 5 and Figure 6).

It should be noted that these reproductions are not perfect matches. Each reproduction has small differences from the original. For example, the profile likelihood reproduction in Figure 6.b does not rise quite as high as the original Figure 6.a (BEIR VII Figure 10-B-4, which has a relative peak likelihood above 0.4). While it is possible that these discrepancies represent meaningful differences between the reproduction and the original BEIR VII analysis, it is improbable. First, the differences are slight. Second, the

same model applied to the atomic bomb survivor and animal carcinogenesis data (described below) also provide very close reproductions. Therefore, it is most probable that these remaining incongruities come from small differences in the analysis or presentation of the data, for example through different approaches to rounding or the density with which θ values were sampled. Whatever the case, the original BEIR VII analysis was reproduced very well and so it is likely that applying this reconstructed model to additional data, the goal in aim 1, should result in a comparable $DDREF_{LSS}$ estimate.

Reproducing the full BEIR VII analysis

After successfully reproducing the BEIR VII's animal mortality analysis, the overall analysis was reproduced. This analysis included animal carcinogenesis data, atomic bomb survivor data, and combination of dataset specific observations into one central $DDREF_{LSS}$ estimate.

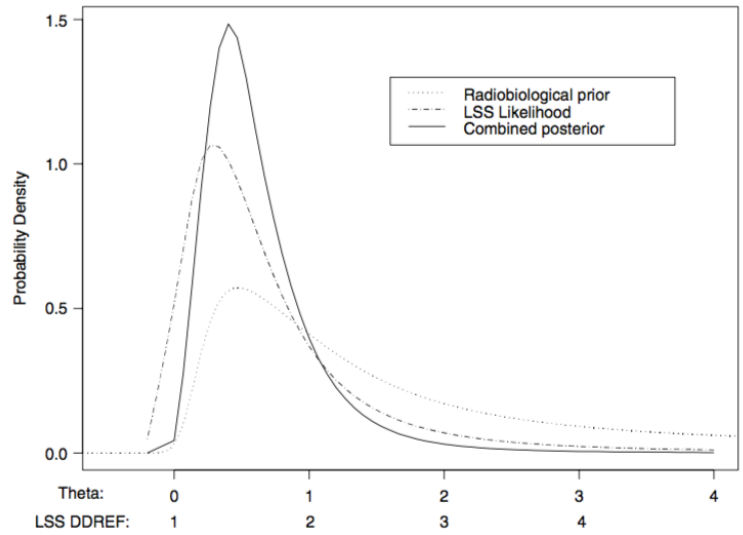
This work was challenging because original data from the data sources used by BEIR VII could not be found. Animal carcinogenesis data came from a technical memorandum published by A.A. Edwards in 1992 for the National Radiological Protection Board [47]. No publically available copy of this memorandum was found nor was one available through the Northwestern University library. Likewise, the atomic bomb survivor data used by BEIR VII are not publically available:

> *"This annex presents details of analyses of data from the LSS cohort of atomic bomb survivors that were conducted to develop these models. Analyses of cancer mortality data were conducted by the committee. Because the most recent cancer incidence data were not yet available outside of RERF, analyses of these data were conducted under the direction of the committee by RERF investigators who served as agents of the Academy."*

- BEIR VII pg. 313 [2]

While the RERF does make atomic bomb survivor data available to the public (at www.rerf.jp/library/dl_e/index.html), BEIR VII's figures could not be reproduced based on publically available data. This was partly because these data are grouped by dose with different cutoffs in the BEIR VII report than in the publicly available data set; also, it is not clear if the observation period for the data in the BEIR VII report is the same as the observation period available for public data sets.

Because original data was not accessible for these parts of the analysis, data were extracted from figures that show dose response for atomic bomb survivors and animal carcinogenesis data (Figures 10-2 and 10B-2 of the BEIR VII report). Using these extrapolated data sets, the overall profile likelihood curves and central estimates of $DDREF_{LSS}$ were reproduced using the same model that had been developed to reproduce the animal mortality data. These reproductions closely approximated the original results and are shown in Figure 7 and Table 1.



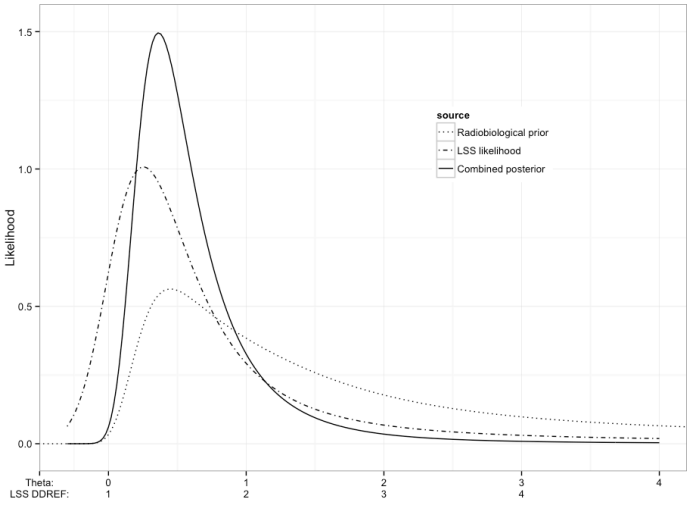


Figure 7: Reproduction of profile likelihood curves used to estimate $DDREF_{LSS}$ from BEIR VII Figure 10-3

Reproduced with permission from Figure 10-3 of "Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2" **a.** BEIR VII's original 10-3 Figure

reproduced with permission, **b.** an attempt to reproduce this figure by applying the BEIR VII dose response model to a combination of original data and data extracted from dose response figures in the BEIR VII report. The likelihood (y-axis) of a range of theta values (x-axis) is shown where theta is the ratio between quadratic and linear coefficients of linear quadratic dose response models fit to animal data (Radiobiological Prior) or atomic bomb survivor data (LSS likelihood). $DDREF_{LSS}$ is approximately equal to theta value plus one. Also shown is the combined posterior, which is a central estimate, formed from these two sub-estimates by Bayesian update. From this central estimate BEIR VII developed their $DDREF_{LSS}$ estimate and credible interval, all values within 14.6% of the maximum likelihood value of the combined posterior.

Estimate of θ	(95 % interval)	LSS DDREF	(95% interval)
Radiobiology animal experiments	0.5 Sv	(0.1, 3.2)	1.5 (1.0, 4.4)
LSS data (0–1.5 Sv dose range)	0.3 Sv	(–0.1, 1.5)	1.3 (0.8, 2.6)
Combined (posterior)	0.5 Sv	(0.1, 1.2)	1.5 (1.1, 2.3)

	Estimate of θ (95% interval)	LSS DDREF (95% interval)
Radiobiology animal experiments	0.5 Sv (0.1, 3.4)	1.5 (1.1, 4.6)
LSS data (0-1.5 Sv dose range)	0.3 Sv (-0.2, 1.4)	1.3 (0.8, 2.5)
Combined (posterior)	0.4 Sv (0.1 1.1)	1.4 (1.1, 2.2)

Table 1: Reproduction of BEIR VII's $DDREF_{LSS}$ estimates from each data source, from Table 10-2

Reproduced with permission from Table 10-2 of "Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2" **a.** BEIR VII's Table 10-2 is presented **b.** an attempt to reproduce this table. Each cell of first column shows central estimates and 95% credible intervals of θ (the ratio of quadratic to linear model coefficients, with units Sv) or (second column) $DDREF_{LSS}$ and 95% credible intervals for animal data, atomic bomb survivor data, or the Bayesian combination of both of these data sources. $DDREF_{LSS}$ is estimated as 1 + 1.08 *θ. Credible intervals correspond to $DDREF_{LSS}$ likelihoods at least 14.6% as large as the maximum $DDREF_{LSS}$ likelihood (see Figure 5.b). Notably these reproductions are not exact, but never differ by more than 0.2 (< 5%)

from the original values. Also note that the columns of the BEIR VII report are misaligned. For example, the column header, “95% interval”, should be “Estimate of θ”. The column headers in the reproduction (b) represent an attempt to guess the original authors intent.

The reproductions developed in this chapter are not perfect, but rather very close to the original curves and estimates. The remaining discrepancies are likely to be trivial and reflect the fact that original data was not accessible and facts about the exact details of the analysis are unknown (e.g. how much precision was used in generating likelihood estimates? How densely were likelihood values sampled?). Regardless of the source of these discrepancies, the method presented here produces results very similar to outcomes reported in BEIR VII report [2]. Therefore, application of these reproduced models to new datasets in aim 1 should be comparable to what they would have been if the original model were known with certainty.

Summary

Attempts to reproduce BEIR VII's analysis exposed two parts of their model that were not clearly specified in their report.

1. Regressions were weighted by the inverse variance of the outcome observed in treatment groups.
2. Animals exposed to protracted radiation over several days were analyzed as if they received infinite fractions and therefore had no component of a quadratic response at all as opposed to some very small

component which would be expected in theory, though is trivial in practice.

Given these two modifications the original $DDREF_{LSS}$ estimates of the BEIR VII report were reproduced with good agreement. Therefore, the new $DDREF_{LSS}$ estimates, as described in Chapter 3 are likely to be comparable to those reported in the original BEIR VII analysis.

Chapter 3: Application of BEIR VII's linear-quadratic model to more animal data

This chapter describes the first aim of this dissertation: Applying BEIR VII's linear quadratic dose response model to additional animal data in order to re-estimate $DDREF_{LSS}$ and therefore the effects of low-dose and protracted exposures. In performing this re-analysis two things were discovered:

1. Protracted exposures induce less risk than BEIR VII's analysis suggested they should. BEIR VII estimated that $DDREF_{LSS}$ is 1.5 and therefore that protracted exposures pose 1.5 less excess relative risk of mortality than acute exposures. This chapter presents evidence that $DDREF_{LSS}$ is higher than 1.5.
2. The linear-quadratic dose response model that BEIR VII used does not fit the observed data. Instead the life shortening data for both protracted and acute exposures appear to have approximately linear dose responses at total doses between 0 and 1.5 Sv, albeit with different slopes.

These findings are elaborated in this chapter. Based on them a new linear-linear model is proposed to estimate the risk of protracted exposures. This new model is applied in Chapter 4.

The expanded animal data set: Included data

28,289 mice in 91 treatment groups from 16 studies were selected to re-estimate $DDREF_{LSS}$ using the BEIR VII dose response model as reproduced in Chapter 2. Inclusion criteria were based on BEIR VII's analysis and are detailed in Table 2 and in the methods of Chapter 6. Treatment groups that were excluded because they could not be validated against published literature are listed in Table 3. In one of the four analyses conducted, the data were further restricted so that groups of mice were only directly compared with each other if they belonged to the same study. This reduced the size of the data set to 20,325 mice in 71 treatment groups as depicted in the last row of Table 2. The survival curves for the animals that were selected for analysis are shown in Figure 8. The details of the study design and lifespan of each treatment group are shown in Table 4.

Studies	Treatments	Animals	Criteria
302	6,810	452,595	All animal data from ERA and Janus archives
124	2,611	205,758	Individual-level animal data available
35	827	116,542	External radiation exposures
35	457	76,096	Low-LET, whole body exposures
34	230	45,730	Total dose equal to or below 1.5 Sv
32	175	43,043	No other treatments (e.g. no chemical exposures)
26	119	34,439	Digitized data on treatment and lifespan confirmed by primary literature
16	91	28,289[a]	At least three distinct treatment groups per stratum so that a linear-quadratic model could be fitted
9	71	20,325[b]	At least three distinct treatment groups after stratifying by study ID

Table 2: Data selection by inclusion criteria

The number of distinct studies, treatment groups, and individual animals that remained eligible for analysis after application of each of the inclusion criteria. Complete definitions of these criteria are elaborated in the methods section.

[a] *dataset used for the "BEIR VII model", "Hormetic correction", and "Heterogeneity correction" models.*

[b] *a more restricted dataset used in the "Stratification by study" and "Survival analysis" models shown in Table 4.*

Treatment group ERA identifier	Reason for exclusion
1003-21-6	This treatment group was abandoned. Cause of death is listed as 'abandon' or 'remove to another experiment'.
11-2-79 11-2-80 11-2-81	Some mice in these treatment groups had impossibly long lifespans, e.g. 6993. This seems to be a coding error in the data. No access to the correct data was available.
1007-3-8 1007-3-16	Mean lifespans differed from those reported in Table 1 of [46] by more than 1 standard deviation. Moreover, there are fewer mice in the ERA dataset than listed by Ullrich and Storer.
3-4 (all treatments)	These groups are identical to those listed in study 3-2.
11-1 (all treatments)	No external data source was found to confirm the treatments and lifespans in this study.
11-2 (all treatments)	No external data source was found to confirm the treatments and lifespans in this study.
3-2 (all treatments)	No external data source was found to confirm the treatments and lifespans in this study. The only source found that details this study [48] was limited to neutron exposures.
1003-xx	No external data source was found to confirm the treatments and lifespans in this study.
9-8	No external data source was found to confirm the treatments and lifespans in this study.

Table 3: Data that could not be confirmed in the literature

Several treatment groups were excluded from the analysis because the ERA or Janus data could not be confirmed in primary literature. The reason(s) for exclusion is listed for each treatment group. ERA study group identifiers denote treatment groups.

Strata in this analysis

Stratum id, sex, strain, quality, age at first exposure, ERA study ids, and references	n	age (μ +/- σ)	Gy	Gy/min	fr.	int.
1 - ♀ RFM/Un Mice ORNL γ-ray at 70 days old 1007-3 [46]	2696	632 +/- 3.2	0.1	0.45	1	-
	930	614 +/- 5.3	0.25	0.45	1	-
	1064	553 +/- 5.3	0.5	0.45	1	-
	237	541 +/- 11.2	0.75	0.45	1	-
	1045	538 +/- 5.4	1	0.45	1	-
	1005	487 +/- 5.8	1.5	0.45	1	-
2 - ♀ B6CF1 Mice ANL γ-ray at 114 days old 1003-20, 21, 22, 24, 25, 26, and 30 [49]	3852	969 +/- 3.1	0	0	1	-
	497	963 +/- 8.7	0.22	0.011	1	-
	346	968 +/- 10.8	0.43	0.022	1	-
	791	919 +/- 6.6	0.86	0.043	1	-
	598	957 +/- 7.7	1	0.001	60	7
3 - ♂ B6CF1 Mice ANL γ-ray at 113 days old 1003-20, 21, 22, 24, 26, 28, 29, and 30 [49]	3275	986 +/- 3.4	0	0	1	-
	585	938 +/- 8.1	0.86	0.043	1	-
	594	971 +/- 7.5	1	0.001	60	7
	160	939 +/- 16.4	1.37	0.069	1	-
4 - ♂ C57BL/Cnb Mice SCK/CEN γ-ray at 84 days old 9-6 [50,51]	467	613 +/- 7.1	0	0	1	-
	241	581 +/- 9.5	0.25	0.3	1	-
	107	605 +/- 15.6	0.25	0.3	10	1
	236	564 +/- 10.2	0.5	0.3	1	-
	109	604 +/- 14.3	0.5	0.3	10	1
	241	550 +/- 9.6	1	0.3	1	-
	104	622 +/- 16.7	1	0.3	8	0.13
	115	615 +/- 13.6	1	0.3	10	1
5 - ♂ RFM/Un Mice ORNL γ-ray at 70 days old 1007-3 [46]	430	711 +/- 7.7	0	0	1	-
	256	720 +/- 10.9	0.1	0.45	1	-
	94	711 +/- 18.1	0.25	0.45	1	-
	247	680 +/- 11.4	0.5	0.45	1	-
	230	673 +/- 11.9	1	0.45	1	-
	199	651 +/- 13.8	1.5	0.45	1	-
6 - ♂ BALB/c/Cnb Mice SCK/CEN γ-ray at 84 days old 9-5 [50–52]	322	766 +/- 8.9	0	0	1	-
	191	745 +/- 13.5	0.25	4	1	-
	111	778 +/- 12.8	0.2	4	10	1

			5			
	194	736 +/- 13.7	0.5	4	1	-
	110	740 +/- 16.2	0.5	4	10	1
	191	732 +/- 10.7	1	4	1	-
	113	751 +/- 15.9	1	4	10	1
7 - ♀ BC3F1 Mice ENEA X-ray at 91 days old 3-1 [53,54]	632	878 +/- 6	0	0	1	-
	100	912 +/- 17	0.04	0.06	1	-
	84	893 +/- 18.3	0.08	0.06	1	-
	53	854 +/- 21.8	0.16	0.06	1	-
	58	874 +/- 24.6	0.32	0.06	1	-
	57	833 +/- 21	0.64	0.64	1	-
	60	707 +/- 23	1.28	0.64	1	-
8 - ♂ C57BL/6Bd Mice ORNL γ-ray at 70 days old 1007-2 [46]	502	906 +/- 6.1	0	0	1	-
	254	909 +/- 8.5	0.5	0.4	1	-
	260	922 +/- 8.2	1	0.4	1	-
9 - ♀ C3Hf/Bd Mice ORNL γ-ray at 70 days old 1007-2 [46]	501	778 +/- 5.8	0	0	1	-
	249	727 +/- 6.9	0.5	0.4	1	-
	250	693 +/- 7.6	1	0.4	1	-
10 - ♀ C57BL/6Bd Mice ORNL γ-ray at 70 days old 1007-2 [46]	491	858 +/- 6.9	0	0	1	-
	253	855 +/- 11.2	0.5	0.4	1	-
	251	865 +/- 10.3	1	0.4	1	-
11 - ♂ C3Hf/Bd Mice ORNL γ-ray at 70 days old 1007-2 [46]	502	732 +/- 5.8	0	0	1	-
	244	713 +/- 7.9	0.5	0.4	1	-
	248	721 +/- 8.7	1	0.4	1	-
12 - ♂ BC3F1 Mice ENEA X-ray at 92 days old 3-5 [53,54]	430	824 +/- 8.8	0	0	1	-
	44	828 +/- 27.4	0.5	0.133	1	-
	48	797 +/- 34.4	1	0.133	1	-
13 - ♂ C57BL/Cnb Mice SCK/CEN X-ray at 7 days old 9-7 [50–52]	105	757 +/- 13.7	0	0	1	-
	72	777 +/- 21.4	0.5	1	1	-
	70	810 +/- 16.1	1	1	1	-
14 - ♂ BC3F1 Mice ENEA X-ray at -4 days old 3-5 [53,54]	34	853 +/- 42.3	0	0	1	-
	48	799 +/- 26.1	0.3	0.133	1	-
	61	822 +/- 27.4	0.9	0.133	1	-
	46	897 +/- 25.4	1.5	0.133	1	-
15 - ♀ BC3F1 Mice ENEA X-ray at -	39	866 +/- 25.7	0	0	1	-

4 days old	40	883 +/- 37.2	0.3	0.133	1	-
3-5	44	850 +/- 22.2	0.9	0.133	1	-
[53,54]	50	872 +/- 30.6	1.5	0.133	1	-
16 - ♂ BC3F1 Mice ENEA X-ray at	41	886 +/- 21.9	0	0	1	-
580 days old	42	901 +/- 21.8	0.5	0.133	1	-
3-5	43	874 +/- 20.2	1	0.133	1	-
[53,54]						

Table 4: Data concordance

A description of data used in this analysis. The first column details the data sources stratified by sex, strain, quality of radiation, and age at first exposure. Strata are organized from most animals (top) to least (bottom) and numbered 1-16 corresponding with the figures in this paper. Also listed are the ERA study IDs corresponding to the data in the strata and references to these studies in the literature. Subsequent columns are further grouped by treatment so that they share the same total dose (Gy), dose-rate (Gy/min), distinct fractions (fr.), and interval between fractions in days (int.). These groups correspond to individual data-points and lines used in the figures in this paper. Total number of animals (n), average lifespan (μ age), and the standard error of the mean lifespan (σ) are shown for each treatment group. Note: In some analysis data were also stratified by study, which excluded several control groups in strata 2 and 3 that came from different studies at Argonne National Laboratory (ANL).

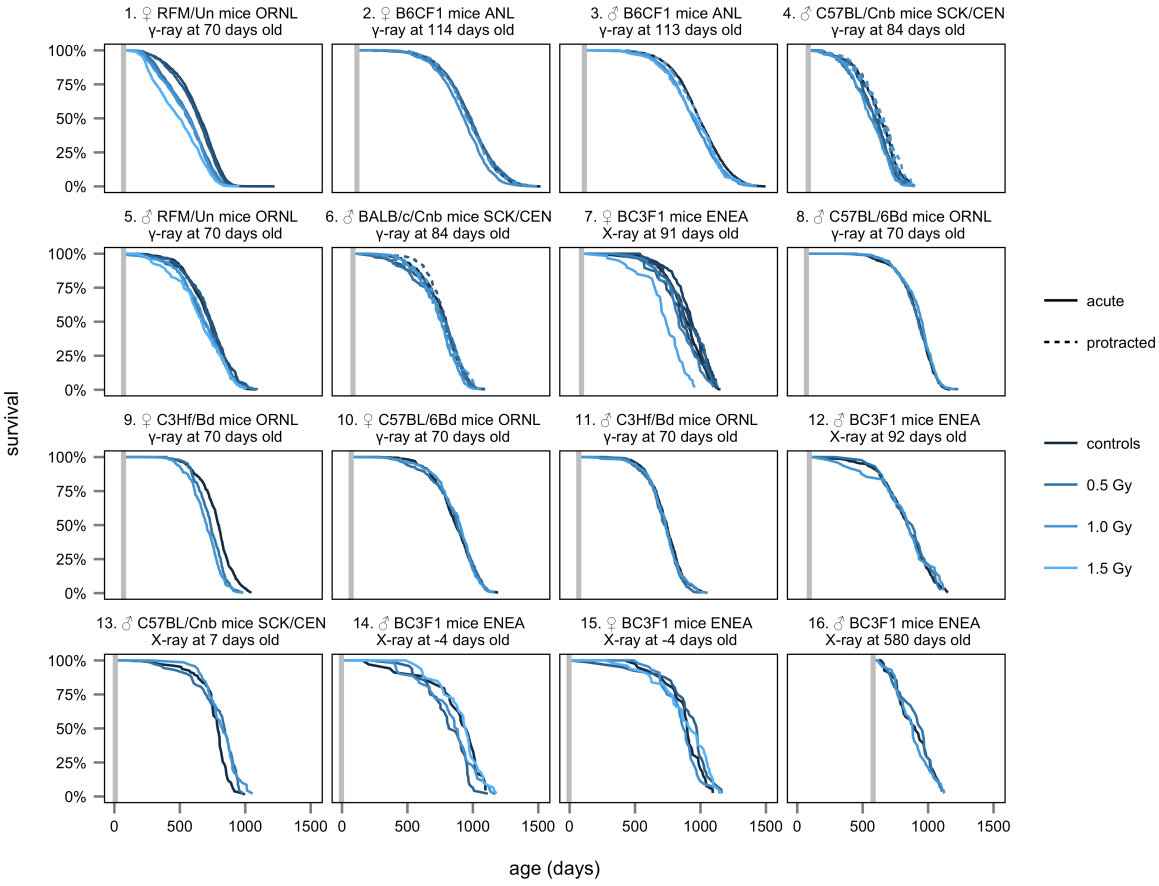


Figure 8: Survival vs. dose

Kaplan-Meier survival curves based on individual animal data show the percent of surviving animals vs. age for each treatment group of the expanded animal data set used in this analysis. The color of each curve indicates total dose, from dark blue (unexposed controls) to light blue (1.5 Gy). A solid line indicates acute exposures. A dashed line indicates fractionated exposures. A vertical gray line indicates age at first exposure. Treatments are stratified by sex, strain, type of radiation, and age at first exposure as labeled. The strata are presented in order of total number of animals included, so that the

1st strata on the top left has the most animals, 6977, and the 16th strata on the bottom right has the least, 126. This same ordering is maintained in all subsequent figures, as are the strata identifiers (e.g. strata labeled #2 always shows data from female B6CF1 ANL animals, 114 days old at the time of first exposure). Please note that the bottom row contains data from studies that investigated the effects of radiation exposure on very young (pre-natal and neonatal) and very old mice (more than 2 years old). In addition, note that the uppermost leftmost stratum contains data used in the original BEIR VII analysis. This stratum however consists of only the acute exposure data from that analysis, as the data from protracted exposures was not available for individual mice.

Re-estimate of $DDREF_{LSS}$ using more data with BEIR VII's method

As in the BEIR VII report, linear-quadratic models were fit to lifespan data according to the function:

$$mortality \sim \alpha \cdot dose + \frac{\beta \cdot dose^2}{fractions}$$

Again, as in the BEIR VII analysis, inverse mean lifespan was used as a proxy for animal mortality. Linear and quadratic coefficients, α and β, were determined for each analysis stratum. Mice within each stratum were of the same strain, sex, and age at exposure – factors that are widely known to affect radiation sensitivity, and therefore linear and quadratic coefficients values [55]. While the values of α and β were allowed to vary by stratum to reflect varying radiation sensitivity, the ratio between quadratic and linear coefficients, β/α, was fixed across all strata of the data during each calculation in order to find a single, best fit $DDREF_{LSS}$ estimate. A range of β/α ratios, corresponding to $DDREF_{LSS}$ from zero to infinity, were fit to the data and the likelihood of each ratio was found in order to establish a 95% confidence interval by the profile likelihood method (see methods section for full details). A 'fraction' was any dose delivered in 1 hour or less.

The best overall fit is shown in Figure 9. Using the replicated BEIR VII method, $DDREF_{LSS}$ was estimated to be infinite with a 95% credible interval from 2.9 to infinity. An infinite value of $DDREF_{LSS}$ would imply that the α term is zero and that protracted

exposures have no effect on lifespan. This $DDREF_{LSS}$ estimate, greater than 2.9, would suggest that BEIR VII's existing $DDREF_{LSS}$ estimate is too low. However, while this conclusion ultimately appears to be true, such a conclusion should not be made on the basis of this estimate alone, because it ignores obvious problems with the fit of this model to the data as discussed in the next section.

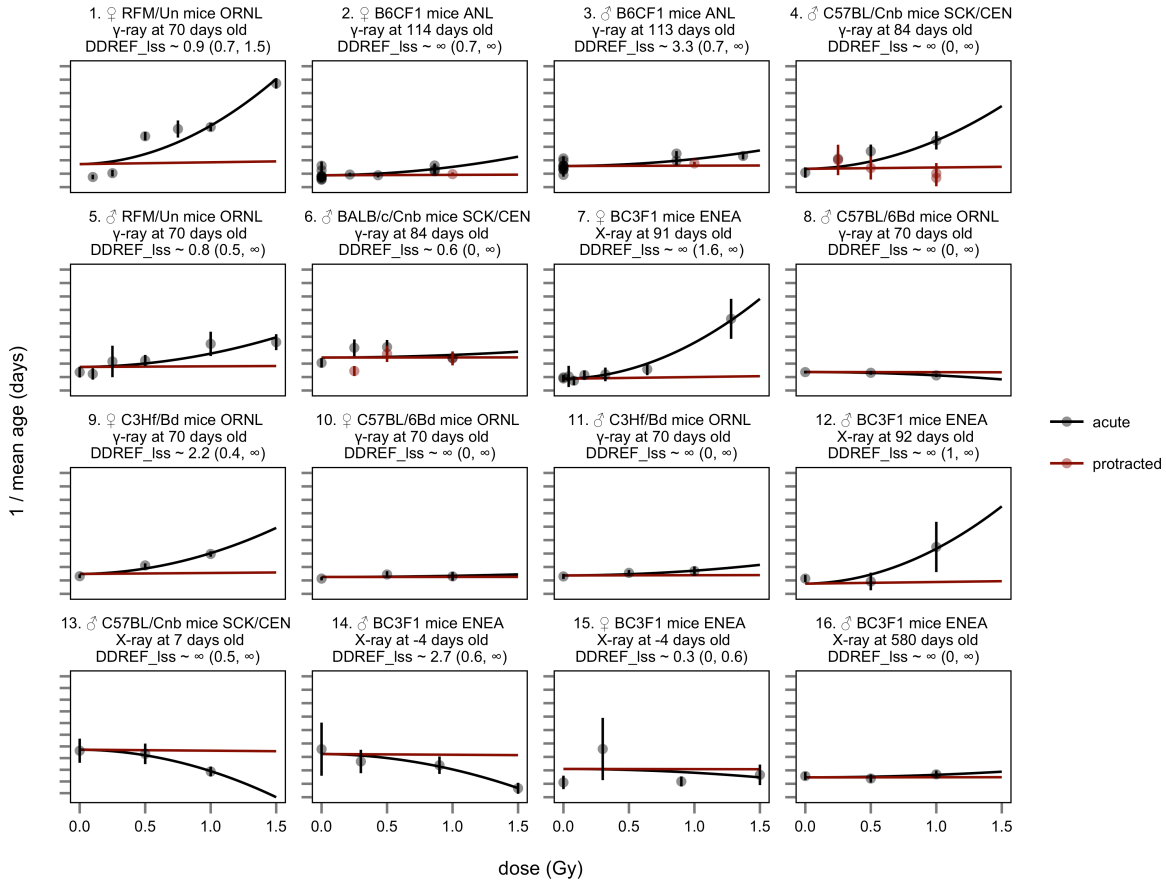


Figure 9: BEIR VII model applied to expanded animal data set

The relationship between inverse mean lifespan, y-axis, and dose, x-axis is shown grouped by analysis strata. Strata are organized and labeled as in Figure 8. The y-axis maintains a constant scale, 0.0001 days per tick with a different baseline for each stratum. Single points indicate results for each treatment group with standard error bars as indicated. Acute exposures and quadratic dose response estimates are shown in black, protracted exposures and linear dose response estimates are shown in red. Please note that protracted exposure data was available in only few cases – strata 2, 3, 4, and 6.

$DDREF_{LSS}$ estimates from each stratum analyzed independently are listed in each facet label with 95% credible intervals in parentheses. The central $DDREF_{LSS}$ estimated from the full data set is infinite with a 95% confidence interval from 2.9 to infinity (see Table 5).

	All data	Acute data	Comparison data
BEIR VII model	∞ (2.9, ∞)	1.3 (0.9, 3.0)	∞ (4.8, ∞)
Hormetic correction	∞ (2.3, ∞)	1.2 (0.9, 3.4)	∞ (4.8, ∞)
Heterogeneity correction	1.3 (0.9, 5.5)	0.9 (0.8, 1.3)	∞ (2.0, ∞)
Stratification by study	1.0 (0.8, 1.6)	1.0 (0.8, 1.2)	∞ (2.2, ∞)
Survival analysis	4.8 (1.5, ∞)	0.9 (0.7, 1.5)	∞ (2.5, ∞)

	Central estimate	Acute atomic bomb survivor data	Acute animal carcinogenesis data	Comparison animal mortality data
Original BEIR VII analysis using only original datasets	1.5 (1.1, 2.3)	1.3 (0.8, 2.4)	1.4 (1.1, 2.6)	2.0 (1.3, 7.7)

Table 5: $DDREF_{LSS}$ estimates from various models

$DDREF_{LSS}$ estimates for a variety of models. Central estimates are shown with 95% credible intervals in parentheses. Estimates from this work are shown on top. Estimates from the original BEIR VII report are shown on bottom for comparison. Note that the animal mortality data in the original BEIR VII report came from data that was more limited than data used in this analysis, although the protracted exposures from BEIR VII's analysis were not used in this analysis because individual level animal data from these studies is not available in public archives. Each model in this report, described in detail in the text, was applied to three different divisions of the data to produce three different $DDREF_{LSS}$ estimates as listed in the three rightmost columns. "All data" refers to $DDREF_{LSS}$ estimates based on all of the available data (as in Figure 9). "Acute data" refers to $DDREF_{LSS}$ estimates based only on the apparent curvature of acute exposure data in each stratum, and wholly excluding protracted exposure data (as in Figure 10).

"Comparison data" refers to $DDREF_{LSS}$ estimates based only on strata that included both acute and protracted exposures, and excluding strata that included only acute exposures (as in Figure 11). Note that the estimates of $DDREF_{LSS}$ based on data that includes acute and protracted exposures are always significantly larger than the estimates extrapolated from acute exposure data alone.

Acute data vs. acute-protracted comparisons

The linear-quadratic model does not fit well to each one of the exposure datasets in Figure 9. For example, the first stratum shows the results from the largest study included in this analysis, animals acutely exposed at Oak Ridge National Laboratory. These data do not fit the quadratic curve that is applied to them (solid black line), and instead appear approximately linear. Similar arguments can be applied to the acute dose-responses in strata 4 and 5. It appears from the figures that these acute dose-responses fit better with linear dose response models rather than linear quadratic models.

To test this supposition, BEIR VII's linear-quadratic dose response model was fit to two subsets of the data independently – in one instance the model was applied to data from acutely exposed animals in each strata, extracting both linear and quadratic coefficients from acute data (Figure 10), in the other, the model was applied only to data from strata that included both acute and protracted exposures, and calculated linear and quadratic coefficients based on both exposure types (Figure 11). The linear-quadratic model predicts that these two subsets should lead to similar $DDREF_{LSS}$ estimates. Instead, $DDREF_{LSS}$ estimates are significantly different.

The results of the two separate fits confirmed the qualitative observations. When linear-quadratic models were fit to acute exposure data (Figure 10), $DDREF_{LSS}$ was estimated to be low, 1.3 with a 95% credible interval from 0.9 to 3.0. When data were restricted to direct comparisons of acute and protracted exposures (Figure 11), $DDREF_{LSS}$

was estimated to be infinite with a 95% credible interval from 4.8 to infinity, significantly higher than the estimate based only on the curvature in acute dose response ($p < 0.01$).

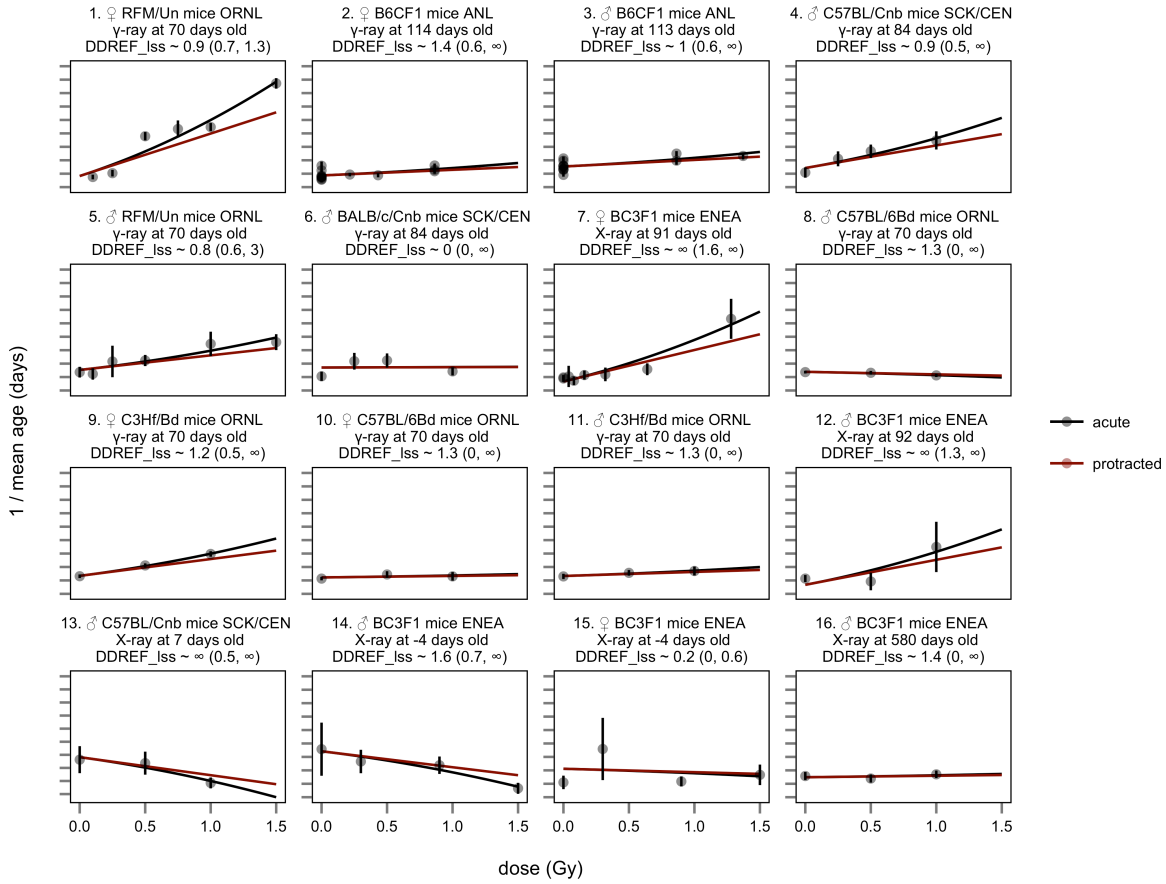


Figure 10: BEIR VII model applied to acute exposures only

Identical to Figure 9, except that, within each stratum, data are restricted to animals that received only acute radiation exposures. Protracted extrapolations, estimated from the linear term of acute exposures, are still shown (red lines). Notably, protracted extrapolations are very similar to acute risk estimates because these dose-responses are nearly linear with only a minimal quadratic curvature. This analysis is similar to BEIR VII's estimates of $DDREF_{LSS}$ based on atomic bomb survivors and animal carcinogenesis data that only included acute exposure data as well (Figure 3).

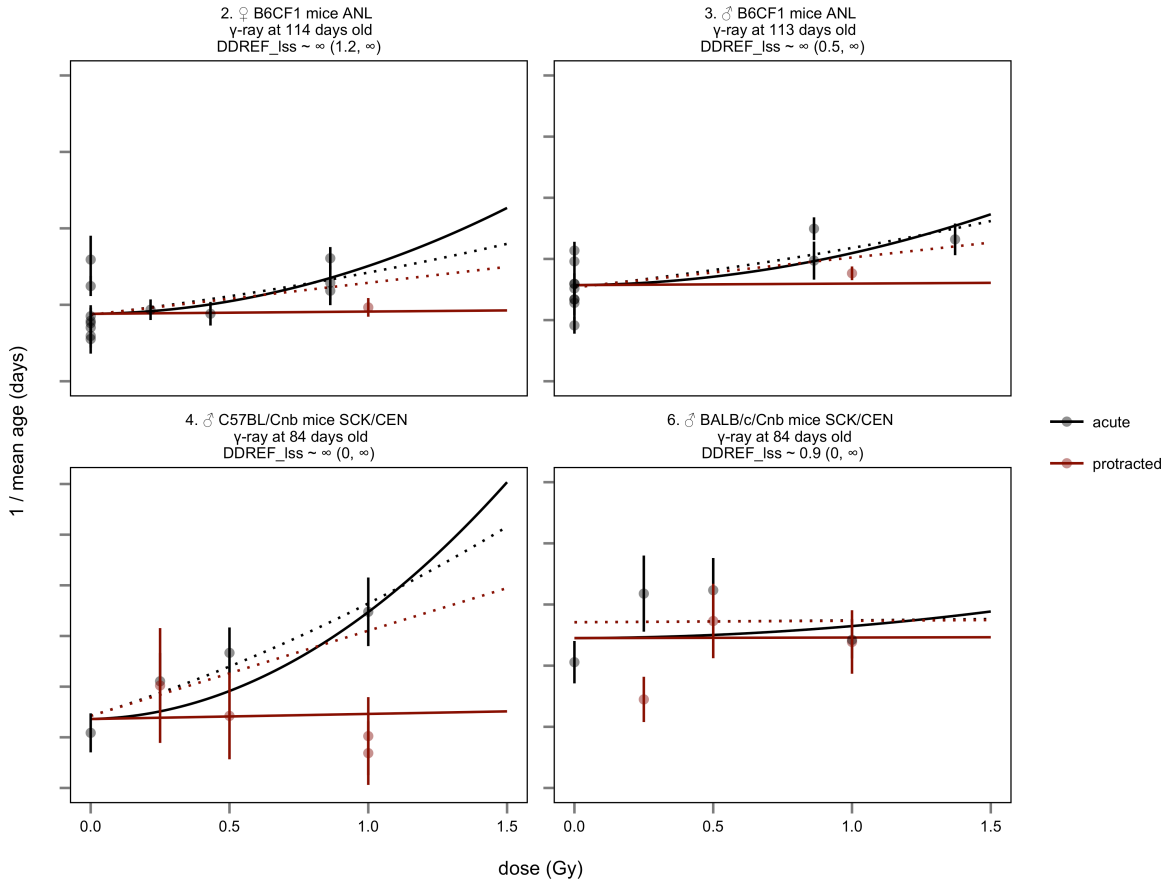


Figure 11: BEIR VII model applied only to protracted-acute comparisons

Similar to Figure 10, except that data are restricted to strata that received both acute and protracted exposures. For comparison, fits based on acute data alone (the same as those in Figure 12) are shown as dotted lines. Note that the real risk of protracted exposure is substantially lower than the risk projected based on acute data. Also, note that stratum 4 includes two protracted exposures accumulating to a total dose of 1.0 Gy corresponding to two different fractionation patterns as described in Table 4.

When the linear-quadratic model is applied to acute exposure data it overestimates the risk of protracted exposures. This is highlighted in Figure 11 where dotted lines show the estimates of dose-response based only on acute data. These estimates fit the acute exposure data reasonably well but overestimate the risk of protracted exposures. This failure of the linear-quadratic model to fit the observed data calls into question not only existing $DDREF_{LSS}$ estimates, but also the conceptual basis used to estimate the relative risk of low dose and protracted exposures.

Variations on BEIR VII's linear-quadratic model

There are many plausible alternatives to the methodological assumptions made by the BEIR VII report. For example, one can argue that animals from distinct studies should not be combined into a single analysis stratum. Perhaps treatment conditions improved between two studies leading to an increase in longevity that was unrelated to changes in radiation exposure.

Several more analyses were conducted to ensure that these results were consistent, even if the data were analyzed with alternative, but still plausible, formulations of the linear-quadratic dose response model. The data was re-analyzed using variations on the reconstructed BEIR VII methodology. Each variation was applied to the full dataset like the analysis depicted in Figure 9. Each variation was also applied to the subsets of the data described above, one consisting only of acute exposures (as in Figure 10) and the other consisting only of strata that included both acute and protracted exposure data (as in

Figure 11). $DDREF_{LSS}$ estimates from these methodological variations are shown in Table 5 and discussed in the sections below.

Regardless of the model variations, the linear-quadratic model never fit the data well. Concretely, $DDREF_{LSS}$ estimates based on curvature in acute exposure data were consistently low, never significantly greater than 1 (Table 5 "acute data"). $DDREF_{LSS}$ estimates based on data from strata that directly compared acute and protracted exposures were consistently high, always significantly higher than 1 and always much higher than the corresponding estimates based on only acute exposure data (Table 5 "comparison data"). These results all contradicted the assumptions of the linear-quadratic model that predicts that these two estimates should lead to the same value.

Central estimates of $DDREF_{LSS}$, based on all of the available data, varied substantially between methodologies and were not consistent when compared to each other (Table 5 "all data"). This is not surprising given the poor fit between linear-quadratic models and data.

Eliminating the hormetic paradox

As shown in Figure 9, several smaller strata (8, 10, 13, 14, and 15) had apparently hormetic responses. Exposed animals lived longer than comparable controls. The concept of DDREF is paradoxical when applied to data that shows a pattern of hormesis. When an exposure is deleterious, a high DDREF indicates that protracted exposure is less *deleterious*. When an exposure is beneficial a high DDREF indicates that protracted

exposure is less *beneficial*. Therefore, one single DDREF value could simultaneously predict that protraction leads to more or less life shortening depending on whether acute exposures induce a hormetic or deleterious response. This paradox is illustrated in Figure 12.

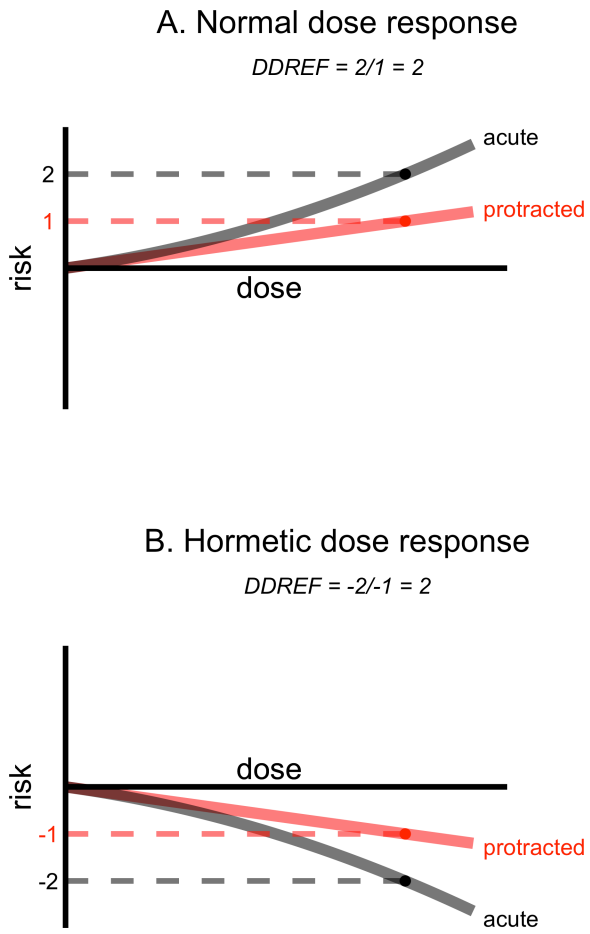


Figure 12: The hormetic DDREF paradox

The same DDREF estimate, 2 in this example, can be obtained when (A) acute exposures appear more damaging than protracted exposures or when (B) acute exposures appear more beneficial than protracted exposures.

Should these studies contribute to the central $DDREF_{LSS}$ estimate? It can be argued that they should not, because:

1. $DDREF_{LSS}$ is applied to atomic bomb survivors who had deleterious, not hormetic, dose responses.
2. The linear-quadratic model, with deleterious chromosomal aberrations as its mechanistic foundation, does not predict that hormetic effects should be possible.
3. Hormetic responses were only observed in a minority of smaller animal studies.

Therefore, in this first model variant and all subsequent models, linear, α, and quadratic, β, coefficients were limited to positive values. This had the effect of eliminating data that showed hormetic dose responses and preventing it from contributing to the $DDREF_{LSS}$ estimate.

$DDREF_{LSS}$ estimates following this 'hormetic correction' were similar to the results based on the BEIR VII model as shown in the first row of Table 2. In spite of possible concerns, apparently hormetic responses did not have a substantial effect on $DDREF_{LSS}$ estimates.

Accounting for heterogeneity among treatment groups

BEIR VII weighted their regression analysis by the variance of the mean lifespan observed in each treatment group. This approach neglects random effects – systematic sources of variance that affect entire treatment groups regardless of their size. For example, entire treatment groups may have been subject to variations in animal living conditions or radiation exposures. These variations are not apparent when individual treatment groups are analyzed in isolation. Rather, they become apparent when multiple treatment groups are compared to each other.

A random effects model estimates unmeasured variance between treatment groups, and adds it to the measured variance within each treatment group. This results in a more balanced weighting between large and small treatment groups.

If the dose response data under analysis included random effects, they might bias the ultimate $DDREF_{LSS}$ estimate. Therefore, in this and subsequent model variants an effort was made to account for random effects in the data.

The DerSimonian Laird method [56] was used to estimate random effects and increase the variance estimates for individual treatment. Details of this adjustment are described in the methods section.

Evidence of random effects was present in each analysis ($p < 0.01$). Accounting for heterogeneity had the effect of lowering the $DDREF_{LSS}$ estimates as shown in Table 5 indicating that the previous estimates may have been biased upwards. Nevertheless, as before, $DDREF_{LSS}$ estimates based on acute data remained significantly lower than

estimates limited to strata that compared acute and protracted exposures. Once again, the latter analysis produced a central $DDREF_{LSS}$ estimate of infinity.

Stratification by study

Like the BEIR VII analysis, the initial analysis combined the results of multiple studies into the same analysis strata. This ignores the possibility that animal mortality and radiation sensitivity varied between studies due to differences in study protocol unrelated to radiation treatment.

Lifespan and radiation sensitivity depend not only on animal strain, age, and gender, but can also be affected by cage crowding, pathogen environment, and even ambient temperature [55]. Because these factors often change between studies, it may be prudent to avoid a direct comparison between treatment groups in separate studies. Therefore, study origin was added to the existing stratification conditions, strain, sex, and age at exposure. As before, strata were excluded from the analysis if they had less than the three distinct treatment groups needed to fit a linear-quadratic model.

This exclusion eliminated an additional ~8000 animals (mostly controls) from the analysis as shown in Table 1 and lowered the overall $DDREF_{LSS}$ estimates again as shown in Table 2. However, $DDREF_{LSS}$ estimates based on the apparent curvature in acute data remained significantly lower than $DDREF_{LSS}$ estimates based on strata that included a direct comparison of acute and protracted exposures, continuing to violate the linear-quadratic assumptions.

Survival analysis

The BEIR VII report used inverse mean lifespan as a proxy for mortality [2]. This is a valid approximation if animal mortality rates increase exponentially with time, as in Gompertz law of mortality [57]. BEIR VII report used a theoretical relationship between animal mortality and age because the committee did not have access to individual level animal data needed to measure mortality rates directly. In all of the analyses described above, inverse mean lifespan values were extracted from the individual animal data as in the BEIR VII report (see Table 4). These values were used for $DDREF_{LSS}$ calculations shown in Table 5.

However, individual level data was available in this study and so it was used to conduct another analysis using this lifespan data directly (see "Survival analysis" in Table 5). Excess mortality was modeled using this individual animal data by using observed mortality rates. Therefore in the final analysis, named "survival analysis correction", Cox proportional hazards modeling was used to fit the change in mortality hazard as a function of age [58].

This modification had the effect of raising the central $DDREF_{LSS}$ estimate relative to the previous analyses. Nevertheless, as in all of the other cases, $DDREF_{LSS}$ estimates based on curvature in acute dose response remained significantly lower than $DDREF_{LSS}$ estimates of strata that directly compared acute and protracted exposures. Therefore, in

all tested variations the data continued to violate the assumptions of the linear-quadratic model.

Summary

After applying the replicated BEIR VII's dose response model to additional animal data, evidence was found that protracted exposures induce less risk than BEIR VII estimated they did, with at least 2 fold less risk than acute exposures at the same total dose. Unfortunately, it was also found that the linear quadratic dose response model used by the BEIR VII committee does not accurately describe the animal data analyzed. There are several reasons why the linear-quadratic model might fail, discussed in detail in Chapter 5. Regardless of the reason(s) for the failure of the linear quadratic model under these circumstances, the result argues that a new dose response model should be used to estimate the effects of protracted exposures.

While the BEIR VII surmises that carcinogenesis and mortality dose responses are linear quadratic based on cellular dose-responses, this conclusion is likely to be wrong. The basic reason, elaborated in Chapter 5, is that the mechanisms that lead from cellular damage to cancer induction are complex and incompletely understood. Instead of attempting to derive the true dose response model based on cellular response, a more conservative approach to risk estimation would determine acute dose-response based on observations of acutely exposed atomic bomb survivors and the effects of protraction based on long-term animal studies which compare acute and protracted exposures. With

few exceptions, radiation responses detected in all studies and observations are well described by linear dose-response models with separate slopes for acute and protracted exposures (see Figures 1 and 9). Since the 'true' dose response model cannot be known with confidence, it is prudent to estimate the effects of protraction from a good fitting model using data that directly compares acute and protracted exposures. This is in contrast to the BEIR VII approach that used acute exposure data to estimate the risk of protracted exposures under the assumption that the true dose response was linear quadratic and that the risk of protracted exposures could be extrapolated from the linear term of models fit only to acute exposure data. This proposed approach avoids the risk of bias that occurs if the wrong dose-response model is assumed. However, it also restricts the set of data that can be used to estimate the risk of protracted exposures, eliminating studies and observations that only include acute exposures.

Therefore, upon concluding this work to estimate $DDREF_{LSS}$ using the BEIR VII dose response model, the linear-linear dose response models were applied to newly curated animal data in order to estimate the effects of protracted radiation exposure. As before, this approach cannot be said to estimate the risk of low-dose exposures. This is because low-dose exposure risk cannot be measured with sufficient statistical precision and therefore estimating the risk of these exposures requires strong assumptions about the true dose response function. Therefore, a dose rate effectiveness factor ($DREF_{LSS}$ as opposed to $DDREF_{LSS}$, a term which implies applicability to low doses) is estimated using a linear dose response model based on the models used to analyze the atomic bomb

survivor data. This $DREF_{LSS}$ value is designed explicitly to estimate the risk of contemporary protracted exposures (several hundred milliSiverts over a typical lifetime) from the acute dose response observed in atomic survivors. The technique used to estimate the parameter, corresponding to Aim 2 of this thesis is described next in Chapter 4.

Chapter 4: Applying Ozasa's RERF model to animal mortality data

Results described in the chapter 3 show that the dose response of animal mortality data does not fit the linear quadratic model that BEIR VII used to estimate the risk of protracted and low dose exposures. Instead, mortality dose responses are well fit by linear dose response models with slopes that vary by species, strain, and whether an exposure was acute or protracted. This chapter presents work to estimate the relative risk of protracted exposures, $DREF_{LSS}$, from mouse and rat exposures using linear models that closely mirror those used to estimate risk from atomic bomb survivor data [10]. There were several main findings:

1. Protracted exposures induce about 2 fold less risk than acute exposures. Specifically, $DREF_{LSS}$ is estimated to be 2.1 with a 95% credible interval from 1.7 to 2.7. This supports the hypothesis of this thesis that $DREF_{LSS}$ is significantly higher than the 1.5 value proposed by the BEIR VII committee.
2. No evidence was found that total dose (within the limits of most inclusive doses used for RERF analyses - up to 4 Sv) or acute radiation toxicity biases $DREF_{LSS}$. Specifically, $DREF_{LSS}$ does not change significantly when dose ranges from 0 to 3 Sv rather than 0 to 4 Sv are considered, nor when animals or treatment groups that showed signs of acute radiation toxicity are excluded from the analysis.

3. No evidence was found that $DREF_{LSS}$ varied by species or gender. $DREF_{LSS}$ estimates from rats (all female) were very similar to estimates from mice (almost all male).
4. Age at exposure, accounted for in the above estimate of $DREF_{LSS}$, generally reduced dose response by an amount similar to the reduction observed in atomic bomb survivor data. But, while age at exposure accounts for some of the risk reduction following protracted exposures, it does not account for all of it.
5. As mentioned in Chapter 3, a linear-linear dose response model fits animal mortality data quite well, although it is important to emphasize that this model is merely a convenient approximation. The true dose response function could be curved in a variety of ways that would produce an apparently linear final result. Still, these results argue that the risks of contemporary exposures, almost always protracted, should be estimated from atomic bomb survivor data after applying a $DREF_{LSS}$ correction of 2. This means that contemporary risk of mortality is 2 fold lower per Gy than the risk to atomic bomb survivors. This argument is justified in detail in Chapter 5.

Evidence to support these findings is presented in the remained of this chapter.

Data selection

11,528 animals (all mice and rats) in 115 treatment groups from 8 studies were selected to estimate $DREF_{LSS}$ using both a model and inclusion criteria closely based on Ozasa's analysis of atomic bomb survivors [10]. Specifically, lifespan studies of animals exposed to low-LET ionizing radiation at total doses less than 4 Sv were included in the analysis. Studies were only included if they directly compared acute and protracted exposures or multiple ages at exposure. The animals that met these criteria are detailed in Table 6. Treatment groups that were excluded because they could not be validated against published literature are listed in Table 7. The survival curves of animals selected for analysis are shown for each stratum separately in Figure 13. Details describing each treatment group are listed in Table 8.

In addition to these basic criteria, $DREF_{LSS}$ was also estimated from several smaller subsets of the same pool of data. These sensitivity analyses were conducted to ensure that $DREF_{LSS}$ estimates were not biased by acute radiation toxicity, the total exposure doses under consideration, or differences between mice and rats. The results of these analyses are listed in Table 9.

One of the critical data selection criteria pertains to maximum total dose allowed in the analysis. Ozasa's analysis was limited to humans who received a 4 Sv surface dose or a 3 Sv colon dose. However, the mice and rats included in this analysis are smaller than humans and the difference between skin and colon doses due to shielding is smaller. Therefore, analyses were done for total exposure dose cutoffs at 3 and 4 Sv both. It

should also be noted that 3 Sv colon dose for humans is frequently fatal without nursing care most rodent species survive at even higher doses. The 4 Sv cutoff was applied to all data, but one analysis additionally restricted data to exposures less than 3 Sv. Any studies that did not compare multiple ages at exposure or acute and protracted exposures in this dose range were eliminated from analysis. Applying these "sensitivity" criteria 9,556 animals were eligible for analysis; this dataset is referred to as "0-3 Sv".

A concern in this analysis was the presence of acute radiation toxicity which can be seen in strata 6 and 7 of the survival curves depicted in Figure 13. These strata show a clear drop in survival closely following exposure indicating some sort of acute radiation toxicity. Such acute effects may originate from failures of the immune system or gastro-intestinal systems when the cell division necessary to keep these systems functional fails or from multiple organ failures (MOF). Acute deaths are an important consideration when acute radiation effects are evaluated, however, this analysis, like the atomic bomb survivor analysis, was designed to estimate the long-term health risks of radiation exposure. This focus is based on the knowledge that most contemporary exposures are too small or protracted to include acute effects.

Consequently, sensitivity analyses designed to eliminate the impact of acute radiation toxicity were tested. One approach excluded the two strata that showed clear signs of acute toxicity in survival curves, strata 6 and 7. The resulting dataset is called the "without toxicity" and it contains 10,399 animal mortality records as eligible for analysis.

Another way of eliminating bias from acute radiation toxicity is to censor data within each stratum so that mortality is calculated from deaths that occur some period of time after the last radiation exposure. Notably the lifespan study of atomic bomb survivors did not begin until 5 years after the atomic bomb exposures (about 6% of an 80-year-old human's lifespan). So a series of sensitivity analyses were conducted that excluded animals that died before the age at last exposure in the stratum plus 6%, 13% or 26% of the mean control lifespan ("censor 6%" - 11,431 animals, "censor 13%" - 11,258 animals, and "censor 26% - 10,594 animals). Because lifespan varies between mice and rats and among strains within each species these values were always calculated relative to the control group for a particular species, strain and study. The survival curves following "censor 26%" are shown in Figure 14. These specific values, 6%, 13%, and 26% were chosen because they correspond to periods of 5, 10, or 20 years in a human population with a life expectancy of 80 years. These life percentages correspond to periods of about 50, 100, or 200 days in the 840 lifespan of the average control mouse and 13% more in control rats, which live about 950 days on average of this study.

A final sensitivity analysis estimated separate $DREF_{LSS}$ values for mice and rats. This was done to ensure that the overall $DREF_{LSS}$ estimates were not driven by just one species – a particularly important check given that these estimates are intended for human application.

Studies	Treatments	Animals	Criteria
302	6,810	452,595	All animal data from ERA and Janus archives
124	2,611	205,758	Individual-level animal data available
35	827	116,542	External radiation exposures
35	457	76,096	Low-LET, whole body exposures
8	115	11,730	Total doses less than 4.0 Sv in studies that directly compare acute and protracted exposures or different treatment ages that could be verified in primary literature (see Table 7)
8	115	11,528	Animals with lifespans longer than the oldest treatment age in the stratum.
Sensitivity analysis subsets			
7	94	9,556	Animals with exposures less than 3.0 Sv. *Used in the "0-3 Sv" analysis.*
6	103	10,399	Animals after exclusion of stratums 6 and 7 which showed signs of acute radiation toxicity. Used in the "without toxicity" analysis.
8	115	$^{6\%}$11,431 $^{13\%}$11,258 $^{26\%}$10,594	Available records after exclusion of animals with lifespans less than the oldest treatment age plus 6%, 13% or 26% of the mean lifespan of the control group in the stratum. Used in the "censor 6%", "censor 13%", and "censor 26%" analyses.
9	66	8,793	Mice only
10	49	14,782	Rats only

Table 6: Data selection by inclusion criteria

The number of distinct studies, treatment groups, and individual animals that remained eligible for analysis after application of each of the inclusion criteria. Complete definitions for each criterion are elaborated in the methods chapter, Chapter 6. The top of the table represents criteria that were applied to all of the data that was analyzed. The

bottom row (11,528 animals) is the primary data used for analysis and development of the central $DREF_{LSS}$ estimate (1.7 to 2.7). Note that animals that died prior to final radiation exposure are excluded from this selection; Table 8 gives details about this animal dataset. The second half of the table represents subsets of the data used for sensitivity analyses. Additional selection criteria were applied to most of these data sets as noted, in order to test the robustness of $DREF_{LSS}$ estimates. However, unlike the criteria in the top of the table, these criteria were applied independently. For example, the row that excludes stratum 6 and 7 considers exposures from 0 to 4 Sv even though the row above it excluded exposures above 3 Sv.

Treatment group ERA identifier	Reason for exclusion
11-2-9	Mean lifespan in this treatment group is much lower than the lifespans reported in published literature [59].
1002-1 (all treatment groups)	The lifespans of ERA data do not match published results [60].
1003-21(all treatment groups)	There were very few mice, only 7 each, in the two treatment groups with doses less than 4 Sv [49].
2-11 (all treatment groups)	Treatment ages (the primary variable under consideration) in the ERA data do not align with those reported in the Gerber 1996 summary, [42], and no primary source was found to address the discrepancies.

Table 7: Data that could not be confirmed in the literature

Several treatment groups were excluded from the analysis because the ERA or Janus data could not be confirmed in primary literature. The reason(s) for exclusion is listed for each treatment group. ERA study group identifiers denote treatment groups. The code used to verify each datasets is available at (github.com/benjaminhaley/janus/blob/master/scripts/exp/data.Rmd) and gives additional information on the rationale for these decisions and small corrections that are not covered in this report.

Strata in this analysis Stratum id, ERA study id, institution, sex, strain, species, quality of radiation, and sometimes age at first exposure	**Cluster in this analysis** cluster number, number of fractions, interval between first and last fraction, dose rate and exposure age (where relevant)	**n**	**age** μ +/- σ	**total** Gy
1. 9-6 SCK/CEN ♂ C57BL/Cnb mice γ-ray at 84 days [52]	Control	467	613 +/- 7	0
	1. acute 0.3 Gy/min	241	581 +/- 9	0.25
		236	564 +/- 10	0.5
		240	552 +/- 9	1
		215	537 +/- 11	2
		142	481 +/- 15	4
	2. 8 over 1 day 0.3 Gy/min	104	622 +/- 17	1
		91	572 +/- 19	2
		110	568 +/- 18	4
	3. 10 over 10 days 0.3 Gy/min	107	605 +/- 16	0.25
		109	604 +/- 14	0.5
		115	615 +/- 14	1
		113	619 +/- 13	2
		117	522 +/- 19	4
2. 1003-22 ANL ♂ B6CF1 mice γ-ray at 120 days [49]	Control	193	1040 +/- 14	0
		186	1000 +/- 13	0
		169	944 +/- 14	0
	1. 24 over 168 days 0.0037 Gy/min	399	909 +/- 9	4
	2. 24 over 168 days 0.0018 Gy/min	595	962 +/- 8	1.98
	3. 120 continuous days 1.3e-5 Gy/min	193	963 +/- 13	2.06
3. 9-5 SCK/CEN ♂ BALB/c/Cnb mice γ-ray at 84 days [50]	Control	322	766 +/- 9	0
	1. acute 4 Gy/min	191	745 +/- 14	0.25
		194	736 +/- 14	0.5
		191	732 +/- 11	1
		149	714 +/- 13	2
		92	610 +/- 22	4
	2. 10 over 10 days 4 Gy/min	111	778 +/- 13	0.25
		110	740 +/- 16	0.5
		113	751 +/- 16	1
		74	726 +/- 16	2
		73	655 +/- 19	4
4. 3-5 ENEA ♂ BC3F1 mice X-ray [53]	Control	30	929 +/- 24	0
		394	860 +/- 7	0
		41	886 +/- 22	0
	1. acute 0.133 Gy/min at -4 days	41	854 +/- 19	0.3
		52	890 +/- 20	0.9
		43	922 +/- 22	1.5
		41	870 +/- 18	2.1
	2. acute 0.133 Gy/min at 92 days	41	856 +/- 23	0.5
		40	884 +/- 22	1

		41	848 +/- 23	2
		39	803 +/- 19	3
		44	821 +/- 21	4
	3. acute 0.133 Gy/min at 580 days	42	901 +/- 22	0.5
		43	874 +/- 20	1
		45	908 +/- 22	2
		47	865 +/- 17	3
		43	853 +/- 18	4
5. 1003-24 ANL ♂ B6CF1 mice γ-ray [49]	Control	305	995 +/- 10	0
	1. acute 0.099 Gy/min at 520 days	148	975 +/- 14	1.98
	2. 60 over 420 days 0.0015 Gy/min at 100 days	133	973 +/- 15	4
6. 9-4 SCK/CEN ♂ C57BL/Cnb mice X-ray at 28 days	Control	131	699 +/- 11	0
	1. acute	100	566 +/- 25	3.29
	2. 4 over 28 days 0.94 Gy/min	100	705 +/- 13	1.88
		98	538 +/- 26	2.82
		143	445 +/- 24	3.76
7. 9-7 SCK/CEN ♂ C57BL/Cnb mice X-ray [51]	Control	105	757 +/- 14	0
	1. acute 1 Gy/min at 7 days	72	777 +/- 21	0.5
		70	810 +/- 16	1
		85	592 +/- 29	3
	2. acute 1 Gy/min at 21 days	66	826 +/- 13	0.5
		76	769 +/- 15	1
		83	590 +/- 31	3
8. 11-2-E TNO ♀ BN/BRIJ rats X-ray at 56 days [59]	Control	140	928 +/- 16	0
	1. acute 0.06 Gy/min	80	892 +/- 21	0.02
	2. 5 over 35 days 0.06 Gy/min	80	926 +/- 19	0.1
		40	898 +/- 29	0.25
		80	895 +/- 21	0.4
		20	878 +/- 42	1
		20	698 +/- 33	4
		80	910 +/- 17	0.4
9. 11-2-F TNO ♀ BN/BRIJ rats X-ray at 56 days [59]	Control	83	923 +/- 24	0
	1. acute 0.06 Gy/min	60	899 +/- 28	0.08
		60	913 +/- 26	0.2
		60	900 +/- 26	0.4
		39	791 +/- 28	1.6
	2. 20 over 28 days 0.06 Gy/min	60	900 +/- 23	0.4
		60	880 +/- 22	1.6
10. 11-2-F TNO ♀ WAG/RIJ rats X-ray at 56 days [59]	Control	99	949 +/- 16	0
	1. acute 0.06 Gy/min	60	900 +/- 18	0.08
		60	860 +/- 23	0.4
		60	766 +/- 20	1.6
	2. 20 over 28 days 0.06 Gy/min	60	849 +/- 20	0.4
		60	835 +/- 21	1.6
11. 11-2-B TNO ♀ WAG/RIJ rats	Control (shared with 11)	59	766 +/- 21	2
	1. acute 0.06 Gy/min	100	959 +/- 16	0

X-ray at 56 days [59]	2. 10 over 305 days 0.06 Gy/min	59	911 +/- 23	0.2
		58	873 +/- 21	2
	3. 20 over 70 days 0.06 Gy/min	58	886 +/- 19	2
	4. 10 over 14 days 0.06 Gy/min	58	865 +/- 22	2
12. 11-2-B TNO ♀ WAG/RIJ rats γ-ray at 56 days [59]	Control (shared with 12)	100	959 +/- 16	0
	1. acute 0.9 Gy/min	40	844 +/- 27	2
	2. 10 over 305 days 0.001 Gy/min	59	926 +/- 18	2
	3. 20 over 70 days 0.001 Gy/min	60	908 +/- 21	2
	4. 10 over 14 days 0.001 Gy/min	60	844 +/- 21	2
13. 1003-24 ANL ♀ B6CF1 mice γ-ray [49]	Control	173	998 +/- 14	0
	1. acute 0.099 Gy/min at 520 days	50	927 +/- 24	1.98
	2. 60 over 420 days 0.0015 Gy/min at 100 days	26	897 +/- 35	4
14. 11-2-E TNO ♀ WAG/RIJ rats X-ray at 56 days [59]	Control	60	956 +/- 19	0
	1. acute 0.06 Gy/min	40	777 +/- 27	2
	2. 10 over 70 days 0.06 Gy/min	40	921 +/- 24	2
		40	774 +/- 25	4
15. 11-2-E TNO ♀ SD/RIJ rats X-ray at 56 days [59]	Control	59	864 +/- 22	0
	1. acute 0.06 Gy/min	40	638 +/- 24	2
	2. 10 over 70 days 0.06 Gy/min	40	785 +/- 26	2
		40	691 +/- 28	4
16. 11-2-D TNO ♀ WAG/RIJ rats γ-ray at 117 days [59]	Control (shared with 18)	40	900 +/- 24	0
	1. acute 0.9 Gy/min	40	949 +/- 25	0.3
		40	867 +/- 22	1.2
	2. 120 over 12 days 0.001 Gy/min	40	886 +/- 21	1.2
17. 11-2-D TNO ♀ WAG/RIJ rats γ-ray at 56 days [59]	Control (shared with 16)	40	900 +/- 24	0
	1. acute 0.9 Gy/min	40	916 +/- 26	0.3
		40	786 +/- 28	1.2
	2. 120 over 12 days 0.001 Gy/min	40	910 +/- 20	0.3
18. 11-2-A TNO ♀ WAG/RIJ rats X-ray at 56 days [59]	Control	44	836 +/- 29	0
	1. acute 0.06 Gy/min	40	712 +/- 21	2
	2. five over 153 days 0.06 Gy/min	40	650 +/- 22	4

Table 8: Data concordance

A description of data used in this analysis. The first column details the data sources stratified by study, sex, strain, and quality of radiation. Strata are organized from most animals (top) to least (bottom) and numbered 1-18 corresponding with labels in Figures 13, 14 and 15. Also listed are the ERA study IDs corresponding to the data in the strata, the abbreviation of institutions where these studies were conducted, and references to

these studies in the literature. Subsequent columns are further clustered by treatment so that they share the same dose rate, age at beginning of exposure, and fractionation pattern. The final three columns are further grouped so that they share the same total dose. These groups correspond to individual data-points and lines used in Figures 13, 14 and 15. Total number of animals (n), average lifespan (μ age), the standard error of the mean lifespan (σ), and dose (Gy) are shown for each treatment group. Note: these treatment groups list data after excluding animals that died before the last treatment in the stratum.

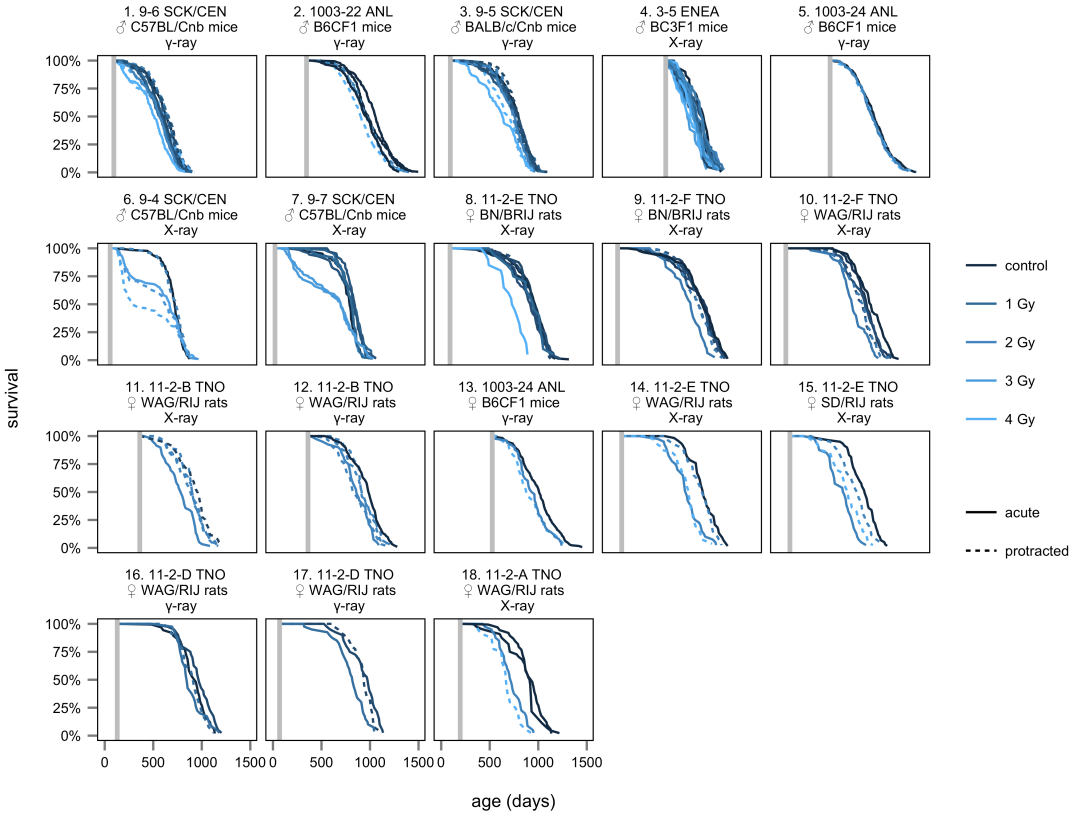


Figure 13: Survival vs. dose

Kaplan-Meier survival curves based on individual animal data show the percent of surviving animals vs. age for each treatment group of the animal data set detailed in Table 8 and used in the "Primary analysis 0-4 Sv". Sensitivity analyses used subsets of this data as described in the text. The color of each curve indicates total dose, from dark blue (unexposed controls) to light blue (4 Gy). A solid line indicates acute exposures. A dashed line indicates fractionated exposures. A vertical gray line indicates the maximum age at last exposure for all treatment groups in the stratum. Animals that died before this

age are censored from the analysis and are not included in these curves. Treatments are stratified by sex, strain, study, and type of radiation as labeled. The strata are presented in order of total number of animals included, so that strata 1 on the top left has the most animals, 2407, and strata 18 on the bottom to the right has the least, 124. This same ordering is maintained in all subsequent figures.

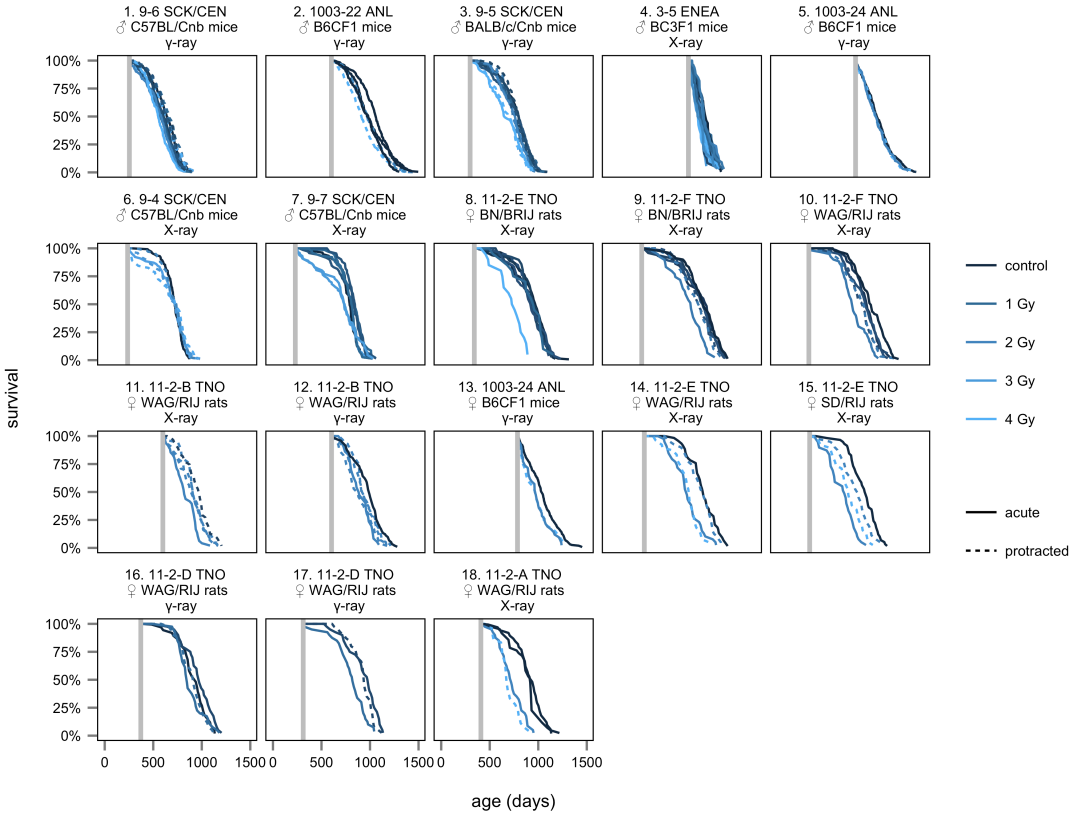


Figure 14: Survival vs. dose with 26% censoring

Identical to Figure 13 except that animals were censored from analysis and removed from survival curves if they died soon after the last radiation exposure in their stratum. Concretely, animals were censored if they died before the sum of days consisting of the last treatment in their stratum plus 26% of the median lifespan of control animals in that stratum. The grey vertical line in each stratum indicates this new cutoff. This analysis was conducted to ensure that acute radiation toxicity did not bias estimates of $DREF_{LSS}$ and instead reflect the long-term health effects of radiation exposure. Also considered

were censoring periods of 6% and 13% in addition to the 26% period shown. These values were chosen to correspond to 5, 10, or 20 year periods for a human population with a median lifespan of 80 years. Because most of the control mice in this analysis live about 840 days, these percentages correspond roughly to 50, 100, and 200 day periods. These periods are about 13% larger in rats that tend to live about 950 days. It should be noted that even the shortest of these time periods (50 days) is longer that the time at which death from hematopoietic syndrome occurs.

Modeling procedure

As previously noted, the aim of this chapter is to estimate $DREF_{LSS}$ from a model similar to the one used to estimate risk from the lifespan study of atomic bomb survivors. Particularly, the approach was based on work by Ozasa and others published in 2012, the 14th annual report of the Radiation Effects Research Foundation (RERF), which conducts the lifespan study [10]. In this report, the risk of cancer mortality is estimated as a linear function of dose adjusted for age at exposure and age at death. Concretely:

$$ERR \sim \lambda_{baseline}\left(1+\left(1+\sigma_{sex}\right)\beta \cdot dose \cdot e^{\tau \cdot age_{at\ exposure}+\nu \cdot \ln\left(age_{at\ death}\right)}\right)$$

Where ERR stands for excess relative risk, relative risk minus 1. $\lambda_{baseline}$ is the risk without exposure stratified by city, sex, birth year, and attained age. σ_{sex} is a sex specific multiplier to dose response. β is the dose response coefficient. The factors τ and ν modify dose response based on age at exposure and age at death respectively using exponential and logarithmic transformations as shown.

This model is similar, though simplified and modified to accommodate the fact that the data comes from multiple strata with different species and strains with differences in average lifespan and dose response. Specifically, the following model was applied:

$$ERR \sim \begin{cases} \lambda_{\text{stratum}} + \dfrac{\beta_{stratum} \cdot dose \cdot e^{\nu \cdot (age\ at\ exposure)}}{1}, & if\ acute \\ \lambda_{\text{stratum}} + \dfrac{\beta_{stratum} \cdot dose \cdot e^{\nu \cdot (age\ at\ exposure)}}{DREF_{LSS}}, & if\ protracted \end{cases}$$

where the data is stratified by study, sex, species/strain, and quality of radiation. Dose response, $\beta_{stratum}$, and baseline risk $\lambda_{stratum}$ are allowed to vary by stratum. Dose response is modified by average age at exposure (as a ratio of average life expectancy for a given species/strain) ν and, if protracted, by $DREF_{LSS}$. Unlike dose response and baseline risks, the value of these moderators are fixed across all strata in order to develop central estimates.

The first difference between the model used in this work and the Ozasa's model is that data is stratified and each stratum is allowed a distinct dose response. While Ozasa analyzed a single (albeit divergent and non-homogeneous) human population, this analysis focuses on multiple species and strains of animals. It is well accepted that distinct species, strains and each gender a show different dose responses, so it is sensible to allow the baseline risk and dose response to take on different values [55]. Data was further stratified on study and quality of radiation as a precaution against the possibility that these factors might influence dose response as well.

The second difference from the Ozasa model is that only moderation by age at exposure was considered instead of both age at death and age at exposure. Lifetime mortality rates were analyzed in this analysis, rather than mortality rates with time as in the Ozasa's analysis. Ozasa's analysis included age specific mortality rates because this value is of general interest to epidemiologists. However, this work is not interested in the age specific mortality rates of mice or rats, but only the effect of protraction on these rates. Therefore, the choice was made to keep the analysis simple and to avoid the

question of how to calculate age specific mortality rates in a way that would be consistent across the different studies, species and strains in this analysis. Specifically, some method would have had to be chosen to divide the data into discrete lifespan intervals as Ozasa did, a step that would add complexity to the analysis without improving the estimates of $DREF_{LSS}$.

Related to this change, regressions were conducted to estimate the mean mortality rate observed in each treatment group, assuming normal error distribution and weighted by the inverse variance of that mortality estimate plus at heterogeneity estimate (which was never significantly greater than zero, because fits were good). This is also a point of departure from Ozasa's analysis, which directly estimated the rate of mortality events for a particular dose, city, sex, age category, and age at exposure category assuming a Poisson distribution.

Unlike Ozasa's work [10], this analysis also had to deal with the fact that different species and strains had different lifespans and mortality rates. In order to put them on a common scale, lifespans, ages at exposure, mortality rates, and the standard error estimates were transformed to make them relative to corresponding values found in control groups for each stratum – a normalization step performed in many previous multi-species analyses [61,62]. For example, if the mean lifespan of a treatment group was 900 days and the mean lifespan of the corresponding control group was 1000 days then the treatment groups mean lifespan was transformed to 90% and the control groups mean lifespan to 100%. The exact details of these transformations are noted in the methods

chapter, Chapter 6. Once completed, this transformation gave the various strata common scales to calculate $DREF_{LSS}$ and the effect of age at exposure.

Finally, unlike in Ozasa's analysis, which dealt only with acute exposures, $DREF_{LLS}$ was explicitly included in this analysis. This term was applied only to protracted exposures. Animals that died before the last protracted exposure in their strata were excluded from analysis.

Having developed this model starting from the Ozasa model as a foundation, the Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm and profile likelihood methods were used to estimate the parameters of interest and 95% credible intervals. The details of these steps are described in the methods chapter, Chapter 6. The results of this analysis are described below.

Aim 2 Results

This work showed that protracted exposures induced approximately two fold less mortality risk than acute exposures. $DREF_{LSS}$ was estimated to be 2.1 with a 95% credible interval from 1.7 to 2.7. Sensitivity analysis agreed with these findings. No evidence was found that acute radiation lethality biased $DREF_{LSS}$ estimate. There was no evidence that $DREF_{LSS}$ changed with the dose range under consideration (see Table 9). Nor was there any evidence that $DREF_{LSS}$ varied by species, at least between those mice and rats which were the subject of this study. These results support the central hypothesis of this thesis that $DREF_{LSS}$ is significantly higher than the BEIR VII estimate of 1.5.

This work further showed that the data fit the linear-linear dose response model well. Figure 15 shows the fit to the "Primary analysis 0-4 Sv" dataset. Notice that most treatment points are within one standard deviation of the best-fit lines of the model, as would be expected. Moreover, no signs of heterogeneity were observed, confirming that the model fit the data well (the methods in Chapter 6 describe the heterogeneity analyses in detail).

The stratum that had the worst fit to the model was stratum 6, the only strata where protracted exposures appeared to induce more risk than acute exposures. This was caused by the fact that the large doses of protracted radiation led to cases of acute radiation syndrome over the period immediately after radiation was concluded. Acutely exposed mice also suffered from radiation syndromes. This stratum was one of the two that showed strong signs of acute radiation toxicity in Figure 13. This suggests that the animals shown here deviate from the general pattern in this analysis because of an unusual amount of acute radiation toxicity. Such toxicity is not found for majority of contemporary exposures where high total dose exposures are uncommon. Still these anomalies did not affect the ultimate $DREF_{LSS}$ estimate. Estimates were 1.7 to 2.7 when stratum 6 was included (“Primary analysis 0-4”) and nearly identical, 1.7 to 2.9, when stratum 6 was excluded (the “without toxicity” sensitivity analysis).

To further test bias from radiation toxicity, another variation to censoring animals was used. In these sensitivity analyses, animals that died soon after the final exposure in a stratum were removed from analysis. The results of these “delayed analyses” are shown

in Table 9 as "censor 6%", "censor 13%", and "censor 26%". In these instances, animals were removed from the analysis if they died before the final exposure plus 6, 13, or 26% of the mean control lifespan in the group, respectively. Because control mice lived about 840 days, this essentially means that they were removed from analysis if they died within 50, 100, or 200 days of the final exposure in the stratum. Control rats lived about 13% longer and so these cutoffs are 13% higher. The survival curves after censoring by 26% of control lifespan are shown in Figure 14. Note that signs of acute radiation toxicity are essentially eliminated in these cases (although some cases of death due to leukemia may have been removed as well). Nevertheless, just as it occurred in the "without toxicity" analyses, the $DREF_{LSS}$ estimates remained substantially the same.

We introduce the term “delayed analysis” in this work to describe this type of sensitivity analysis where reduction of data was conducted in such a manner as to remove from analysis animal deaths occurring over the “censored” period immediately following exposure to radiation. This approach fulfilled dual role – on the first hand it made the analysis similar to RERF work (where first five years following A-bomb exposure were not followed fully); on the second hand, it is fair to assume that all animal deaths associated with radiation syndromes (including multiple organ failure) are excluded from the analysis. In conjunction with the latter alteration in the analysis procedure, we introduce the term “late effects life shortening” to refer to animal deaths that are associated with late effects of radiation, but without differentiating between deaths caused by cancer or late tissue toxicities.

As noted above, a dose cutoff of 0-3 Sv instead of 0-4 Sv was also considered in the sensitivity analysis because both total doses are compatible with Ozasa's atomic bomb survivor analysis. The lower dose range (0-3 Sv) lead to a slightly higher and less certain estimate of $DREF_{LSS}$, 2.6 (1.8, 4.4), than the higher (0-4 Sv) dose range 2.1 (1.7, 2.7). This difference is small and not statistically significant. Either range could be used to estimate $DREF_{LSS}$.

$DREF_{LSS}$ was consistent in both mice (mostly male), 2.0 (1.4, 4.1), and rats (all female) 2.0 (1.5, 3.1). No evidence was found to suggest that the effects of protraction varied between these two groups despite the fact that they differed by species and mostly differed by gender. Though, importantly, this analysis only included two rodent species with relatively similar total lifespans. Moreover, most mice were male while all rats were female and so the effects of gender could not be tested independently of the effects of species. Future work would do well to integrate other species, like dogs, and members of both sex in each species to test whether protraction effects are similar across a wind range of mammalian species in both genders and ensure that results of animal studies are applicable to human populations.

Data	**$DREF_{LSS}$ estimate**	**Effect of age at exposure per 13% increase in age**
Primary analysis 0 – 4 Sv	2.1 (1.7, 2.7)	0.80 (0.55, 1.01)
Sensitivity analysis 0 – 3 Sv	2.6 (1.8, 4.4)	0.78 (0.29, 1.13)
Sensitivity analysis without strata showing radiation toxicity	2.1 (1.7, 2.9)	0.80 (0.54, 1.03)
Delayed analysis: censor 6%	2.2 (1.7, 2.8)	0.82 (0.61, 1.09)

Delayed analysis: censor 13%	2.2 (1.7, 2.9)	0.97 (0.72, 1.23)
Delayed analysis: censor 26%	2.2 (1.8, 2.8)	0.97 (0.73, 1.25)
Mice only (mostly male)	2.0 (1.4, 4.1)	0.84 (0.54, 1.09)
Rats only (all female)	2.0 (1.5, 3.1)	0.72 (0.38, 1.37)

Table 9: $DREF_{LSS}$ estimates and sensitivity analysis

The $DREF_{LSS}$ and effect of age at exposure estimates were derived from the model based on Ozasa's and applied to different subsets of the data. Subsets are noted in the left column and described in the text. $DREF_{LSS}$ estimates are shown with 95% confidence intervals in parenthesis, determined by the profile likelihood method. Age at exposure effects (a moderator) are also shown with 95% confidence intervals. Specifically, this table shows how a 13% increase in age at exposure moderates dose response – this too is further explained in the text. The top row represents the main $DREF_{LSS}$ estimate of this work. Subsequent rows represent $DREF_{LSS}$ estimates for sensitivity analyses. This table indicates that protracted exposures induce about one half the risk of "late effects life shortening" compared to acute exposures. The rightmost column of this table also indicates that risk may decline as a function of age at exposure, although this reduction is not significant at 95% confidence level. Increase in age of 13% was chosen to show how age at exposure moderates dose, because 13% corresponds to one decade of life increase in humans. Ozasa's report shows that a decade increase in age reduces dose response by 10 to 30% in atomic bomb survivors, a reduction that is compatible with the findings presented here.

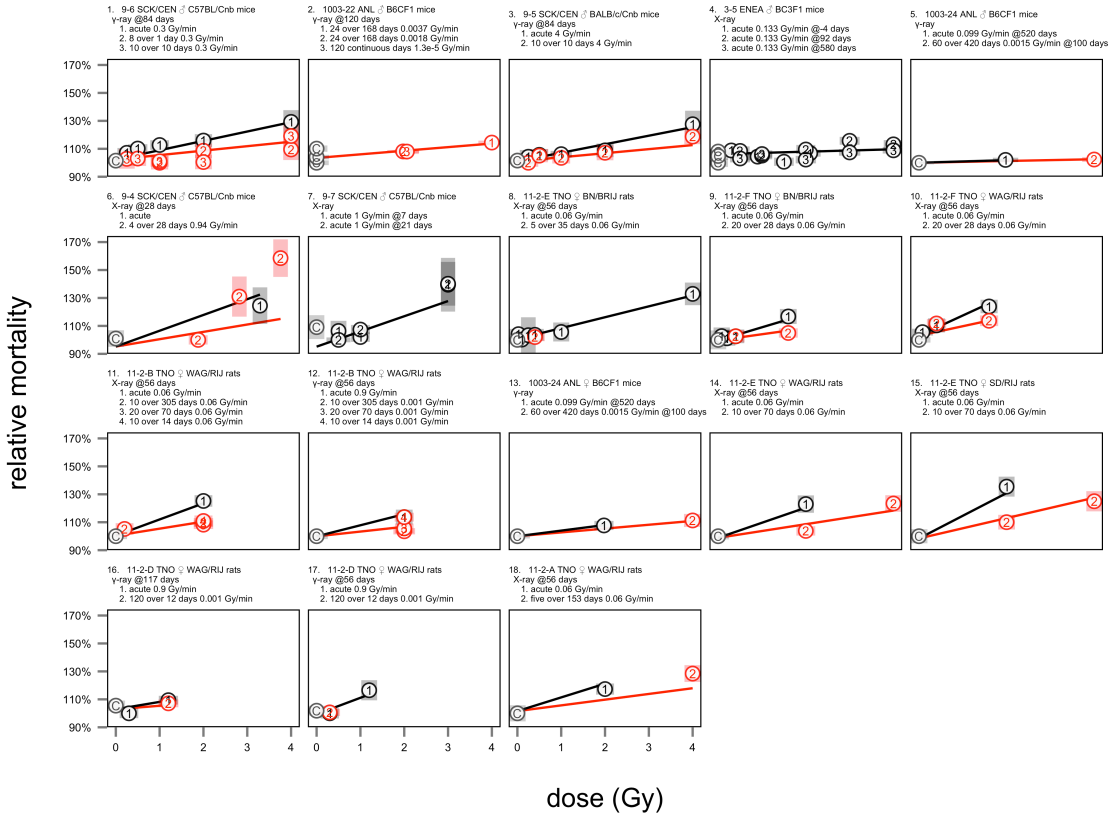


Figure 15: Relative mortality changes in response to different total doses, radiation protraction and animal age.

Dose response for all animals in the "Primary analysis 0-4 Sv". Each facet (1-16) shows mortality rates relative to mean control mortality (Y axis) vs. dose in Gy (X axis) for treatment groups in the analysis stratified by study, sex, strain, species, and quality of radiation exposure. The order of these facets is the same in Figures 13 and 14, sorted by the total number of animals in each stratum. The mean values of individual treatment groups are depicted as circles. The number within the circle indicates the cluster that a

treatment belongs to, detailed in the facet label. The letter “C” indicates control animals; their relative mortality was set to 100%. Vertical bars depict standard error. Black numerals and curves represent acute exposures and red numerals protracted exposures. Thin solid lines correspond to best fit projections from the “Primary analysis 0-4 Sv” without the influence of average age at exposure and determining a single best fit estimate for all acute and all protracted exposures. Note that strata 4 and 7 have no protracted exposure estimates. In these strata all animals were acutely exposed but average age at exposure varied within treatment groups in these strata. Note also that most datapoints (average relative mortality) fit linear-linear model well with the exception of stratum 6. This stratum included highest total doses and a corresponding increase in acute radiation toxicity (see Figure 13).

In addition to estimating $DREF_{LSS}$, the effects of age at exposure were tested for. Studies of atomic bomb survivors show that dose response declines with age at exposure in by about 10-30% per decade [10]. For example, a 1 Gy exposure to a 30-year-old induces 10-30% less cancer mortality than the same dose to a 20-year-old. This fact is relevant to animal studies that compare acute and protracted exposures because usually protracted exposures sequences begin at the same age as acute exposures and subsequent treatments in the protraction groups are given to animals that are older. This means that the average exposure age of protracted treatment groups is higher than that of acutely exposed treatment groups. Therefore, protracted exposure may induce less risk simply because the exposed animals are older and this may bias estimates of $DREF_{LSS}$, making protracted exposures appear safer than they are.

Dose response did decline with age at exposure, as expected, but that the decline did not fully account for the differences in acute and protracted dose responses. On average, dose-response declined by about 20% per 13% increase in lifespan. 13% was chosen because it corresponds to a decade of lifespan in a human population with an average age of 80. Therefore, this estimated increase is comparable to that observed in atomic bomb survivors. However, it is important to note that these age related estimates were not statistically significant. 95% credible intervals ranged from 0.29 on one extreme to 1.25, a range that corresponds to a 70% decrease in dose response per “animal decade” up to a 25% increase. It is likely that these results failed to be statistically significant,

only because there was too little data found that directly compared multiple ages at exposure. Future researchers might integrate more such data into their analyses in order to more accurately assess the impact of age at exposure.

Most important for this analysis, age at exposure did not fully account for the protraction effect, which is why $DREF_{LSS}$ estimates all remain significantly above 1 in Table 9. Also note that stratum clusters, 1.2, 1.3, 3.2, 12.4, 16.2, and 18.2, aged very little (less than 15 days) between first and last exposures in their protracted exposure sequence, yet still showed signs of a lower dose response than corresponding acute exposure stratum (Figure 15).

In toto these results show that age at exposure may decrease dose response, as in atomic bomb survivor analysis, but that protracted exposures induce less risk regardless of average age at exposure.

Summary

The findings presented in this chapter support those of Chapter 3. Protracted exposures induce less risk than acute exposures by 1.7 to 2.7 with 95% confidence. This, in turn, supports the hypothesis that $DREF_{LSS}$ is higher than BEIR VII's estimate of 1.5.

The analysis also supports the findings of Chapter 3 that a linear-linear model fits dose response data well and is an appropriate model to use to estimate the risk of acute exposures. Several major open questions remain:

1. What is the true dose response model that is well fit by a linear-linear model?
2. Is there a range of total doses or dose rates that should be described by independent liner models? (In other words – is a series of linear fits for different, with regard to dose and dose rate, subsets of data functionally better for predicting radiation responses than a more complicated "fits all" model?)
3. Why do the results presented here contradict the findings of Jacob et al. 2009 [6].
4. What recommendations can be made about contemporary radiation exposure from these findings?

These questions are discussed in detail in the following chapter.

Chapter 5: Discussion

This dissertation has provided evidence to support two arguments:

1. Protracted exposures induce less mortality risk than acute exposures. $DREF_{LSS}$ is between 1.7 and 2.7 with 95% confidence (1.8 to 4.4 in one analysis), confirming the overall hypothesis of this work that $DREF_{LSS}$ is higher than BEIR VII's central estimate, 1.5.
2. Animal mortality response following radiation dose is not linear quadratic. Instead, it conforms well to a linear-linear model (illustrated in Figure 16).

These two arguments have implications for the mechanism of radiation induced life-shortening, radiation protection policy, and evaluation of the risk of low dose exposures. They also contradict the results of Jacob 2009, who found that protracted human exposures pose equal risk to acute exposures [6]. This contradiction could be explained in several ways. Perhaps human and rodent responses to radiation exposure are fundamentally different? Perhaps one or another of the two analyses is flawed? Or perhaps the exposures are not comparable for other reasons? Each of these possibilities is discussed in this chapter along with corresponding recommendations of future analysis that might better explain the contradictions, especially by including data from more animal experiments.

Dose response is not linear quadratic, but it's "true" shape is not known

Results presented in Chapter 3 showed that the linear-quadratic model employed by the BEIR VII report does not fit the observed animal data. Specifically, the risk of protracted exposures cannot be extrapolated from the curvature in dose response following acute exposures, because there is no apparent curvature in these data (see Figures 9, 10, and 11). The data do not conform to a linear-quadratic model. Instead they fit well to a linear-linear model with separate slopes for acute and protracted exposures (see Figure 15). It is important to note that this finding does not imply that the "true" underlying dose response function is linear-linear, only that the "true" dose response function (within the dose range used) approximates a linear-linear response, and rejects a linear-quadratic response. So what is the true dose response function?

One possibility is that cell reproductive death, assumed by the BEIR VII report to attenuate the dose response at doses higher than 1.5 Sv, attenuates dose response even at exposures equal to or less than 1.5 Sv. This is plausible: At 1.5 Sv ~10% to 50% of cells succumb to reproductive death [63]. Moreover, Little and others [30] have shown that the linear-quadratic exponential dose-response, the "S" shaped curve that is expected to result from reproductive cell death, fits atomic bomb survivor data (slightly) better than quadratic models. Notably, Little's linear-quadratic exponential fit would imply substantial reproductive cell death (~50%) at 1.5 Sv [30]. If this hypothesis were true, then acute responses would be "S" shaped, even in the range from 0 to 1.5 Sv, and a quadratic fit could appear approximately linear even if dose response curved upwards at

the lowest doses. In this case, $DREF_{LSS}$ would be greater than existing estimates, and, correspondingly, the risk of protracted exposures would be lower than existing estimates. Figure 16 illustrates this possibility.

Another possibility is that the true dose response function includes an adaptive effect. Many biological systems adapt to stresses, becoming more resistant following a first encounter with the insult. Radiation response is no exception. Multiple studies suggest that cells and whole organisms can adapt to radiation exposure and become more radioresistant [35]. In some cases, acute exposures induce less chromosomal aberrations when preceded by a small priming dose that initiates radioresistant adaptation. Priming doses have also been shown to activate the immune system and increase the rate at which damaged cells are eliminated through apoptosis. Notably, the authors of this report are not aware of any study that has shown strong evidence that a priming dose can reduce radiation induced life-shortening or carcinogenesis, although protection from "short term" tissue injury has been noted *in vivo*. For example, epidermal protection was observed in female BALB/c mice when a 5Gy exposure was preceded by a well-timed 100mGy priming dose [64]. If adaptive effects do apply to long-term health outcomes as well, then the first exposure of a protracted series could induce adaptation and subsequent exposures would be induce less mortality risk. This kind of response might also approximate a linear-linear model as illustrated in Figure 16.

Still another possibility is that radiation exposure only induces mortality above some threshold dose. Doses below that threshold have no impact on lifespan, but above

the threshold lifespan is shortened. This kind of dose response is seen for many radiation endpoints, for example, acute radiation toxicity only occurs above a threshold dose. When doses are high enough, critical numbers of cells in the gut or immune system become nonviable, this in its turn makes the whole systems non-functional, leading to the death of an organism. If carcinogenesis or the other long-term life-shortening effects of radiation also require simultaneous damage of many cells at the same time (e.g. resident stem cells or a given organ), then it is plausible carcinogenesis and life-shortening are only increased after a threshold dose has been reached. The individual fractions that make up a protracted exposure series would be largely sub-threshold, and therefore induce less risk than corresponding acute exposures. As with the other models, this possibility could also explain an apparently linear-linear response again illustrated in Figure 16.

Each one of the dose response functions detailed above describe the effects of radiation exposure under some conditions and could, in principle, explain the risk of carcinogenesis and mortality following low-LET radiation exposure [31]. Each could (on its own) also produce the results observed, dose responses that are not linear-quadratic, but approximately linear-linear. However, it is important to emphasize that there are many more possible explanations for the observed dose response pattern as well. The effects of radiation exposure at the cellular level are complex. In addition to chromosomal rearrangements, ionizing radiation exposure leads to epigenetic changes [33], adaptive responses [35], hypersensitivity [36], and off-target effects [37,38]. Any of these factors might influence the shape of the dose-response function.

Moreover, the progression from cellular damage to whole organism diseases that affect mortality, like cancer, involves complex processes that are not completely understood [39,40]. Even if the most relevant forms of cellular level damage have linear-quadratic or sigmoidal dose responses, it is not clear that the long-term dose-responses would take the same form. Consider for example, that acute radiation syndrome is a consequence of cell reproductive death. This cell death can occur at even the smallest radiation doses, sometimes as a result of a single radioactive particle or photon. By contrast acute radiation syndrome never occurs from a single radioactive particle or photon. Instead a threshold dose (~3-4 Gy) is necessary for acute radiation poisoning. This is because many cell deaths are required to cause acute radiation syndrome. Even though cell death causes acute radiation syndrome it has a different dose response curve. Similarly, the unknown mechanisms that lead to cancer development may transform linear-quadratic or sigmoidal cellular responses to myriad of differently shaped whole-organism dose responses.

Finally, it is entirely possible that the mortality increase following radiation exposure is driven by multiple mechanisms with their own different dose response functions. Therefore, the final function may be a summation of multiple differently shaped functions.

At present, there is enough evidence to reject the linear-quadratic model used by BEIR VII and other reports, but not enough evidence to confidently extrapolate the true

shape of long term organism-level dose-responses based on existing knowledge of cellular and tissue level responses.

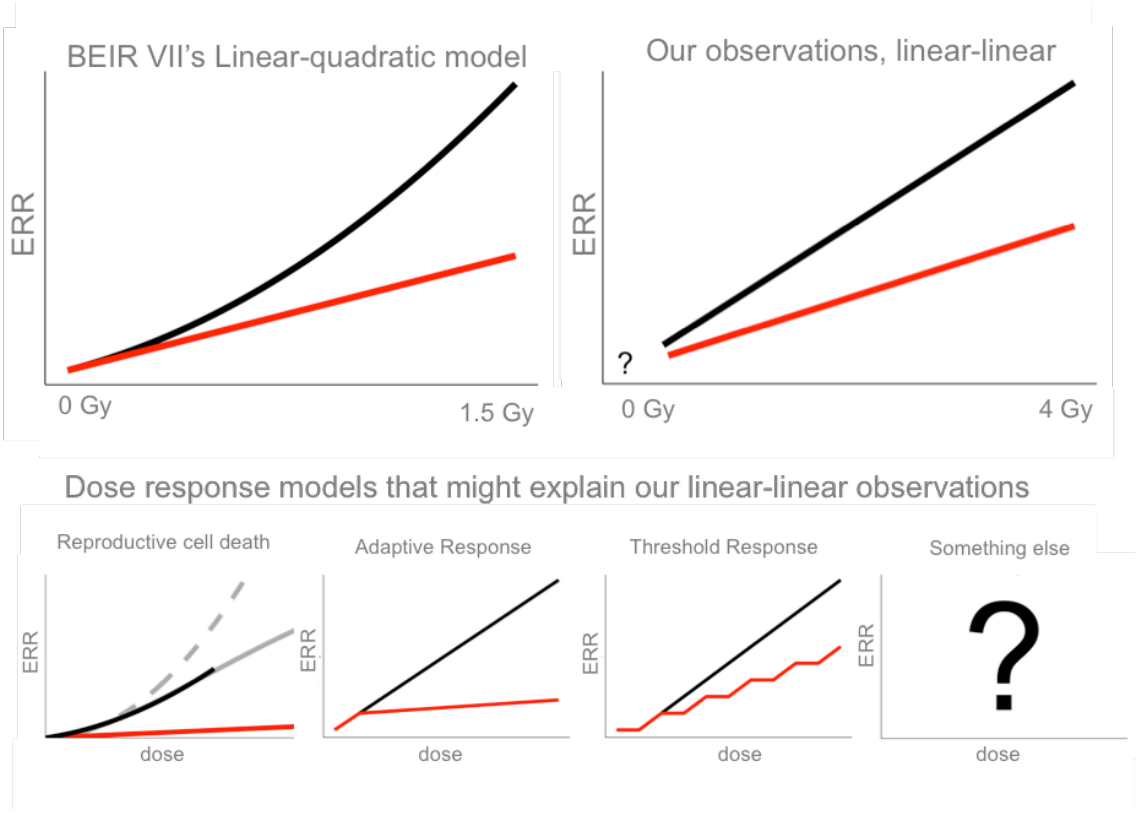


Figure 16: Possible dose response models

Various dose response models discussed in this thesis. Dose is plotted on the X-axis vs. excess relative risk of animal mortality (ERR) on the Y-axis. Black lines correspond to acute exposures. Red lines correspond to protracted exposures. The BEIR VII dose response linear-quadratic dose response model is shown on the top right. According to the BEIRVII report this graph is only applicable to doses less than 1.5 Gy (or 2 Gy in some cases). This model did not fit the complete animal mortality data collected (see Chapter 3). Instead, data fit well to a linear-linear model shown on the top right (see

Chapter 4). However, the "true" dose response model is not necessarily linear-linear. We simply insist that animal mortality as a function of the dose response is not linear quadratic and that a linear-linear model fits the data better. Several alternate "true" dose response functions that might explain these linear-linear observations are shown in the bottom row. Each of these might produce a dose response that appears linear-linear when only a few exposure doses are available (as in this analysis). Cell reproductive death would imply that the "true" dose response function is linear-quadratic-exponential. The grey dashed line depicts the quadratic component of dose response. The true dose response would deviate down from this quadratic fit due to an exponential component that results from cell reproductive death. The ultimate dose response shape would be "S" shaped. Notably BEIR VII believed that the true dose response function is linear quadratic exponential, but that only the linear-quadratic portion was relevant at doses below 1.5 Sv (see e.g. **Figure 1**). An adaptive response would imply that after an initial exposure subsequent exposure induce less risk, represented by the kink in the red, protracted exposure line. A threshold response would imply that damage only occurs when doses are above some threshold. Protracted exposures are made of a series of small exposure fractions. Only the part of a fractionated exposure that was above the threshold would cause damage, and so protracted exposures would be safer. Finally, the "Something else" panel acknowledges that there are many other dose response models that might explain these linear-linear observations.

The relative risk of protracted exposures should be estimated using protracted exposure data

How can radiation protection agencies estimate the relative risk of low dose and protracted exposures using atomic bomb survivor data given the existing uncertainty about the relationship between radiation exposure and long-term health responses? In the BEIR VII report these risks were extrapolated, in part, from acute exposure data after assuming a linear-quadratic dose response model. This approach is prone to error because the true dose response shape is unknown. Rather than attempting to derive the most plausible dose-response model and base all estimates upon it, a more conservative approach would develop protracted risk estimates based on direct comparisons of acute and protracted exposures. This approach makes minimal assumptions about the dose response in order to minimize the possibility of making wrong assumptions.

With this in mind, $DREF_{LSS}$ was estimated using only studies that directly compared acute and protracted exposures, as presented in Chapter 4. The linear-linear dose response model was used, not because it represents the true dose response function, but because it fits the data well and is sufficient to find a central $DREF_{LSS}$ estimate, 1.7 to 2.7, regardless of the true underlying dose response function.

Estimating the relative risk of low dose exposures, $LDEF_{LSS}$, on the other hand, continues to be challenging. Ideally, $LDEF_{LSS}$ would be based on direct comparisons between acute high dose exposures and acute low dose exposures. Unfortunately, the

statistical power of such analyses is low because the effect of such low dose exposures is small. Therefore, the estimated risk of low dose exposures will likely continue to depend on the assumption of some theoretical dose response function. Most radiation protection policy is based on the argument that the risk following such low dose exposures is collinear with the risk following protracted exposures ($DREF_{LSS} = LDEF_{LSS}$). This is founded on the idea that protracted exposures are composed of many low dose exposures separated in time. However, one could also plausibly argue that the risk following low dose exposures should be collinear with the acute dose response or fit a model that is entirely unique (see Figure 16 for some possibilities). This work does not make a specific recommendation, except to note that all humans are exposed to and cope with a continuous protracted radiation from natural background sources [1]. Starting with this background, any occasional additional low dose exposures (e.g. CT scans) could be considered just one fraction in a lifetime of protracted irradiation exposures. In this case $DREF_{LSS}$ would be applicable to these low dose exposures. This, however, is not necessarily so – intermittent radiation exposure "spikes" above environmental thresholds could also be considered as isolated (and hence dose for dose) acute exposures. If some low dose exposures do have a different risk than protracted exposures, it can only be because events like CT-scans represent small exposures spikes that could carry different risk than the ongoing protracted exposures all humans receive.

Finally, it is not impossible that environmental radiation thresholds predispose whole organisms to certain level of "around the clock" DNA and cell repair. If more

repair is needed (because of a small additional exposure and damage), success of such a repair and capacity of cells to execute it may entirely depend on ability of cells to recognize that they have been damaged. Thus, perhaps, a small dose that could "go unnoticed" could cause some extra damage, while a "noticeable" small dose may induce better overall repair (and have a "hormetic" effect). Such two scenarios which do not exclude each other would fit different models as well.

Why do the results presented here contradict Jacob et al. 2009?

In 2009 Jacob and others [6] showed evidence that protracted exposures received by radiation workers in 15 countries induced as much excess risk of cancer development as similar acute exposures in atomic bomb survivors. If this finding is true, it implies that there should not be a $DREF_{LSS}$ correction and contradicts the findings presented here. What could cause this contradiction?

There are several reasons these findings could be in conflict:

1. The analysis presented here is misleading.
2. Jacob's analysis is misleading.
3. The difference is due to chance.
4. Animal mortality and human excess risk of cancer incidence dose responses react differently to protraction.

The analysis presented here may be flawed, though, of course, rigorous effort was made to ensure that it was not. The best validation of the findings of this study is that it agrees with previous analyses of acute and protracted animal studies, which suggest that protracted exposures induce 2-10 fold less risk than acute exposures [23]. Naturally, both the $DREF_{LSS}$ analysis presented here and the previous work by others may be in error.

The Jacob's analysis [6] could also be flawed. While INWORKS follow-up studies have continued to support Jacob's findings [65], other epidemiological work contradicts the 15-country worker study [66] that Jacob's estimate is based on. A study of nuclear weapons plant workers in the United States [29] estimates an excess relative risk of solid cancer development, 0.14 (-0.17, 0.48) per Sv, significantly lower than the estimate from the 15 country study [66], 0.97 (0.14, 1.97). Both of these studies observed hundreds of thousands of workers for millions of person years. There is no compelling reason to believe that they should come to different risk estimates. Yet, while one supports a $DREF_{LSS}$ correction the other does not.

Jacob's analysis relies on epidemiological observations rather than controlled experiments. Human population exposures are not randomly assigned and therefore it is difficult to find an unbiased control population to compare exposed populations to [67]. While epidemiologists do their best to deal with such biases, these problems make the study of human populations inherently more error prone than processing of controlled animal studies data.

Based on these arguments, it is more likely that Jacob's analysis is flawed instead of the one presented here. If either (or both) are flawed, a likely reason is because they underestimate the uncertainty of their $DREF_{LSS}$ estimates. Every statistical model makes assumptions about the distribution of data. If these assumptions are wrong, this leads to over-confidence.

Still, it is not necessary for either analysis to be flawed to explain the contradiction between Jacob's results [6] and the results of this work. They could differ by chance alone. The central estimate of $DREF_{LSS}$ in this work, 2.1, is outside of the 90% confidence interval of Jacob's estimate, 0.5 to 2.0. However, 10% of the time a re-estimate should fall outside of a 90% confidence interval. Moreover, this $DREF_{LSS}$ estimate is just outside of Jacob's range. It is, therefore, entirely possible that the observed difference is due to chance. It is worth noting that the $DREF_{LSS}$ estimate from this work has a tighter confidence interval than Jacob's analysis and is strongly confident that $DREF_{LSS}$ is greater than Jacob's central estimate of $DREF_{LSS}$, 0.8 ($p < 0.00001$). Thus, even if the difference between this result and Jacob's is by chance alone, it is most likely that $DREF_{LSS}$ is substantially higher than Jacob's central estimate.

Finally, it is possible that these results differ from Jacob's because animal mortality and human excess risk of cancer development respond differently to radiation protraction. The analysis presented here was limited to mouse and rat mortality data because these were the species in the set of studies that met the specified inclusion criteria (i.e. lifespan animal studies comparing acute and protracted exposures with doses

less than 4 Sv). The radiation-induced mortality in these rodents is associated with fatal cancers and other fatal complications that respond very differently to acute vs. protracted exposures. The incidence of all cancers, fatal and non-fatal alike, which formed the basis for most of Jacob's meta-analysis [6] may be more equally induced by protracted and acute radiation exposures.

In order to resolve the conflicts between this dissertation work and Jacob's work, more studies need to be performed. An independent party should repeat Jacob's analysis to help confirm that it is not flawed and/or do a companion analysis focused on fatal cancers alone. The analysis presented here should be extended to additional, not necessarily fatal outcomes (most especially cancer incidence) and on additional animal species, especially the data available on dogs known to have radiation responses more like humans than rodents have. Beagle dogs have been extensively studied in US National Laboratories. These added analyses would ensure that the $DREF_{LSS}$ estimates developed from rodents extend to other species as well. This is especially relevant because our ultimate goal is the see if these findings do or do not apply to humans. Such work will also require extending existing radiobiology archives by adding more animal data to them, a goal that is elaborated below.

Does $DREF_{LSS}$ take on multiple values and can these be estimated?

As noted above it is entirely possible that the relative risk of protracted exposures varies depending on treatment conditions and the endpoint under considerations. Some

cancers may be equally induced by acute and protracted exposures. Others may be much more responsive to acute exposures. The protraction effect could vary by age at exposure. The work presented here has only focused estimating a single $DREF_{LSS}$ value, the one applicable to all causes mortality and the treatment conditions represented by studies in Chapter 4. Is it likely that $DREF_{LSS}$ varies by endpoint and treatment conditions? If so, how should this affect a mechanistic understanding of dose response? How should it affect radiation protection policy?

It would be surprising if $DREF_{LSS}$ did not vary by endpoint. In humans radiosensitivity varies by cancer type (if cancer incidence is the endpoint), gender, and age at exposure [10]. Different animal species and strains also show different radiosensitivities with regard to mortality as exemplified in Chapter 4, as well as for cancer induction as shown by our lab [62]. Likewise, the radiosensitivity of cells and their response to protraction vary by cell type and cell stage in cell cycle [17–19]. Given that radiosensitivity is so prone to variation, and that the effects of protraction vary in cell studies, it would be surprising if the moderating effect of protraction, $DREF_{LSS}$, did not also vary for different species, strains, genders and age for long term health outcomes like carcinogenesis and mortality.

Exploring this variation is a worthwhile, though challenging exercise. There is no theory that can accurately predict relative dose response of cells to acute and protracted exposure from first principals. Dose response must instead be measured empirically. Neither is there a theory that can accurately predict what cancers types are most sensitive

to protraction effects. These too are estimated based on direct observations. However, identifying, quantifying, and building predictive models that reliably predict dose response based on fundamental principles, rather than direct measurement, is a critical goal in radiobiology and cancer research. Therefore, it is worth attempting to estimate the ways in which $DREF_{LSS}$ may be moderated by factors such as age at exposure, species, strain, sex, and endpoint in order to assist efforts to fit these variations into a consistent and predictive theoretical framework.

The problem of accurately estimating endpoint specific or treatment specific values of $DREF_{LSS}$ is challenging and because of this is of limited use for the purposes of radiation protection policy.

This dissertation focused on all causes mortality, arguably the most straightforward and un-ambiguous endpoint that can be studied. Every animal dies, and it is easy to identify and record when the death event occurred. By contrast, many cancers are rare and not as easy to identify. Studies vary in rigor, identification procedures, and terminologies that they use to identify endpoints like cancer development.

Despite the fact that this work focused on the straightforward endpoint of life-shortening, developing a consistent estimate of $DREF_{LSS}$ was challenging. An ill-fitting linear quadratic dose response model can bias the final estimate as shown in Chapter 3. Unexpected levels of acute radiation toxicity obscured $DREF_{LSS}$ estimates in one-analysis strata in Chapter 4. There is still too little data to develop a high certainty estimate of how age at exposure affects dose response in rodents, a factor which might further moderate

$DREF_{LSS}$ estimates. If this work had instead focused on the multitude of cancer endpoints and considered factors that might moderate $DREF_{LSS}$ estimates, the challenges would also have multiplied, and the uncertainty associated with the final results would have increased.

Therefore, when developing radiation protection standards, one should be cautious before developing different $DREF_{LSS}$ estimates varying by endpoint or moderator. Such estimates would complicate protection policy and increase the risk that such policy was based on erroneous analyses.

More data and additional analysis should be used to further improve the $DREF_{LSS}$ estimate

This dissertation work is part of the incremental process of scientific advancement that has, with time, improved the estimate of $DREF_{LSS}$ and radiation protection more generally. While, in the opinion of the author, this work represents the most thorough and accurate attempt to estimate $DREF_{LSS}$ from animal mortality studies, ongoing efforts could improve these estimates further and provide additional benefits to radiation biology.

The work presented here is the best estimate of $DREF_{LSS}$ from animal mortality for several reasons. As discussed in the introduction, previous estimates of $DREF_{LSS}$ have been flawed, either because they are based on a linear quadratic model fit to acute exposure data [2,22], or because they are based on exposure doses outside of the range of

those applicable to atomic bomb survivors or because they were conducted without corrections for age at exposure [23]. This work, by contrast, considers exposures in a dose range applicable to atomic bomb survivors, adjusts for age at exposure, and tests for bias from acute radiation toxicity. Moreover, this work considered a larger number of studies that compared acute and protracted exposures than any of the previous work. The uncertainty range suggested by the animal data analysis in this thesis, 1.7 to 2.7, is narrower than previous estimate 2-10 based on animal data analyzed by the NCRP in 1980. This narrowing likely reflects an improved $DREF_{LSS}$ estimate, due to the careful control for the factors detailed above.

However, the estimate of $DREF_{LSS}$ and other radiation protection factors could be further improved. An important step in that process is expanding the set of data available for analysis. This work would not have been possible without the public archiving efforts of the European Radiobiology Archives, the Janus archives, and the International Radiobiology Archives before them [42,43,45]. The exemplary work of the Radiation Effects Research Foundation (RERF) has also made the most important parts of the atomic bomb survivor data available for public analysis [10]. However, there are many important data sets that are still not publically available.

Roughly half of the historic radiation biology studies conducted by national laboratories during the cold war and documented by the International Radiation Biology Archives (IRBA) are still not available as individual level data. Approximately 200,000 animals listed in the IRBA documentation are not part of a publically accessible archive.

Moreover, the public archives that do exist provide very little data on any contemporary study conducted after 1990. Radiation protection policies and radiobiology science would be better informed if these datasets were added to publicly accessible archives.

The availability of human exposure data is similarly difficult. De-identified dose response data is provided for atomic bomb survivors from the RERF (http://www.rerf.jp/library/dl_e/index.html). Similar data are available from studies of radiation workers at the US department of energy (https://www3.orau.gov/CEDR/). Still a good deal of the data analyzed in Jacob et al. 2009 is not available for download.

Of course, data does not have to be publically accessible to contribute to radiation biology. It is possible and common to perform meta-analyses based on published results as Jacob 2009 did. The downside of this approach is that raw data often provides critical information necessary for accurate assessments of radiation effects. For example, the work presented in Chapter 4 showed that animal mortality of stratum 6 did not fit the usual pattern. In these animals, due to high total dose radiation toxicity and the fact that death records were counted only when an animal died at the conclusion of protracted exposure, protracted exposures lead to apparently higher mortality rates than acute exposures (Figure 15). However, survival curves (which necessitate individual animal data) (Figure 13) clearly reveal that acute radiation toxicity is at the basis of this "unusual finding". This kind of careful inspection is much more difficult using published data.

Another important step in improving $DREF_{LSS}$ estimates is to independently re-evaluate the analyses used to develop the estimates. Analysts make mistakes and have

differences in opinions that bias their final estimates. Re-analysis is the best tool available to expose these biases and develop the better estimates. Therefore, revisiting Jacob's 2009 analysis is imperative. Jacob's result has the potential to impact radiation protection policy dramatically, effectively doubling the radiation risk estimate from the ICRP if $DREF_{LSS}$ estimates are changed from 2 to 1 as Jacob's results suggest they should be. However, it would be imprudent to make this change without independently repeating such an analyses to be sure that the results reflect the reality of the data rather than a bias of the analysis.

Together these two steps, making data public, and exposing it to independent analysis are powerful approaches to improve radiation protection policy over the long term.

Other problems that archival data could be applied to

This work has focused on $DREF_{LSS}$ in large part because Jacob's study made it a point of interest in the field of radiation biology. However, the same basic approach that was used here re-estimate $DREF_{LSS}$, using historic publically available radiobiology studies, could be applied fruitfully to re-estimate other protection factors and also to gain a better fundamental understanding of the effects of radiation on life.

One important avenue to explore is the effect of age at exposure. According to atomic bomb survivor data the risk of radiation exposure declines with increasing age at exposure by 10-30% per decade [10]. Therefore, it is believed that children are

substantially more impacted by radiation exposure than adults. Programs like Image Gently (http://www.imagegently.org/) focus especially on reducing medical exposure doses to children precisely because of this perception of greater risk.

A large number of the animal studies that have been conducted tested the effects of multiple ages at exposure on dose response. Studies that were relevant to this analysis were included in Chapter 5. However, only exposures less than 4 Gy and exposures to low-LET radiation were considered in this work. A better estimate of the relationship between age and risk could be developed by looking also at higher total exposure doses, high-LET radiation, and/or by including additional studies which are not yet available in publically available archives. Perhaps the range 10-30% for dose response decline with a 13% increase in age (roughly equivalent to a human decade) could be narrowed to produce a more precise estimate of how risk changes with age.

Also worth revisiting is the question of the relative biological effectiveness of low-LET radiation like X-rays and γ-rays vs. high-LET exposures to, for example, neutrons. It is widely believed that high-LET radiation is more damaging per Gy than low-LET exposures [18], but, like $DREF_{LSS}$, the exact value of RBE is subject to substantial uncertainty. The existing archive contains many comparisons of high-LET and low-LET exposures that might be re-analyzed. Considering that proton irradiation, a cutting edge clinical procedure, generates secondary neutrons, importance of these studies becomes even more immediate.

Finally, it is worth considering the possibility of developing a "complete" model of radiation mortality risk. In the investigations presented in Chapter 4 $DREF_{LSS}$ was quite stable across studies once a fitting data set had been selected. Moreover, a good deal of the variation in dose response between studies could be explained by differences in age at exposure, rather than species or strain specific differences in dose response. This observation has not been perused in depth, but some future project might endeavor to find a cross species dose response model with reasonably high total predictive power. This kind of model would be extremely useful, both for predicting risk in humans and understanding radiobiology and cancer development.

None of these goals are new, but instead, as the $DDREF_{LSS}$ re-evaluation in this work, they are becoming tenable. The changes in practice that enable new analyses are access to raw radiobiology data thanks to archiving efforts and development of less laborious best-fit modeling approaches. Previous efforts to estimate the effects of age at exposure and relative biological effectiveness have generally taken the form of structured reviews. These efforts are very worthwhile, but analysis of combined raw data is even better as the investigator can ensure that all data are treated the same way and that estimates are combined in a rigorous quantitative manner. Therefore, the general approach taken in this dissertation should help to incrementally improve radiobiology understanding in the future. With good luck, this increased understanding will enable theoretical insights that push forward science's basic understanding of biology, just as radiation studies have in the past [68].

Chapter 6: Methods

Data selection for Chapter 3

Chapter 3 sought to revisit the BEIR VII animal mortality analysis with additional animal data from archival studies. This new data included in this work came from animal irradiation experiments conducted in the United States and Europe and deposited into or connected with the Janus (http://janus.northwestern.edu) or ERA (https://era.bfs.de) databases. These databases are part of an ongoing effort to aggregate and make public all of the data generated in studies of animals exposed to radiation [42]. Most of the material in these archives comes from large, lifespan animal studies conducted by national laboratories during the cold war period.

Data were selected to adhere to the same criteria used in the BEIR VII report so that the results of this study could be directly compared to the results of BEIR VII. Steps were taken to ensure the reliability of archived data by cross validating it against primary literature. Initially, all of the individual level animal data available from the ERA and Janus archives were considered. As in BEIR VII's animal mortality analysis, animals that received whole body, external beam, low-LET radiation exposure at total doses less than 1.5 Sv were selected. Animals were excluded if they received additional non-radiation treatments like hormones or radioprotectors.

Once these data were selected, they were verified against the original literature, to ensure that treatment conditions and mean lifespans matched published results. In several cases data were excluded from the analysis because they were irreparably different than

published results. These exclusions and a detailed rationale for each of them are shown in Table 3.

Finally, data were only included if they contained 3 distinct treatment groups per stratum necessary to fit a linear-quadratic dose response model. The specific stratification criteria are addressed below. The number of animals that were eligible for analysis after applying each selection criterion is shown in Table 2.

Notably only mouse data met all the selection criteria specified. Therefore, even though ERA and Janus databases contain information on rats, dogs, and other species, these data were not included in this analysis, usually because their exposures were greater than BEIR VII's 1.5 Sv cutoff.

Data selection in Chapter 4

$DREF_{LSS}$ estimates are applied to atomic bomb survivor data to estimate contemporary risks of ionizing radiation. Therefore, these selection criteria were developed to isolate a set of animal mortality data with exposures comparable to those in the lifespan study (LSS) of atomic bomb survivors. Particularly, the criteria were based on work by Ozasa and others from 2012 [10], the 14th official report from the Radiation Effects Research Foundation (RERF), the organization which conducts the lifespan study.

As in Chapter 3, data was selected from the lifespan studies in the European Radiobiology Archives (ERA) and Janus Archives where individual level animal data was available. Animals were selected if they had been exposed to low-LET ionizing

radiation, either X-rays or γ-rays, because most of the exposure to atomic bomb survivors came from low-LET radiation. 76,096 animals met these criteria as shown in Table 6.

Chapter 3 argues that the risks of protracted exposures should be estimated from direct comparisons of acute and protracted exposures with a correction for age at exposure. Therefore, in Chapter 4 studies were only considered for analysis if they directly compared acute and protracted exposures or multiple ages at average exposure. Ozasa's atomic bomb survivor analysis was limited to exposures less than 4 Sv surface doses, and so exposures greater than 4 Sv were also excluded from all analysis.

All of the data that fit the afore mentioned inclusion criteria was cross-checked against primary literature to address concerns that data from the Janus or European Radiobiology Archives might not be completely reliable due to errors in the data encoding, transfer, or storage. Treatment and lifespan outcomes calculated from raw data were checked to ensure that they closely matched published results. Specifically, species, strain, sex, age at treatment, number of fractions, interval between fractions, dose, dose rate, quality of radiation exposure, number of animals, mean lifespan, and standard deviation of lifespan found for each treatment group in the selected data were verified with published results.

Small discrepancies were ignored (e.g. if mean lifespan differed by a few days or less) and obvious corrections were made (e.g. if a treatment group was missing information on the number of fractions this was added from published results). The details of these corrections are in

https://github.com/benjaminhaley/janus/blob/master/scripts/exp/data.Rmd. Each ignored item is marked with the word “warning”, each modification is marked with the word “fix”.

If studies varied substantially from published results they were excluded. For example, studies with ERA ids of 1002-1, 1003-21, and 2-11 and the study group 11-2-9 were completely excluded from the analysis because they differed substantially and irreconcilably from published results as summarized in Table 7. After applying all of these criteria, 11,528 animals remained eligible for analysis.

A small number of animals died at a younger age than the oldest age at which other animals in the same study where exposed to irradiation. This result can bias mortality analyses. If an animal does not receive the complete sequence of exposures assigned to its treatment group because of early death, then it has a different total exposure dose than the rest of the treatment group. If an animal in one condition dies at an age younger than the treatment assignment age of animals in another condition, then censoring will bias the mean lifespan of this treatment group. While there are many statistically sophisticated ways to deal with censoring, this work adopted a simple approach, excluding animals that died at an age younger than the final age at exposure of any animal in their analysis stratum. This conservative approach seemed best because it is simple, completely avoids bias by early death, and did not eliminate many animals from the analysis. After application, 202 animals were excluded and 11,528 animals remained eligible for analysis.

Finally, there were several criteria that were only applied to subsets of the data in order to perform a sensitivity analysis. These criteria are fully elaborated on in Chapter 4.

Data stratification in Chapter 3

As in the BEIR VII report, data were initially stratified by strain, gender, and age at exposure. Data were not specifically stratified by research institution, but, as a consequence of the chosen stratification conditions, each stratum in the end contained data from only one institution.

The dose response observed in individual treatment groups was fit to linear-quadratic models. It is known that radiation sensitivity varies by age, species, strain, and gender, among other factors. Therefore, as in the BEIR VII analysis, the linear, α, and quadratic, β, coefficients were allowed to take on different values within each stratum, while the ratio between quadratic and linear coefficients, β/α, was constrained across all strata. In this way all strata could contribute to one central $DDREF_{LSS}$ estimate despite variations between groups (e.g. if different genders or different mouse strains have different linear and quadratic coefficients).

Finally, one analysis was performed wherein data were additionally stratified by study to determine if the results remained consistent without the assumption of between-study comparability.

Data stratification in Chapter 4

As in Chapter 3, the analysis presented in Chapter 4 assumed that dose response might vary by sex, species, strain, and type of radiation exposure. Therefore, data was stratified so that each stratum shared all of these features in common. Animals were further stratified by study, as done in one of Chapter 4's analyses, under the assumption that there might be systematic difference in animal treatment between studies. In regression analyses dose response was allowed to vary within each of these strata as described below.

Unlike the Chapter 3 analyses, data was not stratified by age at first exposure. Instead, average age at exposure was used as a parameter in regression. This change was made because most of the exposures in a protracted sequence are delivered to animals that are older than the acutely exposed animals they are being compared to. Therefore, it is important to estimate how age at exposure moderates dose response and which part of the protraction effect is attributable to the age of the exposed animals versus the effects of protraction.

BEIR VII linear-quadratic model in Chapter 3

The linear-quadratic model as developed by the BEIR VII report was applied to lifespan data. Concretely:

$$\frac{1}{mean\left(lifespan\right)} = \alpha_{stratum} \cdot dose + \frac{\beta_{stratum} \cdot dose^2}{fractions} + intercept_{stratum} + \epsilon$$

where lifespan, dose, number of fractions and the residual, ε, took on distinct values for each treatment group and quadratic, β, linear, α, and intercept coefficients were determined separately for each stratum. The β/α ratio was fixed across all strata. Concretely:

$$\frac{\beta_{stratum}}{\alpha_{stratum}} = \theta$$

where θ took on a single value for the entire data set. A range of β/α ratios from -1 to infinity, corresponding to $DDREF_{LSS}$ from 0 to infinity, were considered to establish 95% credible intervals as described below. Likelihood was estimated for each ratio using the ordinary least squares maximum likelihood estimator, assuming normal error distribution, and weighted by inverse variance in lifespan for each treatment group. Concretely:

$$\log\left(likelihood\right) = -\frac{n}{2}\left(\log\left(\frac{2\pi}{n} \right) + \sum \log\left(v\right) + \log\left(\sum w\epsilon^2\right) + 1 \right)$$

where v is the variance in the outcome, n is the number of treatment groups, and ε is the residual difference between observed outcomes and those predicted by the model.

Linear-linear dose response model in Chapter 4

A dose response model similar to the one described by Ozasa and others in 2012 [10] was applied to data. However, this model was simplified and modified to accommodate the fact that the data comes from multiple strata of species and strains with differences in average lifespan and dose response. Specifically, the following model was applied:

$$ERR_{group} \sim \begin{cases} \lambda_{stratum} + \frac{\beta_{stratum} \cdot dose \cdot e^{\nu \cdot (age\ at\ exposure)}}{1}, & if\ acute \\ \lambda_{stratum} + \frac{\beta_{stratum} \cdot dose \cdot e^{\nu \cdot (age\ at\ exposure)}}{DREF_{LSS}}, & if\ protracted \end{cases}$$

where ERR_{group} was the excess relative risk of mortality per treatment group. Mortality per treatment group was the mean of inverse lifespan for all animals in the treatment group normalized by dividing them by mean control mortality rates in each data. Therefore, control groups had an average mortality of 100% and all other mortalities were relative to these values. Dose response, $\beta_{stratum}$, and baseline risk $\lambda_{stratum}$ were allowed to vary by stratum. These dose response values are further modified by age at exposure ν and, if protracted, by $DREF_{LSS}$. Unlike dose response and baseline risks, the value of these moderators are fixed across all strata in order develop central estimates. Average age at exposure was also normalized by dividing them by the mean lifespan of controls in the stratum.

The free parameters in this model were estimated by maximizing a likelihood function where mortality was assumed to fit the above model plus a Gaussian error term with variance equal to the variance in mortality measured in each treatment group plus random effects (which were always estimated to be zero). Concretely the likelihood function that was optimized was:

$$\sum_{n}\left(\log\left(\frac{1}{\sqrt{2\pi}\cdot\sigma_i}\right)-\frac{\left(y-\hat{y}\right)^2}{2\sigma_i^{\,2}}\right)$$

where n is the number of distinct treatment groups, i, represents a single treatment group, y is the measured relative mortality of the treatment group, $\hat{y}$ is the predicted mortality value from the equation defined above, and σ_i is the measured standard error of the treatment group with a correction for standard error from the function:

$$\sigma_i=\sqrt{\sigma_i^{\,2}+\tau^2}$$

where τ represents a standard error estimate that's value is estimated through maximum likelihood just like the other parameters in the regression.

Broyden Fletcher Goldfarb Shanno (BFGS) algorithm as implemented in R's bbmle package [69] was used in order to find parameter values that maximized the likelihood function.

Credible intervals for β/α ratios in Chapter 3

Likelihood was estimated for each β/α ratio as noted in the sections describing each particular model. A 95% credible interval was determined by the profile likelihood method [70]. Concretely, the 95% credible interval consists of all β/α ratios tested with likelihoods within 1.92 fold of the likelihood for the optimal β/α ratio.

Credible intervals for $DREF_{LSS}$ and age at exposure in Chapter 4

Credible intervals for $DREF_{LSS}$ and the moderator for age at exposure were estimated using the same profile likelihood method used to find credible intervals for β/α ratios in Chapter 3.

Conversion between β/α and $DDREF_{LSS}$ in Chapter 3

Using the linear-quadratic model $DDREF = 1 + (\beta/\alpha) \cdot dose$. As in the BEIR VII model, DDREF at 1.08 Gy, $DDREF_{1\,Gy}$ was used to approximate $DDREF_{LSS}$. Therefore, $DDREF_{LSS} = 1 + 1.08 * \beta/\alpha$.

Alternative models in Chapter 3

Several adjustments to the BEIR VII linear-quadratic model were also considered as discussed in the Chapter 3. These are elaborated below.

Eliminating the hormetic paradox

Coefficients were restricted to positive values to avoid the contribution of hormetic-type observations. The justification for this choice is detailed in the results section. Concretely:

$$\alpha_{stratum} > 0$$
$$\beta_{stratum} > 0$$

This had the effect of preventing hormetic-type observations from contributing to the $DDREF_{LSS}$ estimate.

Accounting for heterogeneity

First meta-regression was performed using a fixed effects model. This regression had the same form as the BEIR VII linear-quadratic model with positive constraints on β and α as described above. Likelihood was calculated, also as described above.

Next, the DerSimonian Laird approach was used to estimate random effects variation between treatments groups, τ^2 [56,71]. Concretely:

$$\tau^2 = \frac{\sum\left(\frac{\epsilon}{\sigma}\right)^2 - \left(n - df\right)}{\sum\left(\sigma^2 - extract\_diagonal\left(V^{-1}X\left(X^TV^{-1}X\right)^{-1}X^TV^{-1}\right)\right)}$$

where ε is the difference between observed outcomes and the predictions of the model, σ is the measured standard deviation of inverse mortality in each treatment group, n is the

number of treatment groups, df is the number of features in the model, X is a matrix of model features, extract_diagonal extracts the diagonal component of a matrix into a vector, and V is the diagonal matrix defined by σ^2.

Finally, the variance estimates for each treatment groups was adjusted to account for the random effects estimate. Concretely, the new variance estimates equal σ^2 plus τ^2. The BEIR VII linear-quadratic model was re-run. Likelihood was calculated as shown above with updated variance and weight estimates. This analysis was performed using the metaphor library in R [72].

Stratification by study

Study was added to the stratification criteria in addition to strain, sex, and age at exposure. As before, a minimum of 3 treatment groups per strata was required for inclusion in the analysis. This requirement eliminated many groups of animals from analysis as shown in Table 2. In all other respects this analysis was the same as the meta-regression analysis described in the previous section.

Survival analysis

Mortality over time was modeled by fitting a Cox proportional hazards model [58] with a linear-quadratic dose response:

$$\lambda_{stratum}(t) = \lambda_{stratum}(t) e^{\alpha_{stratum}\left(dose+\theta\frac{dose^2}{fractions}+Z\right)}$$

where $\lambda_{stratum}(t)$ is the hazard rate over time for a particular stratum, the rate at which animals are expected to die at any time, t. As before, the linear coefficient for each stratum, $\alpha_{stratum}$, was restricted to positive values. Z is an estimate of the random distribution of organism mortality rate by group estimated as described above. All other factors are the same as in previous models. This analysis was performed using the coxme library in R [73].

Tools and scripts in Chapter 3

The scripts used to perform these analyses are available in the Janus github repository, https://github.com/benjaminhaley/janus. Data from the ERA and Janus archives was consolidated and validated using https://github.com/benjaminhaley/janus/blob/master/scripts/exp/radiation.R. The resulting data, used in this analysis, is in https://github.com/benjaminhaley/janus/blob/master/data/external5.rds. This data was filtered and analyzed using https://github.com/benjaminhaley/janus/blob/master/scripts/exp/ddref.Rmd. Ongoing analyses are online at http://rpubs.com/benjaminhaley/ddref.

The analysis was performed in R [74] using plyr [75], dplyr [76], ggplot2 [77], survival [78], metafor [72], reshape2 [79], xtable [80], pander [81], lme4 [82], and coxme [73] libraries.

Tools and scripts in Chapter 4

The scripts used to perform the analyses in Chapter 4 are also available in the Janus github repository, https://github.com/benjaminhaley/janus. Data from the ERA and Janus archives was consolidated and validated using https://github.com/benjaminhaley/janus/blob/master/scripts/exp/data.Rmd. The resulting data, used in this analysis, is in https://github.com/benjaminhaley/janus/blob/master/data/thesis.rds and https://github.com/benjaminhaley/janus/blob/master/data/thesis_controls.rds. This data was further filtered and analyzed using https://github.com/benjaminhaley/janus/blob/master/scripts/exp/thesis.Rmd.

The analysis was performed in R [74] using plyr [75], dplyr [76], ggplot2 [77], scales [83], and bbmle [69], libraries.

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Vita

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