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Pathology Effects at Radiation Doses below those Causing Increased Mortality

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Mortality data from experiments conducted at the Argonne National Laboratory (ANL) on the long-term effects of external whole-body irradiation on B6CF$_1$ mice were used to investigate radiation-induced effects at intermediate doses of ^{60}Co γ rays or fission-spectrum neutrons either delivered as a single exposure or protracted over 60 once-weekly exposures. Kaplan-Meier analyses were used to identify the lowest dose in the ANL data (within radiation quality, pattern of exposure, and sex) at which radiation-induced mortality caused by primary tumors could be detected (approximately 1–2 Gy for γ rays and 10–15 cGy for neutrons). Doses at and below these levels were then examined for radiation-induced shifts in the spectrum of pathology detected at death. To do this, specific pathology events were pooled into larger assemblages based on whether they were cancer, cardiovascular disease or non-neoplastic diseases detected within the lungs and pleura, liver and biliary tract, reproductive organs, or urinary tract. Cancer and cardiovascular disease were further subdivided into categories based on whether they caused death, contributed to death, or were simply observed at death. Counts of how often events falling within each of these combined pathology categories occurred within a mouse were then used as predictor variables in logistic regression to determine whether irradiated mice could be distinguished from control mice. Increased pathology burdens were detected in irradiated mice at doses lower than those causing detectable shifts in mortality—22 cGy for γ rays and 2 cGy for neutrons. These findings suggest that (1) models based on mortality data alone may underestimate radiation effects, (2) radiation may have adverse health consequences (i.e. elevated health risks) even when mortality risks are not detected, and (3) radiation-induced pathologies other than cancer do occur, and they involve multiple organ systems. ©

INTRODUCTION

A constantly growing number of astronauts participating in progressively longer missions has given the identification, characterization and quantification of radiation-induced risks a high priority on the research agenda of the National Aeronautics and Space Administration (NASA). Numerous obstacles stand in the way of investigating the radiation-induced risks that are relevant to NASA. The mixed radiation environment of space is exceedingly complex (*1*). The sample size requirements needed for experiments designed to detect and accurately quantify the subtle biological responses that occur at lower doses cannot be achieved realistically (*2*, *3*). Finally, although laboratory animals have frequently been used to investigate radiation effects, there has been considerable reluctance to use data from these studies to predict radiation-induced health effects in humans even in those cases in which human data are either sparse or nonexistent.[3] These issues are not new; they have been intensively studied and discussed for decades.

Historically, a wide range of quantitative methods have been used to model radiation effects. These methods can be classified into two broad categories, empirical or data-driven models and models grounded in theory. Empirically based models have a long history in radiation biology and have generated discussions over such issues as competing risks and the merits of relative risk models compared to absolute risk models (*4–6*). Theoretical models also have a long history and have given rise to debates over cell killing, latency, wasted radiation, hormesis and the stages of carcinogenesis (*7–11*).

In recent years, empirical models have been described as phenomenological, and many investigators have switched to models that attempt to capture the behavior of biological mechanisms within the mathematics of their equations (e.g. *12–14*). Safe doses or thresholds in the dose response, either practical or real, are issues that have played prominent roles in the long and unresolved history of debates over the appropriate models to use when studying effects at low doses (*15–21*). Although models that fit data poorly can be easily identified, determining the best model is a difficult if not impossible task, especially at low doses (*3*, *22*, *23*). Despite these complexities, an organization like NASA must identify and quantify radiation risks to ensure the health and safety of their astronauts.

Animal studies were conducted at the Argonne National Laboratory (ANL) for nearly 50 years to study the biological consequences of whole-body exposure to external sources of radiation (*24–26*). The young adult mice used in these studies were irradiated under conditions intended to reveal the chronic effects associated with occupational levels of exposure (*27*). The ANL data have been examined

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extensively for radiation-induced life shortening and cancer mortality within a dose–response framework [e.g. see refs. (*24*, *25*, *28*, *29*) for comprehensive citations]. Very few of the experimental animals in the ANL studies received what has been arbitrarily defined (*30*) as either a low dose (below 20 cGy) or a low dose rate (below 0.05 mSv/min). There are, however, numerous exposure groups in the ANL database that straddle or slightly exceed the low-dose range. It is this small subset of the ANL data that is the focus of this study. This is not a dose–response study. Instead, our goal was to examine dose groups where radiation-induced effects on mortality could not be detected and to determine whether mice exposed at these levels have a spectrum of pathology (type and frequency) that differs from the pathology recorded for control mice.

DATA

A continuous series of large-scale animal studies was conducted between 1970 and 1992 in the Biological and Medical Research Division, ANL—a research effort generally referred to as the JANUS program (*25*, *27*). From these studies, a large database was compiled on the responses of both sexes of an F_1 hybrid mouse, the $B6CF_1$ (C57BL/6 X BALB/c), to external whole-body irradiation by ^{60}Co γ rays and fission neutrons [see ref. (*25*) for detailed documentation]. Three primary patterns of exposure were investigated: single exposures, 24 once-weekly exposures, and 60 equal once-weekly exposures. All irradiations were terminated at predetermined total doses, with dose calculated in centigrays at the midline of the mouse (*31*). Because we were interested only in the lowest doses available within the ANL data, γ-ray doses above 3 Gy and neutron doses above 30 cGy were not considered—a restriction that eliminated the data for 24 once-weekly exposures. Similarly, males receiving a single dose of neutrons were excluded from these analyses because of a paucity of relevant data. Although the ANL program that generated these data no longer exists, the data have been deposited in the National Radiobiology Archives created by the Department of Energy at Pacific Northwest National Laboratory in Richland, WA and are available to the public and interested researchers.

PATHOLOGY

Animal husbandry practices at ANL met or exceeded the federal standards established to ensure the humane treatment of laboratory animals. The experimental protocol of the JANUS studies required that animals be allowed to die a natural death (i.e. no medical interventions to delay death). Pathology data from the JANUS database come in two forms—Macro and Micro (*25*). Data derived from pathology judgments based on macroscopic examination (autopsy) performed by experienced prosectors are referred to as Macro data—data that exist for nearly every animal in the database. Pathology data derived from histological examinations performed by certified veterinary pathologists are referred to as Micro data (a random subset of the Macro data).

When a mouse died, a single cause of death was established at autopsy (designated "L") and pathologies that were simply observed at death (designated "N") were also recorded. For animals receiving a histological examination (i.e. Micro data), a board-certified pathologist also identified pathologies that were not the primary cause of death but were judged to have contributed to death (designated "C"). An independent external audit of these data confirmed 92% of the pathology diagnoses made by ANL pathologists (*29*). When diagnostic disagreements were found, the audit acknowledged that they were minor and subject to interpretation (*29*).

Every observed pathology was assigned a unique four-letter JANUS pathology code—translations to SNOMED (systematized nomenclature of medicine) and SNOVET (systematized nomenclature of veterinary medicine) codes also exist (*25*). Although overlaps between Macro and Micro pathology codes exist, the codes for the Micro data reflect the far greater diagnostic specificity that derives from histological examination (*25*). Over the years, subsets of the JANUS pathology codes have been organized (and formally incorporated into the database) into dozens of larger assemblages (combined pathology codes) in response to the analysis needs of the investigators working with these data (e.g. *29*). One of these combined pathology codes, all primary tumors (PR_T), was used in the mortality component of the analyses presented in this paper. When Macro and Micro pathology records for an animal are compared, the judgments made about a pathology having either caused or contributed to death are in agreement 98% of the time whenever primary tumors are involved (*25*). This extremely high concordance suggests that the Macro database with its larger sample size can be used reliably. PR_T is the mortality end point of interest.

METHODS: DETECTING AGE SHIFTS IN MORTALITY

Mortality analyses were performed only to identify the lowest doses within the ANL data at which a statistically significant shift in radiation-induced mortality (i.e. earlier deaths) could be detected. This was accomplished by applying standard Kaplan-Meier analyses (*32*) within strata defined by sex, radiation quality, exposure pattern, and experiment. The event of interest for these analyses was defined as a death where a PR_T was judged to have either killed or contributed to the death of an animal. All other causes of death (principally accidental) were classified as censored observations (*33*). The Wilcoxon (sensitive to early deaths) and log-rank (sensitive to late deaths) tests were used to test for the homogeneity (equality) of survival functions (*34*). Whenever multiple control groups existed within a stratum, Kaplan-Meier analyses were performed to verify that they could be pooled. Next, Kaplan-Meier analyses were used to compare the lowest-dose group within a stratum to its within-experiment (concurrent) control or pooled control. These pairwise comparisons performed within stratum were repeated at progressively higher doses until a statistically significant shift in mortality was detected. This entire series of Kaplan-Meier analyses was done twice—once for Macro data and once for Micro data. The informal logic used in these analyses was that a mortality shift was probably real when it could be detected in both the Macro data with its larger sample sizes and the Micro data with its more detailed pathology diagnoses.

METHODS: DETECTING SHIFTS IN THE SPECTRUM OF PATHOLOGY

The strata structure used for the mortality analyses was retained for the core objective of this study—an investigation of radiation-induced shifts in pathology. Within a given stratum (i.e. radiation quality, exposure pattern, experiment, sex), only those dose groups less than or equal to the lowest dose associated with a statistically significant shift in mortality were of interest. For this phase of the study, analyses were restricted to Micro data. Mice almost always die with multiple pathologies (an average of four or five per mouse), and it was deemed essential that the pathology diagnoses used for these analyses be based upon histological examinations made by board-certified pathologists.

For the investigation of pathology shifts, the JANUS pathology codes described previously were organized into nonoverlapping combined pathology groups. Two of the groups (cancer and diseases of the cardiovascular system) were chosen because they account for the vast majority of human deaths (*35*). Because of their abundance and importance to mortality, cancer and cardiovascular disease were further partitioned into their L, C and N subgroups. The remaining combined pathology groups used in these analyses were based on organ systems: liver and biliary tract, lungs and pleura, reproductive organs, and urinary tract. The small number of pathology codes that failed to fall into any of the previously mentioned categories were assigned to a miscellaneous group referred to

TABLE 1
Summary of Kaplan-Meier Pairwise Mortality Comparisons between Each Dose Group (Exposed to γ Rays) and its Control where Causes of Death (COD) from All Primary Tumors (PR_T) are the Events of Interest

			Macro		W	LR	Micro		W	LR
Experiment	Sex	Dose	PR_T	*N*	*P*	*P*	PR_T	*N*	*P*	*P*
1	Female	0 cGy	791	1026			220	248		
		22 cGy	354	453	0.38	0.43	159	177	0.38	0.38
		43 cGy	229	314	0.78	0.60	108	121	0.81	0.62
		86 cGy	131	177	0.08	0.04	60	73	0.16	0.33
		2.06 Gy	163	188	0.00	0.00				
	Male	0 cGy	149	191			120	142		
		86 cGy	153	189	0.47	0.55	131	138	0.20	0.11
		1.37 Gy	114	150	0.38	0.33	100	113	0.29	0.16
		1.98 Gy	262	308	0.00	0.00	118	122	0.00	0.00
60	Female	0 cGy	450	534			188	207		
		1 Gy	468	545	0.00	0.01	196	217	0.39	0.98
		2 Gy	132	162	0.03	0.01	111	124	0.07	0.12
		3 Gy	65	73	0.00	0.00	53	57	0.00	0.00
	Male	0 cGy	483	558			171	192		
		1 Gy	508	562	0.06	0.01	192	206	0.57	0.29
		2 Gy	139	164	0.11	0.05	95	113	0.80	0.67
		3 Gy	68	76	0.00	0.00	52	54	0.01	0.01

Notes. *N* is the total number of animals in the group. Experiment is weeks of exposure (single or 60 once-weekly), Macro is cause of death based on gross autopsy, Micro is cause of death based on histopathology, W is the Wilcoxon test, LR is the logrank test and *P* is the significance level of the test where $P = 0.00$ means $P < 0.01$.

as ''other''. Finally, the total number of pathologies (both neoplastic and non-neoplastic) identified within an animal was represented by a variable referred to as ''total''. The smaller sample sizes of the organ-based category and the two other aggregate categories led to the decision not to partition them into their L, C or N subgroups. For every mouse, the number of pathologies that fell within each of the 12 pathology categories just described was determined. In other words, the multiple pathology records that exist within the Micro database for an individual mouse were collapsed into a single record containing counts for each of the pathology categories (subsequently referred to as counting bins).

The 12 counting bins arising from the above pathology classification were used as explanatory variables in logistic regression analyses (*36*). Unlike ordinary regression, the response variable in logistic regression is dichotomous. For example, in these analyses the response variable was set to 0 when a mouse came from a control group and it was 1 when it came from an exposed group. The logistic model then uses the explanatory variables to predict the probability (p_i) that the response variable for a particular mouse is either 0 or 1:

$$\text{logit}(p_i) = \log[p_i/(1 - p_i)] = \sum \beta_i x_i,$$

where β_i are regression coefficients, x_i are the explanatory variables (i.e. the 12 pathology counting bins), $p_i/(1 - p_i)$ is the odds ratio, and $\log[p_i/(1 - p_i)]$ is the log odds ratio or logit (*36*). An intercept was not included in the logistic models. In summary, the logistic model uses the frequency and identity of pathologies observed within a mouse (i.e. the combined pathology counting bins) to predict a probability that a given mouse is either a control or exposed animal. All 12 explanatory variables were included in the initial logistic model, and a final model was achieved by the progressive elimination of nonsignificant variables.

RESULTS

Tables 1 and 2 provide sampling statistics for the dose groups used in this paper. In addition, these tables summarize the Kaplan-Meier analyses used to identify the lowest dose within a radiation quality, pattern of exposure, and sex where radiation-induced mortality could be detected. For γ rays (Table 1), there are indications (*P* values below 0.10) in the Macro data for females that animals exposed to a single dose of 86 cGy and higher die from PR_T at younger ages than do control animals. A significant shift in the age distribution of death from PR_T for males does not emerge until approximately 2 Gy. Minimum doses are higher in the data for protracted γ-ray exposures (Table 1). Nevertheless, the Macro data with its better sampling statistics gave indications of mortality effects at protracted doses of 1 to 2 Gy and were clearly evident for both sexes in the Macro and Micro data by 3 Gy—results generally consistent with those reported for single exposures (Table 1). For neutrons (Table 2), earlier mortality could be detected at a single dose of 9 cGy. The responses of animals receiving protracted exposures to neutrons were consistent with those just described for single exposures. A shift toward earlier mortality from PR_T was clearly evident in both the Macro (both sexes) and Micro (females) data by 14 cGy (Table 2).

Tables 3 and 4 summarize the behavior of the cancer variables in the logistic regression models. These tables reveal that shifts in the spectrum of pathologies found within an animal at death occur at doses lower than those reported above for mortality effects. For both γ rays (Table 3) and neutrons (Table 4), animals exposed to these lower doses were found at autopsy to have (relative to controls) significantly more (i.e. odds ratio > 1) tumors that were judged to have neither caused nor contributed to death, and significantly fewer (i.e. odds ratio < 1) tumors that were judged by a pathologist to have caused death—see the Tum_N and Tum_L columns, respectively.

Tables 5 and 6 summarize the interpretation of the non-

TABLE 2
Summary of Kaplan-Meier Pairwise Mortality Comparisons between Each Dose Group (Exposed to Neutrons) and its Control where Causes of Death (COD) from All Primary Tumors (PR_T) are the Events of Interest

Experiment	Sex	Dose (cGy)	Macro PR_T	Macro *N*	W *P*	LR *P*	Micro PR_T	Micro *N*	W *P*	LR *P*
1	Female	0	791	1026			220	248		
		1	501	661	0.26	0.29	223	253	0.29	0.17
		2	314	411	0.70	0.69	152	169	0.27	0.57
		5	222	312	0.31	0.50	115	132	0.65	0.52
		9	160	230	0.11	0.10	87	91	0.02	0.05
		19	148	183	0.00	0.00	672	78	0.00	0.00
60	Female	0	452	538			191	211		
		2	431	524	0.56	0.94	196	215	0.78	0.95
		8	174	205	0.19	0.02	78	87	0.23	0.17
		14	181	221	0.00	0.00	90	103	0.01	0.00
		22	195	228	0.00	0.00	92	110	0.00	0.01
	Male	0	485	561			171	192		
		2	459	537	0.13	0.27	151	174	0.67	0.91
		8	220	251	0.41	0.26	78	90	0.43	0.93
		14	202	230	0.02	0.03	67	78	0.88	0.68
		22	210	227	0.00	0.00	79	91	0.01	0.01

Notes. N is the total number of animals in the group. Experiment is weeks of exposure (single or 60 once-weekly), Macro is cause of death based on gross autopsy, Micro is cause of death based on histopathology, W is the Wilcoxon test, LR is the logrank test and *P* is the significance level of the test where $P = 0.00$ means $P < 0.01$.

TABLE 3
Summary of the Importance of Tumor Variables in Logistic Regression Models Used in Pairwise Comparisons between Each Dose Group (Exposed to γ Rays) and its Control

Experiment	Sex	Dose	Model *P* value	Tum_L OR (*P* value)	Tum_C OR (*P* value)	Tum_N OR (*P* value)
1	Female	22 cGy	0.00	0.66 (0.00)	NS	1.54 (0.00)
		43 cGy	0.00	0.36 (0.00)	NS	2.34 (0.00)
		86 cGy	0.00	0.38 (0.00)	NS	2.39 (0.00)
	Male	86 cGy	0.00	NS	1.57 (0.00)	1.24 (0.02)
		1.37 Gy	0.00		2.27 (0.01)	1.99 (0.01)
60	Female	1 Gy	0.00	0.80 (0.06)	NS	1.83 (0.00)
		2 Gy	0.00	0.41 (0.00)	NS	2.11 (0.00)
		3 Gy	0.00	0.24 (0.00)	NS	1.90 (0.00)
	Male	1 Gy	0.12	NS	NS	1.26 (0.10)
		2 Gy	0.00	0.65 (0.04)	NS	1.56 (0.11)
		3 Gy	0.00	0.66 (0.12)	NS	NS

Notes. Listing includes weeks of exposure (Experiment), sex, total dose (cGy/Gy), significance of the overall model, and odds ratios (OR) and their *P* values for the tumor variables. $P = 0.00$ means $P < 0.01$, NS means *P* value > 0.15. Tumor pathologies were identified as lethal (L), contributing to death (C) or simply observed at death (N).

TABLE 4
Summary of the Importance of Tumor Variables in Logistic Regression Models Used in Pairwise Comparisons between Each Dose Group (Exposed to Neutrons) and its Control

Experiment	Sex	Dose (cGy)	Model *P* value	Tum_L OR (*P* value)	Tum_C OR (*P* value)	Tum_N OR (*P* value)
1	F	1	NS	NS	NS	NS
		2	0.00	0.72 (0.03)	NS	1.28 (0.09)
		5	0.00	0.58 (0.00)	NS	1.28 (0.12)
		9	0.00	0.61 (0.03)	NS	1.66 (0.02)
60	F	2	0.02	NS	NS	1.40 (0.01)
		8	0.00	0.39 (0.00)	NS	NS
		14	0.00	0.42 (0.00)	NS	1.44 (0.06)
	M	2	0.02	0.66 (0.00)	1.48 (0.05)	1.38 (0.07)
		8	0.00	0.46 (0.00)	NS	NS
		14	0.00	0.50 (0.00)	1.54 (0.08)	1.46 (0.15)

Notes. Listing includes weeks of exposure (Experiment), sex, total dose (cGy), significance of the overall model, and odds ratios (OR) and their *P* values for the tumor variables. $P = 0.00$ means $P < 0.01$, NS means *P* value $>$ 0.15. Tumor pathologies were identified as lethal (L), contributing to death (C) or simply observed at death (N).

cancer variables in the logistic regression models. The only pathology variables that appear in these tables are those that were retained in one or more of the final models. For animals exposed to γ rays, detectable shifts in the spectrum of pathology observed at death were restricted almost exclusively to animals that received a single exposure (Table 5). Unlike the tumor variables, increased numbers of pathologies (odds ratio $>$ 1) were not detected until dose levels that also resulted in radiation-induced mortality. Once detected, these pathologies involved multiple organ systems (cardiovascular, kidney, lungs and pleura, and reproductive organs), and their relative effect (as measured by odds ratios) was equal to or greater than that reported above for tumors. The most noticeable result in this phase of the study was the almost complete absence of elevated nontumor pathologies in animals that received protracted exposure to γ rays (Table 5) and in animals receiving either pattern of neutron exposure (Table 6)—only three instances (2 Gy γ rays 8 and 14 cGy neutrons), all of which were of borderline significance.

DISCUSSION

The difficulties of studying phenomena at low and intermediate dose levels have been known and debated for decades, and they have not been solved because there are no solutions. At these exposure levels, empirical models bump into inescapable data limitations and theoretical models are limited by the complexity, lack of knowledge, and immense variability that exist at microscopic levels of biological organization. Although the inherent uncertainties of responses that exist at lower dose levels can be neither avoided nor eliminated, efforts have been made to quantify them[2,3] (*37*). This effort can be enhanced by a comparison of interpretations from different modeling approaches, especially when applied to the same data (*38*). These issues are extremely important to organizations like NASA that must identify, characterize and anticipate the radiation risks for astronauts as space travel becomes more common and space flights become longer.

The analyses presented in this paper were not done in a dose–response framework. We wanted to avoid the documented modeling problems that exist at lower doses. Further, most quantitative methods used in dose–response research (e.g. hazard models, Kaplan-Meier analyses) were designed for events that are assumed to be rapidly lethal (i.e. mortality effects). Our objective was to look for biological responses at dose levels at which radiation-induced mortality could not be detected. To identify the lowest dose associated with detectable mortality in the ANL data, we took advantage of the much larger sample sizes available in the Macro pathology data (see Tables 1 and 2). Potential

[2] P. G. Groer and B. A. Carnes, Bayesian estimation of dose thresholds for lung tumors in mice after single exposure to ^{60}Co Gamma radiation or fission neutrons, manuscript submitted for publication.

[3] NCRP, *Extrapolation of Risks from Nonhuman Experimental Systems to Man.* National Council on Radiation Protection and Measurements, Bethesda, MD, in preparation.

TABLE 5[a]
Summary of the Importance of Organ System Variables in Logistic Regression Models Used in Pairwise Comparisons between each Dose Group (Exposed to γ Rays) and its Control

Experiment	Sex	Dose	Cv_L OR (*P* value)	Cv_C OR (*P* value	Cv_N OR (*P* value)	Kid OR (*P* value)	Lvr OR (*P* value)	Pul OR (*P* value)	Rep OR (*P* value)	Tot OR (*P* value)
1	Female	22 cGy	NS	NS	NS	NS	0.33 (0.05)	NS	0.60 (0.06)	NS
		43 cGy	0.12 (0.11)	NS	NS	NS	NA	0.71 (0.08)	NS	NS
		86 cGy	0.33 (0.13)	NS	NS	2.01 (0.00)	NS	0.66 (0.00)	1.28 (0.03)	NS
	Male	86 cGy	NS	1.15 (0.10)	6.96 (0.07)	NS	0.71 (0.10)	NS	NS	NS
		1.37 Gy	NS	NS	5.98 (0.14)	NS	NS	3.03 (0.00)	5.94 (0.02)	0.60 (0.00)
60	Female	1 Gy	NS	NS	NS	NS	NA	NS	NA	NS
		2 Gy	NS	2.39 (0.14)	NS	NS	NS	NS	NS	NS
		3 Gy	NS	NS	NS	NS	NS	NS	0.36 (0.03)	NS
	Male	1 Gy	NS	NS	NS	NS	NS	NS	NS	NS
		2 Gy	NS	NS	NS	NS	NS	NS	NS	0.89 (0.11)
		3 Gy	NS	NS	NS	NS	NS	NS	NS	0.74 (0.00)

Notes. Listing includes weeks of exposure (Experiment), sex, total dose (cGy/Gy), odds ratios (OR) and their *P* values for the organ system variables. $P = 0.00$ means $P < 0.01$, NS means P-value > 0.15. Cv = cardiovascular disease [lethal (L), contributing to death (C), observed at death (N)], Kid = urinary tract pathologies, Lvr = liver disease, Pul = pathologies of the lungs and pleura, Rep = pathologies of the reproductive organs, and Tot = sum of all pathologies observed in an animal.

[a] Extension of Table 3.

TABLE 6[a]
Summary of the Importance of Organ System Variables in Logistic Regression Models Used in Pairwise Comparisons between each Dose Group (Exposed to Neutrons) and its Control

Experiment	Sex	Dose (cGy)	Cv_L OR (*P* value)	Cv_C OR (*P* value)	Cv_N OR (*P* value)	Kid OR (*P* value)	Lvr OR (*P* value)	Pul OR (*P* value)	Rep OR (*P* value)	Tot OR (*P* value)
1	Female	2	NS	NS	NS	NS	NS	0.76 (0.10)	NS	NS
		5	NS	NS	NS	NS	NA	0.66 (0.03)	NS	NS
		9	NS	NS	NS	NS	NS	NS	NS	0.80 (0.01)
60	Female	2	NS	NS	NS	NS	0.30 (0.14)	NS	NA	NS
		8	NS	2.90 (0.12)	NS	NS	NS	NS	NS	NS
		14	NS	NS	NS	NS	NS	NS	NS	NS
	Male	2	NS	NS	NS	NS	NS	NS	NS	NS
		8	NS	NS	NS	NS	0.18 (0.11)	NS	NS	NS
		14	NS	NS	NS	NS	2.31 (0.10)	NS	NS	0.82 (0.03)

Notes. Listing includes weeks of exposure (Experiment), sex, total dose (cGy), odds ratios (OR) and their *P* values for the organ system variables. $P = 0.00$ means $P < 0.01$, NS means *P* value > 0.15. Cv = cardiovascular disease [lethal (L), contributing to death (C), observed at death (N)], Kid = urinary tract pathologies, Lvr = liver disease, Pul = pathologies of the lungs and pleura, Rep = pathologies of the reproductive organs, and Tot = sum of all pathologies observed in an animal.

[a] Extension of Table 4.

diagnostic errors were not a concern in this case because it had been determined previously (*26*) that these errors were below 5% for the PR_T end point (primary tumors) used in these analyses. In combination, the parallel analyses of the Macro and Micro data provided a reasonably consistent message. Radiation-induced mortality could be detected for both γ rays (1–2 Gy, Table 1) and neutrons (10–15 cGy, Table 2) at doses near the lower end of the intermediate dose range.

Historically, protraction effects in the ANL data have been investigated with dose–response models. An augmentation of neutron injury and a diminishment of γ-ray injury with dose protraction has been detected by changes in the slope coefficients of the dose term in regression models used to describe life shortening (*28*) and a variety of neoplastic end points (*29*). The effects of the exposure pattern in those analyses did not emerge until doses higher than those used in this paper were included within the dose range used for analysis (e.g. 40–60 cGy for neutrons). In that respect, the general lack of apparent protraction effects for the tumor variables presented here for low and intermediate doses (Tables 3 and 4) are consistent with the results reported previously for life shortening.

Two messages emerge from the analyses of the full spectrum of pathologies observed at death. First, radiation-induced injury is revealed at doses below those causing detectable shifts in the age distribution of mortality. As a consequence, response thresholds estimated from mortality models may underestimate potentially important health effects. Second, analyzing all the observed pathologies revealed that effects are not limited to neoplastic events, which is consistent with observations for atomic bomb survivors (*39*). Although the tumor burden was invariably elevated, especially for tumors that neither caused nor contributed to death, excess injury was also seen in the major organ systems studied. This was particularly the case for mice receiving single exposures to γ rays (Table 5). However, the less frequent occurrence of significant non-neoplastic pathology at either low neutron doses or protracted γ irradiation is also notable. Although not accompanied by a greater risk of death, the elevated pathology burdens detected in irradiated individuals suggests that increased health problems may occur at dose levels otherwise considered to be insignificant.

In summary, detectable shifts in mortality at doses straddling the low and intermediate dose boundary, a significantly elevated burden of nonfatal tumors at even lower doses, and a spectrum of radiation-induced pathologies other than cancer involving numerous organ systems are findings that have important implications for radiation safety and protection.

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