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Transgenerational Effects on Lifespan and Pathology of Paternal Pre-conceptional Exposure to Continuous Low-dose-rate Gamma Rays in C57BL/6J Mice

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The present work investigates the multigenerational effects of paternal pre-conceptional exposure to continuous low-dose-rate gamma rays in C56BL/6J mice. Male C57BL/6J (F0 sires) mice were exposed to low dose rates of 20, 1, and 0.05 mGy/day for 400 days, to total accumulated doses of 8,000, 400, and 20 mGy, respectively. Upon completion of the radiation exposure, the F0 male mice were immediately bred to non-irradiated 8-week-old C57BL/6J females (F0 dams) to produce the first-generation (F1) mice. Randomly selected F1 males and females were then bred to produce the second-generation (F2) mice. All the mice, except the F0 dams, were subjected to pathological examination upon natural death. Reproductive parameters, lifespan, causes of death, neoplasm incidences and non-neoplastic disease incidences were used as parameters to evaluate the biological effects of continuous pre-conceptional exposure of the sires (F0) to continuous low-dose-rate radiation. There were no significant differences in the pregnancy and weaning rates among the parent (F0) generation. Average litter size and average number of weaned pups (F1) from dams bred to males (F0) exposed to 20 mGy/day were significantly decreased compared to the non-irradiated controls. Significant lifespan shortening in the sires (F0) was observed only in the 20 mGy/day group due to early death from malignant lymphomas. Life shortening was also observed in the F1 progeny of sires (F0) exposed to 20 and 1 mGy/day, but could not be attributed to a specific cause. No significant differences in the causes of death were found between dose groups in any generation. The number of primary tumors per mouse was significantly increased only in the F0 males exposed to 20 mGy/day. Except for the increased incidence rate for Harderian gland neoplasms in sires (F0) exposed to 20 mGy/day, there was no significant difference in neoplasm incidences and tumor spectra in all 3 generations in each sex regardless of radiation exposure. No multi- or transgenerational effects in the parameters examined were observed in the F1 and F2 progeny of sires exposed to 0.05 mGy/day for 400 days.

INTRODUCTION

Multigenerational and/or transgenerational effects are observed in offspring born after one or both parents have been exposed to radiation prior to conception (*1*, *2*). Although the possibility of human germ cell mutation following radiation exposure is recognized and considered (*3*, *4*), it has been assumed that should transgenerational effects occur in humans, incidence rates are too low to be detected epidemiologically (*5*, *6*).

In humans, reports on transgenerational effects after pre-conceptional radiation exposure have been controversial, with much debate on some of the endpoints studied (*1*), many studies have failed to provide clear evidence of heritable mutations (*7*, *8*). Similarly, the likelihood of adverse pregnancy outcomes due to pre-conceptional exposures have not been demonstrated in children of exposed parent(s) (*9*). Multiple studies report no evidence of ncreased cancer rates in children whose fathers were exposed to the atomic bomb (*10–12*), but a recent epidemiological re-analysis by Yamada et al. (*13*) showed consistent, but not significant, associations between pre-conceptional exposures and increase in the risk of major congenital malformations and perinatal death. Although some evidence shows increased germline mutation rates at microsatellite loci in offspring of parents exposed to radioactive fallout after the Chernobyl nuclear accident (*14*), recent evidence from that accident suggests no association between parental radiation exposure and frequency of genetic mutations (*15*). Mutagenic doses of chemotherapy and radiotherapy to the gonads have not been associated with genetic defects in offspring in a Danish cancer survivor case-cohort study (*16*).

Radiation induces mutations in somatic cells of humans, rodents, and microorganisms, while heritable (transgenerational) effects have been reported in irradiated mice. Studies

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on parental (F0 sire) exposures to acute high-dose X rays (100–1,000 r) in various strains of mice have been associated with other reproductive outcomes in offspring (mostly in F1 only) such as embryonic lethality (200, 400 or 800 R of X rays) (*17*), heritable chromosomal translocations and dominant lethal mutations in embryos (200 R X rays) (*18*), heritable gene mutations based on the specific locus test (3 Gy X rays) (*19*) and genomic instability (0.5 to 1 Gy gamma rays) (*20*). Spermatogonia of CD1 mice irradiated with gamma rays at an acute dose of 1 Gy have also been shown to transmit heritable genomic instability up to F4 (*21*). X-ray exposure of ICR male mice exposed to 2.06 Gy (*22*) and of LT and N5 male mice exposed to 5.04 Gy resulted in increased incidence of lung cancers and lymphocytic leukemias in F1 progeny (*23*), respectively. On the other hand, no evidence of radiation-induced genetic injury, based on growth and mortality rates, was found in a multigenerational mouse study using male RFM mice (10 to 35 generations) exposed to X rays [acute high-dose rate (HDR) of 50 rads/min to a total dose of 200 rads/generation] prior to sibling-mating with non-irradiated females (*24*).

Since mice oocytes are extremely radiosensitive and are killed even after exposure to low doses of radiation, most data on hereditary effects of radiation have been conducted in male mice (*25*). A study by Dasenbrock et al. (*26*) on pre-conceptional maternal exposure of C57BL/6N exposed to acute HDR X rays (2 fractions of 2 Gy) showed no effect on the survival of F1 progeny, but an increased incidence of hepatocellular carcinomas and total incidence rates of hepatocellular and bronchiolo-alveolar tumors in F1 males born from irradiated dams. Maternal exposure of BALB/c and CBA/Ca mice to an acute dose of 1 Gy of X rays at HDR did not affect the frequency of mutations at extended simple tandem repeats in F1 progeny (*27*).

Experiments that demonstrate transgenerational effects involve paternal irradiation and showed a linear dose-response curve for paternal mutations induced at premeiotic but not at post-meiotic stages (*28*). Recent studies also show that the mutation yields may differ depending on the stage susceptibility of the mouse strain used, as reported by Barber et al. (*29*). In vivo and in vitro studies suggest that radiation exposure of males, at high doses (mostly over 1 Gy), lead to genetic and epigenetic effects in the somatic cells of offspring over several generations that could not be attributed to the inheritance of simple mutation through the parental germ line (*7*, *30*). Analysis of maternal mutation rates after paternal irradiation at various stages of spermatogenesis by Dubrova et al. (*31*) did not find any significant difference. Dubrova et al. (*32*) hypothesized that ionizing radiation could indirectly lead to a persistent genetic instability that is manifested as transgenerational increases in mutation rates in F2 offspring of irradiated male mice by continuing to exhibit an increase in tandem repeat mutation rates despite being a generation removed from the exposure. Another study by Mughal et al. (*33*) showed that paternal exposure of BALB/c mice to 1 Gy of gamma rays delivered chronically does not destabilize the F1 genomes, whereas an acute exposure significantly affected the mutation rates in the germline and somatic tissues in F1 progeny.

The present study describes the effects of paternal (F0) exposure to continuous very low-dose-rate gamma rays on the F1 and F2 progeny of C57BL/6J Nirs mice using various parameters including reproduction criteria, incidences of neoplastic and non-neoplastic diseases, causes of death and lifespan.

MATERIALS AND METHODS

Mice and Animal Husbandry

A total of 720 specific pathogen-free (SPF) C57BL/6J Nirs males (F0) used as sires in the study were bred in-house at the Low-Dose Radiation Effects Research Facility (LERF) of the Institute for Environmental Sciences (IES). The 8-week-old F0 males were randomly divided into 4 groups (180 animals each): 1 non-irradiated control and 3 irradiated groups. After grouping, the non-irradiated F0 males were moved to standard animal rooms within the SPF facility while the irradiated groups were moved into the irradiation rooms. The mice were group-housed in plastic cages (4 mice/cage, 218 × 320 × 133 mm), except during breeding when they were housed individually for cohabitation with females. The F1 and F2 progeny were similarly group-housed from weaning, as described previously (*34*), except at the time of breeding F1 males or until the F2 pups were weaned at 21 days of age in the case of the F1 females (dams). Virgin 8-week-old C57BL/6J Nirs females, also bred in-house, were used as F0 dams.

The entire study was conducted (in 6 staggered batches of 20–40 F0 males/dose group) under similar SPF environmental conditions and husbandry practices (12 h light-dark cycle, weekly cage change, ad libitum feed and water supply, daily health monitoring or clinical inspection and monthly monitoring of SPF status) described previously (*35*).

The C57BL/6J mouse strain was chosen because it is widely available, commonly used as a general-purpose strain in a wide variety of research areas, and is used as a genetic background for congenic and mutant mice for use as human disease models. It is the first mouse to have its genome sequenced and is also known for easy breeding and robustness. The C57BL/6J Nirs mice used in the study were bred in-house (over F20) at the IES and is the same strain used as parent stock of B6C3F1 mice used in IES experiments. All experiments were conducted according to legal regulations in Japan and following the Guidelines for Animal Experiments of the Institute for Environmental Sciences.

Irradiation

The three irradiated groups were continuously exposed to whole-body low-dose-rate (LDR) gamma rays (^{137}Cs) for 22 h/day, at daily doses of 0.05, 1.0 and 20 mGy, from 8 weeks of age for approximately 400 consecutive days, to total accumulated doses of 20, 400 and 8,000 mGy, respectively, as in the lifespan study (*35*). Based on previous work (*34–36*), LDRs of 0.05, 1 and 20 mGy/day were selected. The absorbed doses are based on measurements made using thermoluminesence dosimeters (TLDs) surgically inserted into the abdominal cavity of the mouse as described by Shiragai et al. (*37*).

Breeding

Male mice, like human males, remain fertile regardless of age but are less likely to breed or breed successfully (*38*) when older (over 6–8 months old), obese or when housed alone or in same sex pairs/groups (*39*). Inbred mouse strains have an expected reproductive lifespan of up to 7–8 months of age, after which they are usually retired from the breeding colony. By the time the radiation exposure is completed, the F0 males (non-irradiated and irradiated)

TABLE 1
Mean Life Spans of Male C57BL/6J Mice (F0) Continuously Exposed to Very Low-Dose-Rate Gamma Rays for 400 Days Pre-conception and their Progeny (F1 and F2)

			F1				F2			
	F0 (Sire)		Male		Female		Male		Female	
Dose-rates for the F0 (Sires)	n	Life span (95% CI)	n	Life span (95% CI)	n	Life span (95% CI)	n	Life span (95% CI)	n	Life span (95% CI)
Non-irradiated	180	866.8 (839.7, 893.9)	278	893.3 (868.7, 917.9)	274	812.6 (790.5, 834.7)	444	888.1 (869.2, 906.9)	389	812.2 (794.4, 829.9)
0.05 mGy/day	180	851.7 (824.1, 879.4)	250	878.0 (851.3, 904.6)	239	794.8 (773.8, 815.8)	346	864.1 (841.4, 886.8)	345	797.8 (777.2, 818.4)
1 mGy/day	180	865.4 (837.3, 893.5)	259	866.4 (842.1, 890.7)[a]	237	810.9 (788.9, 832.9)	425	882.7 (864.2, 901.1)	373	810.2 (792.2, 828.1)
20 mGy/day	180	808.6 (781.1, 836.0)[b]	218	855.5 (827.4, 883.6)[a]	215	802.9 (777.4, 828.5)	326	880.0 (858.6, 901.3)	292	815.7 (794.8, 836.6)

[a] $P < 0.05$.
[b] $P < 0.01$ (Steel test) vs. non-irradiated control.

will be approximately 15 months old (approximately 456 days) at the time of mating and well over the 7–8 month expected reproductive lifespan. To prevent loss of reproductive (mating or copulatory) behavior and libido, sexual experience was provided by mating them to virgin 8-week-old C57BL/6J Nirs females (bred in-house but not counted into the study), every 3 months during the irradiation period (total of 7 matings). Reproductive performance (pregnancy rates, number of implantation sites and dead/live pups born) from these matings were monitored, as described previously (*34*) and all progeny were sacrificed at weaning (21 days). All matings were done at a ratio of 1 male and 1 female (1:1) except for the first batch of mice (bred at a ratio of 1 male:2 females).

After completion of radiation exposure, the F0 males were moved out of the irradiation rooms to the standard animal rooms in individual cages (218 × 320 × 133 mm) for cohabitation with virgin 8-week-old C57BL/6J Nirs females allowing them to mate for 7 days (1 week). At the same age (456 days), the non-irradiated F0 males were also transferred to individual cages for cohabitation as described above. After mating, the F0 males were moved back to group housing with their former cage mates and the females were transferred out and housed individually throughout the pregnancy until the pups were weaned. Increasing the time interval between radiation exposure and conception/mating has been shown to greatly reduce the hereditary consequences of a given radiation dose, since the stage of spermatogenesis at which the animal is exposed to radiation is correlated to the number of mutations produced (*25*) where exposed mature spermatozoa show greater induction of mutations compared to sperm exposed in the primitive state (suggesting recovery/repair). Based on this premise and taking into consideration the age of the F0 sires, we chose to mate immediately after completion of radiation exposure (no recovery period) to increase the probability of observing hereditary effects.

Randomly selected F1 males and females were bred at 8 weeks (56 days) of age to produce the F2 generation by allowing them to cohabit and breed in the same manner as the F0 generation, after which the F1 males were re-housed with their original cage mates. Pregnant F1 females were housed individually until their pups were weaned at 21 days of age and then re-housed with their original cage mates. Reproduction parameters (including pregnancy rates, numbers of implantations sites and resorbed fetuses, litter sizes at birth and at weaning, and weaning rates) were monitored, as described previously (*34*). A total of 5630 C57BL/6 mice (excluding the females used to maintain reproductive/copulatory behavior and F0 dams) were used in the study.

Monitoring and Pathological Examination

Pups were carefully counted (total n = 4910, see Table 1) as soon as possible after birth and were weaned at 21 days (3 weeks) of age, at which time they were individually identified with ear notches, weighed, separated by sex, and group-caged (4 mice/cage). Pre-weaning (postnatal day 0–21) mortalities were low and were not significantly different among groups and between generations. Dead pups, if found, were either cannibalized (some partially) or in advanced states of decomposition making the determination of the cause of death (COD) difficult. Pre-weaning mortalities were therefore not included in the calculation of the survival curves and were not included in the total n subjected to pathological diagnosis.

All the mice (F0 sires, F1 and F2 progeny) were allowed to die a natural death upon which they were subjected to necropsy (gross examination), and organs were collected, weighed, and fixed in 10% neutral buffered formalin for histopathological examination based on a standard protocol (*36*). When deemed necessary, additional tissue samples were collected from neoplasms and from organs or tissues with gross abnormalities, and special histochemical procedures performed for diagnostic purposes.

Histopathological examination was performed blind and neoplasms were classified based on proposed nomenclatures of World Health Organization/International Agency for Research on Cancer (WHO/IARC) (*40*) and the National Toxicology Program (NTP) (*41*) as described previously (*36*). A COD was assigned to all animals as described by Tanaka et al. (*36*) in the lifespan study.

Multiple primary neoplasms and pathologies were treated as in the previous lifespan study (*36*) wherein multiple (including multiple or metastatic foci) neoplasms of the same type were counted only once. All neoplasms were counted into the overall incidence.

After the F1 pups were weaned at 3 weeks of age, all the F0 dams were humanely sacrificed with an overdose of isofluorane (IsoFlo®, Abbott Laboratories, Chicago, IL), necropsied, examined for gross pathological changes and the uteri collected. The uteri were clarified (*42*), and the number of implantation sites counted and recorded accordingly.

Statistical Analyses

Fischer's exact tests were used to analyze the crude mortality rates, causes of death, non-neoplastic lesion and neoplasm incidence. Reproductive performance and neoplasm multiplicity was analyzed using the Wilcoxon test. The male-to-female sex ratio of F1 and F2 generations were analyzed by chi-square (χ^2) test. Analyses of mean lifespan and body weights were done using the Steel test. Levels of significance for mortality rates and incidence rates of non-neoplastic lesions and neoplasms were chosen as $P = 0.05$ and $P = 0.01$.

RESULTS

Reproduction Parameters

Reproduction parameters from the 7 matings (data not shown) initiated to maintain reproductive behavior during the irradiation period were not significantly different from the final mating post-irradiation at 456 days. Reproduction

Table 2A
Reproductive Parameters of Male C57BL/6J Mice (F0) Exposed Continuously to Very Low-Dose-Rate Gamma Rays for 400 Days Pre-conception

	Non-irradiated	0.05 mGy/day	1 mGy/day	20 mGy/day
	0 mGy	20 mGy	400 mGy	8000 mGy
Number of F0 sires	180	180	180	180
Number of virgin females for breeding	194	194	197	190
Number of pregnant dams	125	119	115	116
Pregnancy rate (%)	64.4	61.3	58.4	61.1
Average no. of implantation sites/dam	7.3 (7.0, 7.7)[a]	7.3 (7.0, 7.6)	7.4 (7.1, 7.7)	7.1 (6.8, 7.5)
Average no. of early resorbed fetus	1.9 (1.6, 2.2)	1.8 (1.5, 2.1)	2.1 (1.8, 2.4)	2.1 (1.8, 2.3)
Litter size (Average no. of pups born/litter)	5.4 (5.0, 5.7)	5.1 (4.7, 5.4)	5.2 (4.8, 5.5)	4.9 (4.5, 5.2)[b]
Average no. of weaned pups/litter	4.4 (4.0, 4.8)	4.2 (3.7, 4.6)	4.3 (3.9, 4.7)	3.7 (3.2, 4.1)[b]
Average no. of weaned male pups/litter	2.3 (2.0, 2.5)	2.2 (1.9, 2.5)	2.2 (2.0, 2.5)	1.9 (1.6, 2.2)
Average no. of weaned female pups/litter	2.2 (2.0, 2.5)	2.1 (1.8, 2.3)	2.1 (1.8, 2.3)	1.9 (1.6, 2.2)
Average no. of pre-weaning loss/litter	1.0 (0.8, 1.2)	0.9 (0.7, 1.2)	0.9 (0.6, 1.1)	1.2 (0.9, 1.5)
Average weaning rate (%)	75.3 (69.4, 81.3)	76.5 (70.0, 83.3)	79.7 (73.9, 85.5)	69.8 (62.5, 77.2)

[a] 95% Confidence interval.
[b] $P < 0.05$ Wilcoxon Test.

parameters of the final mating postirradiation are shown in Table 2A for the F0 generation and Table 2B for the F1 generation. In the F0 generation, the number of implantation sites were significantly higher in the non-irradiated group dams compared to those bred to irradiated mice regardless of dose (Table 2A). While the average number of pups born (litter size = 4.9 pups/litter) and weaned (3.7 pups/litter) from dams bred to F0 males exposed to 20 mGy/day were significantly decreased ($P < 0.03$, Table 2A) compared to the non-irradiated controls (litter size = 5.4 pups/litter; weaned = 4.4 pups/litter), there was no significant difference in these same parameters in the F1 progeny of the 20 mGy/day group (litter size = 5.7 pups/litter; weaned = 4.8 pups/litter; Table 2B) compared to the F1 descendants of the non-irradiated group (litter size = 5.9 pups/litter; weaned = 5.0 pups/litter; Table 2B). In the F1 generation (Table 2B), there was no significant difference in the parameters examined between those born from non-irradiated and irradiated F0 except for the significant increase in the number of pups born/litter in the non-irradiated group (5.9 pups/litter). Pregnancy rates and average litter sizes at birth were slightly higher in the F1 generation (Table 2B) than the F0 generation (Table 2A) since the F1 generation were bred at a significantly younger age (8 weeks of age) than the F0 sires. The average weaning rate in the F1 generation (72.9%, Table 2B) in the 0.05 mGy/day group was significantly ($P<0.02$) lower than the non-irradiated group (83.2%). At weaning, there was no difference in the ratio of male to female pups within the F1 and F2 generations regardless of the sires' (F0) radiation exposure.

Body Weights

Body weights were measured by weighing a representative number (varies at each weighing point, and mean weights

TABLE 2B
Reproductive Parameters of the F1 Progeny of C57BL/6J Mice (F0) Exposed Continuously to Very Low-Dose-Rate Gamma Rays for 400 days Pre-conception

	Non-irradiated	0.05 mGy/day	1 mGy/day	20 mGy/day
	0 mGy	20 mGy	400 mGy	8000 mGy
Number of F1 males	262	230	234	199
Number of F1 females	262	230	234	199
Number of pregnant F1 dams	168	158	164	128
Pregnacy rate (%)	64.1	68.7	70.1	64.3
Litter size (Average no. of pups born/litter)	5.9 (5.6, 6.1)[a]	5.6 (5.3, 5.9)	5.7 (5.5, 6.0)	5.7 (5.4, 6.0)
Average no. of weaned pups/litter	5.0 (4.7, 5.3)	4.4 (4.0, 4.8)	4.9 (4.5, 5.2)	4.8 (4.4, 5.2)
Average no. of weaned male pups/litter	2.7 (2.4, 2.9)	2.2 (2.0, 2.5)	2.6 (2.4, 2.8)	2.5 (2.3, 2.8)
Average no. of weaned female pups/litter	2.3 (2.1, 2.6)	2.2 (2.0, 2.5)	2.3 (2.0, 2.5)	2.3 (2.0, 2.5)
Average no. of pre-weaning loss/litter	0.9 (0.7, 1.1)	1.1 (0.9, 1.4)	0.8 (0.6, 1.1)	0.9 (0.7, 1.1)
Average weaning rate (%)	83.2 (79.1, 87.3)	72.9 (67.2, 78.6)[b]	82.2 (77.6, 86.9)	81.5 (76.5, 86.4)

[a] 95% Confidence interval.
[b] $P < 0.05$ Wilcoxon Test.

were calculated accordingly) of animals/group from the start of irradiation (8 weeks of age for F0) or from weaning (3 weeks of age for all F1 and F2 progeny) to a maximum of 180 weeks (Fig. 1). No significant difference in body weights nor weight gain (growth rate) were observed between dose groups within each generation or sex.

Survival Curves

Survival curves of sires (F0, Fig. 2A) exposed to 20 mGy/day show a significant shift to the left and their mean lifespan was significantly ($P = 0.0031$) shorter at 808.6 (781.1–836.0) days than the non-irradiated F0 controls at 866.8 (839.7–893.9) days (Table 1). F1 male progeny of sires exposed to 1 and 20 mGy/day (Fig. 2B) also show a slight shift to the left with significantly shorter mean lifespans at 866.4 ($P = 0.0485$; 842.1–890.7) and 855.5 ($P = 0.0105$; 827.4–883.6) days, respectively, compared to the male F1 progeny of non-irradiated sires at 893.3 (868.7–917.9) days (Table 1). There was no significant difference in the survival curves of the male F2 and female (F1 and F2) progeny born or descended from non-irradiated and irradiated F0 sires.

Causes of Death (COD)

The causes of death with their corresponding incidence rates are shown in Table 3A for the males (F0, F1 and F2) and Table 3B for the females (F1 and F2). Since most of the incidence rates for causes of death were low, the neoplasms are listed according to tissue/organ of origin (in alphabetical order), except those originating from the hematopoietic system. Neoplasms remain the major cause of death across generations regardless of sex (53.6–57.8% in males; 59.0–66.5% in females) or radiation exposure, followed by inflammatory diseases. Inflammatory diseases were most frequently respiratory (acidophilic macrophage pneumonia), vascular (arteritis) and skeletal (degeneration/osteoarthritis of the temporo-mandibular joint) in origin and those classified as "Others" include dental dysplasia, intestinal torsion (digestive), amyloidosis (systemic) and are commonly seen in aging animals (*43*).

Overall, the major neoplastic causes of death in males (F0 to F2, Table 3A) were hematopoietic in origin and consisted mainly of malignant lymphomas and histiocytic sarcomas with no significant difference in incidence rates between non-irradiated and irradiated groups within each generation. Across generations within the same dose groups, the frequency at which malignant lymphomas caused death decreased in males from F0 to F2, while that of histiocytic sarcomas increased from F0 to F2, regardless of radiation exposure. The frequency of vascular neoplasms causing death was highest in the non-irradiated F0 sires but was not significantly different between dose groups in each generation. The frequency of inflammation as COD in males was not significantly different regardless of radiation exposure within each generation, but was significantly increased in

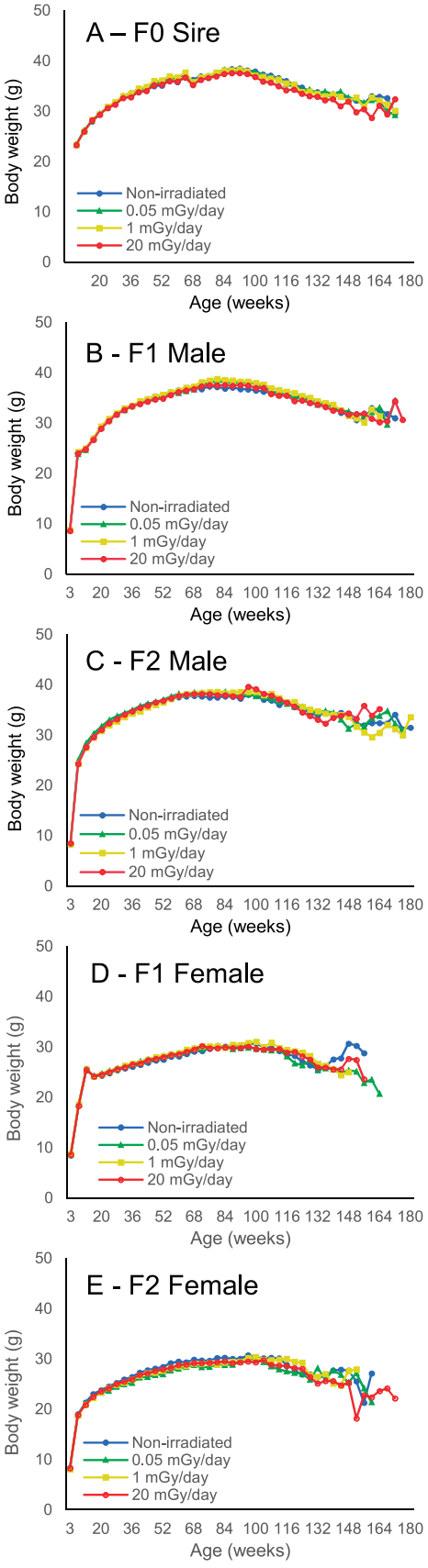

FIG. 1. Average body weights of C57BL/6J mice continuously exposed to very low-dose-rate gamma rays: F0 sires (panel A) and their F1 (panel B, panel D) and F2 (panel C, panel E) progeny.

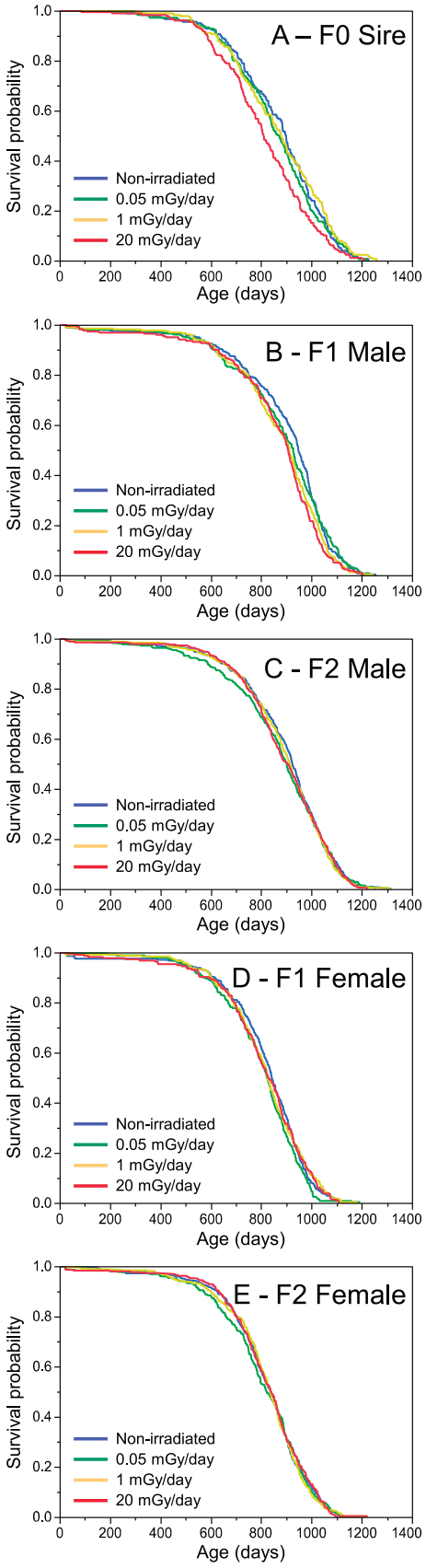

FIG. 2. Survival curves of C57BL/6J mice continuously exposed to very low-dose-rate gamma rays: F0 sires (panel A) and their F1 (panel B, panel D) and F2 (panel C, panel E) progeny.

the F1 and F2 generations born in the 20 mGy/day group compared to the F0 generation.

As with the male progeny, female mice in the F1 and F2 generations (Table 3B) died mostly from hematopoietic neoplasms, mainly malignant lymphomas with slightly higher incidence rates (not significant) in the F2 generation born from irradiated sires. There was also no significant difference in the frequency of inflammatory causes of death in females between generations regardless of the F0 radiation exposure.

Undetermined/unknown CODs comprised 2.1–8.5% of all deaths in all generations. For other causes of death, no significant differences were observed in the incidence rates between the non-irradiated and irradiated groups in neither sex nor between generations.

Mean Survival Times for Causes of Death

Kaplan-Meier estimates of mean survival times for the major causes of death are shown in Tables 4A (males) and 4B (females). Overall mean survival for the sires (F0) exposed to 20 mGy/day (808.6 ± 13.9 days) was significantly ($P < 0.01$) shorter compared to the non-irradiated controls (886.8 ± 13.7 days). Mean survival times for all fatal neoplasms was also significantly ($P < 0.01$) shorter for sires (F0) exposed to 20 mGy/day (887.3 ± 14.5 days) compared to the non-irradiated controls (953.1 ± 15.0 days). Sires (F0) exposed to 20 mGy/day that died from malignant lymphomas (969.8 ± 16.7 days, $P<0.01$) and histiocytic sarcomas (1,039.3 ± 7.1 days, not significant) had shorter mean survival times than the non-irradiated controls. Overall mean survival times of male (F1) progeny of born from sires exposed to 1 mGy/day (866.4 ± 12.3 days) and 20 mGy/day (855.5 ± 14.3 days) was significantly ($P < 0.05$) shorter than the male progeny of non-irradiated sires (893.3 ± 12.5 days) but could not be attributed to a specific COD. No significant life shortening was observed in other major CODs in males in all generations. For female progeny, F1 and F2 (Table 4B), there was no significant difference in mean survival times for all CODs.

Neoplasm Incidence

All neoplasms, fatal (COD) and incidental, are listed in Tables 5A (males) and 5B (females), classified according or tissue/organ (in alphabetical order) of origin. As most of the incidence rates are low (as in the COD Tables 3A and 3B), the neoplasms are listed according to tissue/organ of origin (in alphabetical order), except those originating from the liver, hematopoietic system, and blood vessels (vascular). Only the incidence rate for Harderian gland neoplasms (Table 5A) in sires (F0) exposed to 20 mGy/day (10.6%, $P <$ 0.01) was significantly increased compared to the F0 non-irradiated controls (2.8%). For other neoplasms, there was no significant difference in the incidence rates between the

TABLE 3A

Causes of Death in Male C57BL/6J Mice Continuously Exposed to Very Low-Dose-Rate Gamma Rays for 400 Days Pre-conception (F0) and their Progeny (F1 and F2)

	F0 (Sire)				F1				F2			
	Non-irradiated 0 mGy n = 180 (%)	0.05 mGy/day 20 mGy n = 180 (%)	1 mGy/day 400 mGy n = 180 (%)	20 mGy/day 8000 mGy n = 180 (%)	Non-irradiated 0 mGy n = 278 (%)	0.05 mGy/day 20 mGy n = 250 (%)	1 mGy/day 400 mGy n = 259 (%)	20 mGy/day 8000 mGy n = 218 (%)	Non-irradiated 0 mGy n = 444 (%)	0.05 mGy/day 20 mGy n = 346 (%)	1 mGy/day 400 mGy n = 425 (%)	20 mGy/day 8000 mGy n = 326 (%)
Neoplasms	104 (57.8)	104 (57.8)	102 (56.7)	117 (65.0)	159 (57.2)	134 (53.6)	144 (55.6)	122 (56.0)	264 (59.5)	201 (58.1)	244 (57.4)	180 (55.2)
Digestive system	9 (5.0)	14 (7.8)	14 (7.8)	9 (5.0)	14 (5.0)	7 (2.8)	18 (6.9)	13 (6.0)	23 (5.2)	12 (3.5)	22 (5.2)	6 (1.8)
Gastro-intestinal tract	2 (1.1)	4 (2.2)	4 (2.2)	2 (1.1)	4 (1.4)	3 (1.2)	6 (2.3)	6 (2.8)	9 (2.0)	4 (1.2)	12 (2.8)	1 (0.3)
Liver	5 (2.8)	10 (5.6)	10 (5.6)	7 (3.9)	10 (3.6)	4 (1.6)	12 (4.6)	7 (3.2)	13 (2.9)	8 (2.3)	9 (2.1)	5 (1.5)
Salivary gland	2 (1.1)								1 (0.2)		1 (0.2)	
Endocrine system	1 (0.6)	1 (0.6)	5 (2.8)	2 (1.1)	1 (0.4)	3 (1.2)	1 (0.4)	2 (0.9)	4 (0.9)	3 (0.9)	2 (0.5)	1 (0.3)
Harderian gland	1 (0.6)				1 (0.4)		1 (0.4)		1 (0.2)	1 (0.3)	2 (0.5)	1 (0.3)
Hematopoietic system	63 (35.0)	75 (41.7)	59 (32.8)	71 (39.4)	105 (37.8)	94 (37.6)	99 (38.2)	73 (33.5)	183 (41.2)	142 (41.0)	175 (41.2)	135 (41.4)
Lymphoma, malignant	40 (22.2)	57 (31.7)	44 (24.4)	58 (32.2)	49 (17.6)	52 (20.8)	64 (24.7)	42 (19.3)	103 (23.2)	71 (20.5)	93 (21.9)	76 (23.3)
Sarcoma, histiocytic	21 (11.7)	17 (9.4)	15 (8.3)	10 (5.6)	53 (19.1)	39 (15.6)	31 (12.0)	31 (14.2)	78 (17.6)	68 (19.7)	77 (18.1)	56 (17.2)
Other	2 (1.1)	1 (0.6)		3 (1.7)	3 (1.1)	3 (1.2)	4 (1.5)		2 (0.5)	3 (0.9)	5 (1.2)	3 (0.9)
Nervous system		1 (0.6)			1 (0.4)	1 (0.4)	1 (0.4)	1 (0.5)	1 (0.2)		2 (0.5)	1 (0.3)
Pancreas											1 (0.2)	
Reproductive system	1 (0.6)		1 (0.6)	1 (0.6)				1 (0.5)	1 (0.2)	1 (0.3)	3 (0.7)	
Respiratory system	7 (3.9)	4 (2.2)	8 (4.4)	9 (5.0)	9 (3.2)	6 (2.4)	6 (2.3)	5 (2.3)	9 (2.0)	6 (1.7)	11 (2.6)	8 (2.5)
Skeletal system and teeth	3 (1.7)	2 (1.1)			2 (0.7)	2 (0.8)		1 (0.5)	4 (0.9)	4 (1.2)	3 (0.7)	2 (0.6)
Skin				2 (1.1)			2 (0.8)			1 (0.3)	2 (0.5)	
Soft tissue	2 (1.1)	1 (0.6)	1 (0.6)	1 (0.6)	6 (2.2)	3 (1.2)	7 (2.7)	6 (2.8)	11 (2.5)	8 (2.3)	9 (2.1)	6 (1.8)
Urinary system			1 (0.6)	1 (0.6)		1 (0.4)			2 (0.5)			2 (0.6)
Vascular	17 (9.4)	6 (3.3)	13 (7.2)	21 (11.7)	20 (7.2)	16 (6.4)	9 (3.5)	20 (9.2)	25 (5.6)	23 (6.6)	12 (2.8)	18 (5.5)
Other						1 (0.4)						
Inflammation	33 (18.3)	28 (15.6)	32 (17.8)	21 (11.7)	73 (26.3)	63 (25.2)	60 (23.2)	52 (23.9)	81 (18.2)	61 (17.6)	98 (23.1)	80 (24.5)
Digestive system	2 (1.1)	2 (1.1)	4 (2.2)	1 (0.6)	2 (0.7)	1 (0.4)	4 (1.5)	2 (0.9)	5 (1.1)	4 (1.2)	3 (0.7)	1 (0.3)
Ear					1 (0.4)			1 (0.5)		1 (0.3)	1 (0.2)	
Heart									1 (0.2)		1 (0.2)	
Nervous system									1 (0.2)			
Reproductive system	1 (0.6)	3 (1.7)	2 (1.1)		2 (0.7)	3 (1.2)		2 (0.9)	2 (0.5)		2 (0.5)	2 (0.6)
Respiratory system	13 (7.2)	7 (3.9)	8 (4.4)	9 (5.0)	23 (8.3)	12 (4.8)	19 (7.3)	19 (8.7)	23 (5.2)	25 (7.2)	30 (7.1)	22 (6.7)
Skeletal system and teeth	10 (5.6)	7 (3.9)	9 (5.0)	9 (5.0)	32 (11.5)	32 (12.8)	25 (9.7)	17 (7.8)	36 (8.1)	18 (5.2)	33 (7.8)	29 (8.9)
Skin	2 (1.1)					1 (0.4)	2 (0.8)	1 (0.5)			2 (0.5)	6 (1.8)
Soft tissue								1 (0.5)				
Systemic	1 (0.6)		1 (0.6)		2 (0.7)	1 (0.4)	1 (0.4)	3 (1.4)		1 (0.3)	1 (0.2)	
Urinary system		1 (0.6)	2 (1.1)				1 (0.4)				1 (0.2)	1 (0.3)
Vascular	4 (2.2)	8 (4.4)	6 (3.3)	2 (1.1)	11 (4.0)	13 (5.2)	8 (3.1)	5 (2.3)	12 (2.7)	12 (3.5)	21 (4.9)	19 (5.8)
Other								1 (0.5)	1 (0.2)		3 (0.7)	
Others	29 (16.1)	34 (18.9)	33 (18.3)	32 (17.8)	29 (10.4)	39 (15.6)	33 (12.7)	34 (15.6)	64 (14.4)	55 (15.9)	57 (13.4)	44 (13.5)
Accidental death						1 (0.4)						
Digestive system	3 (1.7)	6 (3.3)	7 (3.9)	6 (3.3)	6 (2.2)	10 (4.0)	7 (2.7)	5 (2.3)	15 (3.4)	11 (3.2)	10 (2.4)	9 (2.8)
Heart	1 (0.6)	2 (1.1)	5 (2.8)	5 (2.8)		3 (1.2)		1 (0.5)	3 (0.7)	9 (2.6)	2 (0.5)	2 (0.6)
Nervous system			1 (0.6)	1 (0.6)		1 (0.4)	1 (0.4)		1 (0.2)		1 (0.2)	2 (0.6)
Reproductive system	15 (8.3)	10 (5.6)	9 (5.0)	2 (1.1)	4 (1.4)	4 (1.6)	3 (1.2)	3 (1.4)	2 (0.5)	5 (1.4)	4 (0.9)	4 (1.2)
Respiratory system	6 (3.3)	12 (6.7)	8 (4.4)	15 (8.3)	8 (2.9)	10 (4.0)	10 (3.9)	12 (5.5)	11 (2.5)	13 (3.8)	14 (3.3)	10 (3.1)
Runt					2 (0.7)	1 (0.4)	2 (0.8)	1 (0.5)	3 (0.7)	3 (0.9)	3 (0.7)	1 (0.3)
Skeletal system and teeth			1 (0.6)			3 (1.2)	3 (1.2)	1 (0.5)	10 (2.3)	6 (1.7)	3 (0.7)	2 (0.6)
Systemic					2 (0.7)		2 (0.8)	3 (1.4)	5 (1.1)	3 (0.9)	6 (1.4)	2 (0.6)
Urinary system	4 (2.2)	3 (1.7)	2 (1.1)	3 (1.7)	7 (2.5)	6 (2.4)	5 (1.9)	8 (3.7)	13 (2.9)	5 (1.4)	14 (3.3)	10 (3.1)
Vascular		1 (0.6)							1 (0.2)			2 (0.6)
Unknown	14 (7.8)	14 (7.8)	13 (7.2)	10 (5.6)	17 (6.1)	14 (5.6)	22 (8.5)	10 (4.6)	35 (7.9)	29 (8.4)	26 (6.1)	22 (6.7)

non-irradiated controls and irradiated groups within each generation, regardless of sex.

Multiple Primary Neoplasms

The frequencies of multiple primary neoplasms are shown in Tables 6A (males) and 6B (females). Over 70.2% of the males and 74.1% of the females had one or more primary neoplasms at the time of death with 1 mouse (male F2, 0.05 mGy/day) having a maximum of 7. The average number of primary neoplasms/mouse was highest in the sires (F0) exposed to 20 mGy/day at 1.49 neoplasms per mouse and was significantly ($P < 0.01$) increased compared to the F0 non-irradiated controls. No significant increase in the number of primary neoplasms/mouse was observed in the F1 and F2 generations in either sex. Analysis of the mean number of

TABLE 3B

Causes of Death in Female C57BL/6J Progeny (F1 and F2) of Male C57BL/6J Mice Continuously Exposed to Very Low-Dose-Rate Gamma Rays for 400 Days Pre-conception (F0)

	F1				F2			
	Non-irradiated 0 mGy n = 274 (%)	0.05 mGy/day 20 mGy n = 239 (%)	1 mGy/day 400 mGy n = 237 (%)	20 mGy/day 8000 mGy n = 215 (%)	Non-irradiated 0 mGy n = 389 (%)	0.05 mGy/day 20 mGy n = 345 (%)	1 mGy/day 400 mGy n = 373 (%)	20 mGy/day 8000 mGy n = 292 (%)
Neoplasms	178 (65.0)	141 (59.0)	151 (63.7)	143 (66.5)	251 (64.5)	223 (64.6)	225 (60.3)	187 (64.0)
Digestive system	4 (1.5)		3 (1.3)	5 (2.3)	7 (1.8)	4 (1.2)	5 (1.3)	2 (0.7)
Gastro-intestinal tract	1 (0.4)		2 (0.8)	4 (1.9)	3 (0.8)	1 (0.3)	4 (1.1)	
Liver	3 (1.1)		1 (0.4)	1 (0.5)	4 (1.0)	3 (0.9)	1 (0.3)	2 (0.7)
Endocrine system	27 (9.9)	15 (6.3)	24 (10.1)	18 (8.4)	44 (11.3)	28 (8.1)	25 (6.7)	29 (9.9)
Pituitary gland	27 (9.9)	13 (5.4)	22 (9.3)	18 (8.4)	42 (10.8)	27 (7.8)	25 (6.7)	29 (9.9)
Other		2 (0.8)	2 (0.8)		2 (0.5)	1 (0.3)		
Harderian gland		1 (0.4)			1 (0.3)	1 (0.3)		1 (0.3)
Hematopoietic system	126 (46.0)	110 (46.0)	113 (47.7)	111 (51.6)	178 (45.8)	171 (49.6)	176 (47.2)	141 (48.3)
Lymphoma, malignant	100 (36.5)	81 (33.9)	83 (35.0)	84 (39.1)	134 (34.4)	134 (38.8)	143 (38.3)	119 (40.8)
Sarcoma, histiocytic	23 (8.4)	25 (10.5)	25 (10.5)	20 (9.3)	38 (9.8)	27 (7.8)	29 (7.8)	17 (5.8)
Other	3 (1.1)	4 (1.7)	5 (2.1)	7 (3.3)	6 (1.5)	10 (2.9)	4 (1.1)	5 (1.7)
Nervous system	1 (0.4)	1 (0.4)						
Reproductive system	2 (0.7)	3 (1.3)	1 (0.4)	2 (0.9)	4 (1.0)	2 (0.6)		
Respiratory system	3 (1.1)			1 (0.5)	1 (0.3)	3 (0.9)	1 (0.3)	1 (0.3)
Skeletal system and teeth	5 (1.8)	1 (0.4)	3 (1.3)	2 (0.9)		1 (0.3)	4 (1.1)	2 (0.7)
Skin		1 (0.4)	1 (0.4)					
Soft tissue	6 (2.2)	3 (1.3)	5 (2.1)	3 (1.4)	4 (1.0)	4 (1.2)	6 (1.6)	3 (1.0)
Urinary system						1 (0.3)		
Vascular	3 (1.1)	6 (2.5)	1 (0.4)	1 (0.5)	12 (3.1)	8 (2.3)	8 (2.1)	8 (2.7)
Other	1 (0.4)							
Inflammation	54 (19.7)	47 (19.7)	47 (19.8)	37 (17.2)	77 (19.8)	64 (18.6)	66 (17.7)	55 (18.8)
Digestive system		2 (0.8)	1 (0.4)	1 (0.5)	3 (0.8)	3 (0.9)	1 (0.3)	
Heart		1 (0.4)						
Reproductive system	1 (0.4)							1 (0.3)
Respiratory system	32 (11.7)	21 (8.8)	22 (9.3)	22 (10.2)	47 (12.1)	29 (8.4)	37 (9.9)	34 (11.6)
Skeletal system and teeth	12 (4.4)	8 (3.3)	11 (4.6)	6 (2.8)	7 (1.8)	13 (3.8)	8 (2.1)	8 (2.7)
Skin	4 (1.5)	2 (0.8)	1 (0.4)	1 (0.5)	2 (0.5)	3 (0.9)	1 (0.3)	5 (1.7)
Soft tissue		1 (0.4)						
Systemic	2 (0.7)	1 (0.4)	4 (1.7)	1 (0.5)	5 (1.3)	3 (0.9)		3 (1.0)
Vascular	3 (1.1)	11 (4.6)	8 (3.4)	6 (2.8)	13 (3.3)	13 (3.8)	17 (4.6)	3 (1.0)
Other							2 (0.5)	1 (0.3)
Others	29 (10.6)	41 (17.2)	30 (12.7)	28 (13.0)	51 (13.1)	43 (12.5)	62 (16.6)	44 (15.1)
Accidental death								1 (0.3)
Digestive system	3 (1.1)	7 (2.9)	9 (3.8)	5 (2.3)	8 (2.1)	6 (1.7)	6 (1.6)	9 (3.1)
Heart	2 (0.7)	1 (0.4)	3 (1.3)	4 (1.9)	1 (0.3)	3 (0.9)	7 (1.9)	4 (1.4)
Hematopoietic system								1 (0.3)
Nervous system	1 (0.4)				1 (0.3)			1 (0.3)
Pancreas								1 (0.3)
Pituitary gland							1 (0.3)	
Reproductive system		1 (0.4)	3 (1.3)		2 (0.5)	1 (0.3)	2 (0.5)	1 (0.3)
Respiratory system	2 (0.7)	3 (1.3)			2 (0.5)	4 (1.2)	1 (0.3)	3 (1.0)
Runt	2 (0.7)	1 (0.4)	2 (0.8)	1 (0.5)	1 (0.3)	4 (1.2)	2 (0.5)	2 (0.7)
Skeletal system and teeth	4 (1.5)	2 (0.8)	1 (0.4)	3 (1.4)	5 (1.3)	2 (0.6)	3 (0.8)	
Systemic	2 (0.7)	11 (4.6)	2 (0.8)	7 (3.3)	13 (3.3)	10 (2.9)	14 (3.8)	12 (4.1)
Urinary system	13 (4.7)	15 (6.3)	10 (4.2)	6 (2.8)	18 (4.6)	13 (3.8)	26 (7.0)	9 (3.1)
Vascular				2 (0.9)				
Unknown	13 (4.7)	10 (4.2)	9 (3.8)	7 (3.3)	10 (2.6)	15 (4.3)	20 (5.4)	6 (2.1)

benign and malignant neoplasms/mouse showed no significant difference within the F1 and F2 generations regardless of F0 radiation exposure.

Non-Neoplastic Diseases

Incidence rates for various non-neoplastic lesions (itemized list not shown) observed in the current study were low. As described above and classified similarly as the COD, frequently observed lesions include acidophilic macrophage pneumonia, non-specific pneumonia, dental caries, degenerative joint disease (temporo-mandibular joint), arteritis (focal or systemic), amyloidosis (focal or systemic) and hyaline glomerulopathy in the kidneys.

In contrast to published information on C57BL/6 (*44*), our in-house bred C56BL/6J mice did not develop ulcerative dermatitis, maybe due, in part, to the SPF environment of the IES animal facility. Mecklenburg et al. (*45*) noted

TABLE 4A

Kaplan-Meier Estimates of Mean Survival of Major causes of Death in Male C57BL/6J Mice (F0) Continuously Exposed to Very Low-Dose-Rate Gamma Rays Pre-conception and their Male (F1 and F2) Progeny

Dose rate (Total dose)	Non-irradiated	0.05 mGy/day	1 mGy/day (400 mGy)	20 mGy/day (8000 mGy)
Sire (F0)				
All causes of death				
Number of mice	180	180	180	180
Mean survival (days ± SE)	866.8 ± 13.7	851.7 ± 14.0	865.4 ± 14.2	808.6 ± 13.9[a]
All fatal neoplasms				
Incidence (%)	104 (57.8)	104 (57.8)	102 (56.7)	117 (65.0)
Mean survival	953.1 ± 15.0	946.6 ± 14.0	967.6 ± 15.7	887.3 ± 14.5[a]
Lymphoma, malignant				
Incidence (%)	40 (22.2)	57 (31.7)	44 (24.4)	58 (32.2)
Mean survival	1026.0 ± 10.5	1025.8 ± 16.2	1032.1 ± 13.6	969.8 ± 16.7[a]
Sarcoma, histiocytic				
Incidence (%)	21 (11.7)	17 (9.4)	15 (8.3)	10 (5.6)
Mean survival	1114.5 ± 14.8	1109.5 ± 12.6	1121.0 ± 11.1	1039.3 ± 7.1
Vascular neoplasms				
Incidence (%)	17 (9.4)	6 (3.3)	13 (7.2)	21 (11.7)
Mean survival	1070.2 ± 9.0	1108.6 ± 7.4	1077.4 ± 7.5	1081.1 ± 15.0
Inflammation				
Incidence (%)	33 (18.3)	28 (15.6)	32 (17.8)	21 (11.7)
Mean survival	1041.6 ± 10.5	1055.5 ± 15.0	1104.6 ± 18.6	1051.0 ± 12.9
F1 offspring				
All causes of death				
Number of mice	278	250	259	218
Mean survival (days ± SE)	893.3 ± 12.5	878.0 ± 13.5	866.4 ± 12.3[b]	855.5 ± 14.3[b]
All fatal neoplasms				
Incidence (%)	159 (57.2)	134 (53.6)	144 (55.6)	122 (56.0)
Mean survival	974.0 ± 11.5	986.8 ± 12.5	948.0 ± 11.1	956.2 ± 12.1
Lymphoma, malignant				
Incidence (%)	49 (17.6)	52 (20.8)	64 (24.7)	42 (19.3)
Mean survival	1073.9 ± 9.6	1082.2 ± 11.8	1021.8 ± 10.0	1017.0 ± 9.0
Sarcoma, histiocytic				
Incidence (%)	53 (19.1)	39 (15.6)	31 (12.0)	31 (14.2)
Mean survival	1096.2 ± 11.3	1110.4 ± 11.4	1085.6 ± 9.8	1101.6 ± 15.9
Vascular neoplasms				
Incidence (%)	20 (7.2)	16 (6.4)	9 (3.5)	20 (9.2)
Mean survival	1049.1 ± 5.1	1043.1 ± 4.7	979.8 ± 3.1	1080.4 ± 9.0
Inflammation				
Incidence (%)	73 (26.3)	63 (25.2)	60 (23.2)	52 (23.9)
Mean survival	1075.2 ± 15.5	1072.5 ± 15.2	1068.1 ± 14.0	1043.3 ± 18.0
F2 offspring				
All causes of death				
Number of mice	444	346	425	326
Mean survival (days ± SE)	888.1 ± 9.6	864.1 ± 11.5	882.7 ± 9.4	879.9 ± 10.8
All fatal neoplasms				
Incidence (%)	264 (59.5)	201 (58.1)	244 (57.4)	180 (55.2)
Mean survival	1093.2 ± 7.8	1098.9 ± 11.4	1069.2 ± 7.0	1082.5 ± 8.9
Lymphoma, malignant				
Incidence (%)	103 (23.2)	71 (20.5)	93 (21.9)	76 (23.3)
Mean survival	1122.0 ± 15.4	1069.2 ± 10.4	1058.8 ± 8.2	1059.2 ± 9.9
Sarcoma, histiocytic				
Incidence (%)	78 (17.6)	68 (19.7)	77 (18.1)	56 (17.2)
Mean survival	1093.2 ± 7.8	1098.9 ± 11.4	1069.2 ± 7.0	1082.5 ± 8.9
Vascular neoplasms				
Incidence (%)	25 (5.6)	23 (6.6)	12 (2.8)	18 (5.5)
Mean survival	1121.5 ± 5.2	1099.7 ± 6.2	1075.6 ± 3.8	1093.4 ± 4.9
Inflammation				
Incidence (%)	81 (18.2)	61 (17.6)	98 (23.1)	80 (24.5)
Mean survival	1099.2 ± 9.2	1117.6 ± 13.9	1088.3 ± 15.1	1071.6 ± 11.3

[a] $P < 0.01$.
[b] $P < 0.05$ (Log-rank test).

TABLE 4B

Kaplan-Meier Estimates of Mean Survival of Major causes of Death in Female Progeny (F1 and F2) of Male C57BL/6J Mice (F0) Continuously Exposed to Very Low-Dose-Rate Gamma Rays Pre-conception

Dose rate (Total dose)	Non-irradiated	0.05 mGy/day	1 mGy/day (400 mGy)	20 mGy/day (8000 mGy)
F1 offspring				
All causes of death				
Number of mice	274	239	237	215
Mean survival (days ± SE)	812.6 ± 11.2	794.8 ± 10.7	810.9 ± 11.2	802.9 ± 12.9
All fatal neoplasms				
Incidence (%)	178 (65.0)	141 (59.0)	151 (63.7)	143 (66.5)
Mean survival	885.3 ± 9.1	876.1 ± 12.0	882.3 ± 11.8	869.4 ± 11.5
Lymphoma, malignant				
Incidence (%)	100 (36.5)	81 (33.9)	83 (35.0)	84 (39.1)
Mean survival	951.0 ± 10.9	963.4 ± 17.8	976.7 ± 16.6	936.1 ± 13.1
Pituitary gland neoplasms				
Incidence (%)	27 (9.9)	13 (5.4)	22 (9.3)	18 (8.4)
Mean survival	1048.5 ± 10.6	1001.2 ± 5.5	1038.6 ± 8.7	1036.1 ± 8.3
Sarcoma, histiocytic				
Incidence (%)	23 (8.4)	25 (10.5)	25 (10.5)	20 (9.3)
Mean survival	1006.8 ± 6.9	1075.0 ± 16.7	1025.5 ± 9.5	999.2 ± 8.6
Inflammation				
Incidence (%)	54 (19.7)	47 (19.7)	47 (19.8)	37 (17.2)
Mean survival	989.3 ± 10.8	952.4 ± 10.3	1011.4 ± 12.4	1011.7 ± 13.5
F2 offspring				
All causes of death				
Number of mice	389	345	373	292
Mean survival (days ± SE)	812.2 ± 9.0	797.8 ± 10.5	810.2 ± 9.1	815.7 ± 10.6
All fatal neoplasms				
Incidence (%)	251 (64.5)	223 (64.6)	225 (60.3)	187 (64.0)
Mean survival	879.5 ± 8.6	870.4 ± 9.7	891.6 ± 8.8	883.4 ± 10.2
Lymphoma, malignant				
Incidence (%)	134 (34.4)	134 (38.8)	143 (38.3)	119 (40.8)
Mean survival	960.4 ± 10.6	932.4 ± 10.8	943.3 ± 9.8	932.6 ± 10.4
Pituitary gland neoplasms				
Incidence (%)	42 (10.8)	27 (7.8)	25 (6.7)	29 (9.9)
Mean survival	1044.0 ± 8.5	1071.0 ± 9.7	1139.2 ± 16.7	1037.9 ± 7.6
Sarcoma, histiocytic				
Incidence (%)	38 (9.8)	27 (7.8)	29 (7.8)	17 (5.8)
Mean survival	1029.1 ± 7.0	943.3 ± 4.2	1029.5 ± 6.4	979.6 ± 4.2
Inflammation				
Incidence (%)	77 (19.8)	64 (18.6)	66 (17.7)	55 (18.8)
Mean survival	999.2 ± 9.4	1005.7 ± 10.5	1013.2 ± 11.4	998.1 ± 8.8

that the severity and prevalence of ulcerative dermatitis are dependent on diet (nutrition) and husbandry practices within the colony/vivarium as well as environmetal allergens (*46*) (pollen or fungal spores) brought into the room by personnel clothing, feed or bedding. None of the F0 mice had inflammatory conditions resulting from fight injuries since they were housed with the same cage-mates for the entire conduct of the study. The F1 males were group-housed except during the breeding period (1 week) when they cohabited with F1 females (ratio of 1:1), after which they returned to group housing with the same cage-mates (from the same litter) they were housed with post-weaning.

DISCUSSION

The current study reports on the effects of continuous pre-conceptional low-dose-rate gamma-ray exposure of male C57BL/6J Nirs (F0) mice on reproduction, neoplasm incidence, causes of death and lifespan in the F1 and F2 progeny. It should be noted that the results of the present study are not directly comparable to our previous lifespan

TABLE 5A

Neoplasm Incidence in C57BL/6J Males Continuously Exposed to Very Low-Dose-Rate Gamma Rays for 400 Days Pre-conception (F0) and their Progeny (F1 and F2)

	F0 (Sire)				F1				F2			
	Non-irradiated 0 mGy n = 180 (%)	0.05 mGy/day 20 mGy n = 180 (%)	1 mGy/day 400 mGy n = 180 (%)	20 mGy/day 8000 mGy n = 180 (%)	Non-irradiated 0 mGy n = 278 (%)	0.05 mGy/day 20 mGy n = 250 (%)	1 mGy/day 400 mGy n = 259 (%)	20 mGy/day 8000 mGy n = 218 (%)	Non-irradiated 0 mGy n = 444 (%)	0.05 mGy/day 20 mGy n = 346 (%)	1 mGy/day 400 mGy n = 425 (%)	20 mGy/day 8000 mGy n = 326 (%)
Digestive system	16 (8.9)	28 (15.6)	29 (16.1)	28 (15.6)	25 (9.0)	16 (6.4)	26 (10.0)	24 (11.0)	42 (9.5)	24 (6.9)	40 (9.4)	14 (4.3)
Gastro-intestinal tract	4 (2.2)	8 (4.4)	9 (5.0)	7 (3.9)	9 (3.2)	8 (3.2)	10 (3.9)	10 (4.6)	18 (4.1)	9 (2.6)	22 (5.2)	6 (1.8)
Liver	10 (5.6)	19 (10.6)	20 (11.1)	20 (11.1)	16 (5.8)	8 (3.2)	16 (6.2)	14 (6.4)	23 (5.2)	15 (4.3)	17 (4.0)	8 (2.5)
Adenoma, hepatocellular	6 (3.3)	10 (5.6)	9 (5.0)	11 (6.1)	7 (2.5)	3 (1.2)	6 (2.3)	5 (2.3)	9 (2.0)	6 (1.7)	8 (1.9)	3 (0.9)
Carcinoma, hepatocellular	4 (2.2)	8 (4.4)	10 (5.6)	9 (5.0)	9 (3.2)	5 (2.0)	7 (2.7)	7 (3.2)	12 (2.7)	8 (2.3)	9 (2.1)	5 (1.5)
Other		1 (0.6)	1 (0.6)				3 (1.2)	2 (0.9)	2 (0.5)	1 (0.3)		
Salivary gland	2 (1.1)	1 (0.6)		1 (0.6)					1 (0.2)		1 (0.2)	
Endocrine system	28 (15.6)	25 (13.9)	34 (18.9)	37 (20.6)	43 (15.5)	35 (14.0)	33 (12.7)	28 (12.8)	58 (13.1)	42 (12.1)	49 (11.5)	34 (10.4)
Adrenal gland	6 (3.3)	9 (5.0)	6 (3.3)	6 (3.3)	11 (4.0)	9 (3.6)	8 (3.1)	10 (4.6)	17 (3.8)	13 (3.8)	16 (3.8)	10 (3.1)
Pancreas (Endocrine)						1 (0.4)					2 (0.5)	
Parathyroid gland											1 (0.2)	
Pituitary gland	1 (0.6)	4 (2.2)	4 (2.2)	1 (0.6)	5 (1.8)	5 (2.0)	5 (1.9)	2 (0.9)	5 (1.1)	3 (0.9)	3 (0.7)	1 (0.3)
Thyroid gland	21 (11.7)	12 (6.7)	24 (13.3)	30 (16.7)	27 (9.7)	20 (8.0)	20 (7.7)	16 (7.3)	36 (8.1)	26 (7.5)	27 (6.4)	23 (7.1)
Harderian gland	5 (2.8)	3 (1.7)	4 (2.2)	19 (10.6)[a]	13 (4.7)	7 (2.8)	5 (1.9)	10 (4.6)	17 (3.8)	9 (2.6)	12 (2.8)	13 (4.0)
Hematopoietic system	68 (37.8)	82 (45.6)	66 (36.7)	81 (45.0)	125 (45.0)	103 (41.2)	111 (42.9)	78 (35.8)	199 (44.8)	154 (44.5)	187 (44.0)	150 (46.0)
Lymphoma, malignant	45 (25.0)	63 (35.0)	50 (27.8)	68 (37.8)	68 (24.5)	60 (24.0)	74 (28.6)	47 (21.6)	114 (25.7)	80 (23.1)	107 (25.2)	87 (26.7)
Sarcoma, histiocytic	21 (11.7)	18 (10.0)	16 (8.9)	10 (5.6)	54 (19.4)	40 (16.0)	33 (12.7)	31 (14.2)	82 (18.5)	71 (20.5)	75 (17.6)	60 (18.4)
Other	2 (1.1)	1 (0.6)		3 (1.7)	3 (1.1)	3 (1.2)	4 (1.5)		3 (0.7)	3 (0.9)	5 (1.2)	3 (0.9)
Mesothelium						1 (0.4)						
Nervous system		1 (0.6)			1 (0.4)	1 (0.4)	1 (0.4)	1 (0.5)	2 (0.5)		2 (0.5)	1 (0.3)
Pancreas (Exocrine)									1 (0.2)		1 (0.2)	
Reproductive system	3 (1.7)	1 (0.6)	4 (2.2)	1 (0.6)		3 (1.2)	4 (1.5)	3 (1.4)	7 (1.6)	3 (0.9)	5 (1.2)	3 (0.9)
Respiratory system	44 (24.4)	37 (20.6)	43 (23.9)	62 (34.4)	60 (21.6)	64 (25.6)	48 (18.5)	52 (23.9)	80 (18.0)	64 (18.5)	89 (20.9)	60 (18.4)
Skeletal system and teeth	4 (2.2)	4 (2.2)	1 (0.6)	2 (1.1)	4 (1.4)	4 (1.6)	1 (0.4)	1 (0.5)	12 (2.7)	5 (1.4)	6 (1.4)	5 (1.5)
Skin		1 (0.6)	1 (0.6)	2 (1.1)	1 (0.4)		4 (1.5)			4 (1.2)	3 (0.7)	
Soft tissue	2 (1.1)	2 (1.1)	1 (0.6)	3 (1.7)	8 (2.9)	4 (1.6)	8 (3.1)	8 (3.7)	14 (3.2)	9 (2.6)	13 (3.1)	8 (2.5)
Urinary	1 (0.6)	3 (1.7)	1 (0.6)	5 (2.8)		2 (0.8)		1 (0.5)	7 (1.6)	1 (0.3)	2 (0.5)	3 (0.9)
Vascular	20 (11.1)	13 (7.2)	17 (9.4)	27 (15.0)	25 (9.0)	25 (10.0)	16 (6.2)	26 (11.9)	39 (8.8)	35 (10.1)	24 (5.6)	31 (9.5)
Hemangioma	11 (6.1)	7 (3.9)	12 (6.7)	8 (4.4)	6 (2.2)	10 (4.0)	7 (2.7)	11 (5.0)	12 (2.7)	16 (4.6)	12 (2.8)	13 (4.0)
Hemangiosarcoma	9 (5.0)	6 (3.3)	5 (2.8)	19 (10.6)	19 (6.8)	15 (6.0)	9 (3.5)	15 (6.9)	27 (6.1)	19 (5.5)	12 (2.8)	18 (5.5)
Zymbal's gland			1 (0.6)	1 (0.6)			1 (0.4)		1 (0.2)	1 (0.3)		

[a] $P < 0.01$ (Fisher's exact test, two-tailed).

studies using outbred B6C3F1 mice exposed to continuous low-dose-rate exposure to gamma rays for 400 days (*36*) or to in utero exposures for 18 days (*34*) because of the strain difference.

Reproductive Parameters

Pregnancy rates were not significantly different between the F0 and F1 generations in non-irradiated controls but was slightly higher (not statistically significant) in the F1 generation of the irradiated groups as compared to the F0 generation in the corresponding dose group. The higher pregnancy rates in the F1 generation could be attributed to the younger ages (at least 8 weeks of age) at the time of mating.

The slight decrease in reproductive capacity in the F0 generation is partly due to physical incapacity (*47*) because of age (approximately 456 days) at the time of mating, and in the case of the irradiated sires, a decrease in sperm counts (*48*) resulting from continuous radiation exposure since they were bred immediately after radiation exposure was completed (mice were continuously exposed to radiation during all stages of spermatogenesis) with no recovery period. Although multiple studies have shown that increases in germ-line genetic damage is associated with paternal aging, it could not exclusively account for the increased mutations in offspring (*49*). Since the current study uses age-matched non-irradiated controls, the age of the F0 generation at the time of mating as a confounding factor also needs to be taken into consideration when comparing between generations.

The transformation of spermatogonial stem cells through the spermatozoa stage in the seminiferous tubules requires 60–70 days in humans and approximately 30 days in mice, before spermatozoa mature in the epididymis (*50*). Grewenig et al. (*48*) showed that the effects of fractionated low-dose radiation on spermatogenesis in C57BL/6 (C57BL/6NCrl) mice (X rays, 10 mGy/day at HDR of 2 Gy/min) is dependent on the cumulative dose since a significant decrease in Ki-67 positive spermatogonia was observed only when the total

TABLE 5B

Neoplasm IIncidence in the Female F1 and F2 Progeny of C57BL/6J Males (F0) Continuously Exposed to Very Low-Dose-Rate Gamma Rays for 400 Days Pre-conception

	F1				F2			
	Non-irradiated 0 mGy n = 274 (%)	0.05 mGy/day 20 mGy n = 239 (%)	1 mGy/day 400 mGy n = 237 (%)	20 mGy/day 8000 mGy n = 215 (%)	Non-irradiated 0 mGy n = 389 (%)	0.05 mGy/day 20 mGy n = 345 (%)	1 mGy/day 400 mGy n = 373 (%)	20 mGy/day 8000 mGy n = 292 (%)
Digestive system	8 (2.9)	6 (2.5)	11 (4.6)	9 (4.2)	23 (5.9)	13 (3.8)	17 (4.6)	6 (2.1)
Gastro-intestinal tract	4 (1.5)	4 (1.7)	8 (3.4)	7 (3.3)	16 (4.1)	5 (1.4)	13 (3.5)	3 (1.0)
Liver	4 (1.5)	2 (0.8)	3 (1.3)	2 (0.9)	7 (1.8)	8 (2.3)	4 (1.1)	3 (1.0)
Endocrine system	128 (46.7)	89 (37.2)	96 (40.5)	83 (38.6)	142 (36.5)	147 (42.6)	135 (36.2)	95 (32.5)
Adrenal gland	2 (0.7)	1 (0.4)	2 (0.8)	2 (0.9)	2 (0.5)	1 (0.3)		1 (0.3)
Pituitary gland	98 (35.8)	69 (28.9)	75 (31.6)	60 (27.9)	112 (28.8)	113 (32.8)	109 (29.2)	78 (26.7)
Thyroid gland	28 (10.2)	19 (7.9)	19 (8.0)	21 (9.8)	28 (7.2)	33 (9.6)	26 (7.0)	16 (5.5)
Harderian gland	5 (1.8)	7 (2.9)	8 (3.4)	6 (2.8)	6 (1.5)	10 (2.9)	8 (2.1)	10 (3.4)
Hematopoietic system	144 (52.6)	121 (50.6)	128 (54.0)	121 (56.3)	208 (53.5)	187 (54.2)	205 (55.0)	159 (54.5)
Lymphoma, malignant	117 (42.7)	90 (37.7)	94 (39.7)	93 (43.3)	163 (41.9)	148 (42.9)	169 (45.3)	133 (45.5)
Sarcoma, histiocytic	23 (8.4)	27 (11.3)	28 (11.8)	21 (9.8)	38 (9.8)	29 (8.4)	32 (8.6)	20 (6.8)
Other	4 (1.5)	4 (1.7)	6 (2.5)	7 (3.3)	7 (1.8)	10 (2.9)	4 (1.1)	6 (2.1)
Nervous system	1 (0.4)	1 (0.4)						
Reproductive system	17 (6.2)	21 (8.8)	13 (5.5)	14 (6.5)	32 (8.2)	20 (5.8)	21 (5.6)	18 (6.2)
Mammary gland	1 (0.4)	8 (3.3)	2 (0.8)	1 (0.5)	2 (0.5)	3 (0.9)		1 (0.3)
Ovary	13 (4.7)	9 (3.8)	11 (4.6)	11 (5.1)	23 (5.9)	17 (4.9)	13 (3.5)	16 (5.5)
Uterus	3 (1.1)	4 (1.7)		2 (0.9)	7 (1.8)		8 (2.1)	1 (0.3)
Respiratory system	18 (6.6)	14 (5.9)	15 (6.3)	9 (4.2)	30 (7.7)	25 (7.2)	29 (7.8)	18 (6.2)
Skeletal system and teeth	8 (2.9)	3 (1.3)	6 (2.5)	2 (0.9)	5 (1.3)	2 (0.6)	4 (1.1)	3 (1.0)
Skin		1 (0.4)	3 (1.3)					2 (0.7)
Soft tissue	8 (2.9)	3 (1.3)	6 (2.5)	5 (2.3)	7 (1.8)	9 (2.6)	7 (1.9)	5 (1.7)
Urinary system						1 (0.3)	1 (0.3)	
Vascular	13 (4.7)	13 (5.4)	11 (4.6)	8 (3.7)	22 (5.7)	18 (5.2)	20 (5.4)	17 (5.8)
Hemangioma	12 (4.4)	8 (3.3)	8 (3.4)	7 (3.3)	13 (3.3)	11 (3.2)	13 (3.5)	9 (3.1)
Hemangiosarcoma	1 (0.4)	5 (2.1)	3 (1.3)	1 (0.5)	9 (2.3)	7 (2.0)	7 (1.9)	8 (2.7)
Other	1 (0.4)				1 (0.3)			

accumulated dose reached 300 mGy (6 weeks), suggesting reduced proliferative capacity despite no observable impact on spermatogenesis (morphometrically and histologically). Surviving spermatogonial stem cells also showed increased levels of 53BP-1 foci (an indicator of double-stranded breaks, DSBs) that persisted up to 10 weeks after exposure to 100 mGy/day (total dose = 5 Gy) suggesting the possibility that acute and long-term effects of radiation on spermatogonial stem cells and their genomic integrity may result in transmissible genetic damage, should these DSBs persist through differentiation to spermatozoa (*48*).

While sterility from radiation exposure has not been shown to alter hormone balance, libido or physical capability in men, doses as low as 0.15 Gy result in reduced sperm counts (oligospermia) 6 weeks after exposure, with azoospermia observed from doses above 0.5 Gy resulting in temporary sterility from which recovery is dose dependent (*25*). In humans, radiation damage to the germinal epithelium of the testes due to direct exposure to 100 mGy or, more frequently, through scattered radiation during radiation therapy to surrounding tissues results in oligospermia, germ cell loss and Leydig cell dysfunction (*51*). Oligospermia has been reported to occur after exposure to 100 mGy (*52*). Meistrich (*53*) reports that a dose >6 Gy or a fractionated exposure to a total dose >2.5 Gy equally results in permanent azoospermia, although another report shows that irreversible damage occurs after a single dose of 4 Gy and above (*51*, *53*). The inverse dose-rate effect reported by Vilenchik and Knudson (*54*) has been presented as a possible explanation for the difference in testicular damage due to LDR and HDR radiation exposure where the mutation frequency may be higher at 6 mGy/h than at 60–600 mGy/h due to diminished activation of DNA repair when the ratio of DNA damage is lower than the spontaneous DNA background damage. A recent report by Bae et al. (*55*) proposed that DNA damage in the BALB/c testis after chronic LDR exposures up to ~3.4 mGy/h for 100 days (total dose = 8 Gy) that is not adequately repaired may result in increased cell death. While the current study also exposed the C57BL/6J F0 males to the same the total dose of 8 Gy used by Bae et al. (*55*), we used a much lower dose rate of approximately 909 μGy/h for a longer exposure period of 400 days.

TABLE 6A

Frequency of Multiple Primary Neoplasms in C57BL6 Male Mice Continously Exposed Pre-conception to Very Low-Dose-Rate Gamma Rays (F0) and their Progeny (F1 and F2)

	Sire				F1				F2			
	Non-irradiated 0 mGy n = 180 (%)	0.05 mGy/day 20 mGy n = 180 (%)	1 mGy/day 400 mGy n = 180 (%)	20 mGy/day 8000 mGy n = 180 (%)	Non-irradiated 0 mGy n = 278 (%)	0.05 mGy/day 20 mGy n = 250 (%)	1 mGy/day 400 mGy n = 259 (%)	20 mGy/day 8000 mGy n = 218 (%)	Non-irradiated 0 mGy n = 444 (%)	0.05 mGy/day 20 mGy n = 346 (%)	1 mGy/day 400 mGy n = 425 (%)	20 mGy/day 8000 mGy n = 326 (%)
Average number/mouse	1.06	1.11	1.12	1.49[a]	1.10	1.06	1.00	1.06	1.08	1.02	1.02	0.99
Number of neoplams												
One or more	127 (70.6)	133 (73.9)	134 (74.4)	147 (81.7)	211 (75.9)	181 (72.4)	184 (71.0)	155 (71.1)	342 (77.0)	249 (72.0)	307 (72.2)	229 (70.2)
0	53 (29.4)	47 (26.1)	46 (25.6)	33 (18.3)	67 (24.1)	69 (27.6)	75 (29.0)	63 (28.9)	102 (23.0)	97 (28.0)	118 (27.8)	97 (29.8)
1	78 (43.3)	81 (45.0)	87 (48.3)	64 (35.6)	134 (48.2)	115 (46.0)	126 (48.6)	91 (41.7)	238 (53.6)	167 (48.3)	202 (47.5)	159 (48.8)
2	35 (19.4)	40 (22.2)	29 (16.1)	54 (30.0)	64 (23.0)	50 (20.0)	44 (17.0)	51 (23.4)	75 (16.9)	67 (19.4)	84 (19.8)	48 (14.7)
3	13 (7.2)	10 (5.6)	15 (8.3)	22 (12.2)	9 (3.2)	14 (5.6)	12 (4.6)	13 (6.0)	27 (6.1)	12 (3.5)	20 (4.7)	20 (6.1)
4	1 (0.6)	2 (1.1)	3 (1.7)	5 (2.8)	4 (1.4)	2 (0.8)	2 (0.8)		1 (0.2)	2 (0.6)	1 (0.2)	2 (0.6)
5				2 (1.1)					1 (0.2)			
6												
7										1 (0.3)		

[a] $P < 0.01$ Wilcoxon Test.

As this was a lifespan study, histopathological examination of the testes immediately after completion of the 400-day irradiation period was not performed, so we can only assume that there was a slight/minimal degree of oligospermia at the time of mating based on the reproductive outcomes. Unpublished observations (personal communication with Dr. S. Nakamura) from a small number (n = 5/group) of 456 days old B6C3F1 male mice sacrificed after exposure to 20 mGy/day for 400 days showed that the combined weight of the testes decreased (mean = 178.86 mg) compared to the non-irradiated controls (mean = 247.18 mg). Histopathological examination of the testes at the time of death showed no detectable morphological differences between age-matched irradiated and non-irradiated F0 by the end of life.

The decrease in the average weaning rate for the 0.05 mGy/day group in the F1 generation was due to a combination of decreased litter size and increased pre-weaning mortality (COD undetermined).

Alterations in sex ratios are not considered as a valid indicator for possible genetic effects (*56*), and there is limited evidence for decreased sex ratio resulting from paternal exposure to non-ionizing radiation from high voltage-electricity (*57*).

Bodyweights

Compared to the male B6C3F1mice in the lifespan study (*36*) where the group exposed to 1 mGy/day weighed significantly more ($P < 0.05$) than the non-irradiated controls, the same chronic low-dose-rate exposures did not affect the body weights (and rate of body weight gain) of the C57BL/6J F0 sires in the current study. The average weights of male F1 and F2 mice were higher (about 40 g) than the female F1 and F2mice (about 30 g) and weighed significantly less (approximately 5 grams less at their heaviest at 83–100 weeks) than age-matched B6C3F1 mice used in the lifespan study (*36*). We also note that our in-house bred (over F20) C57BL/6JN mice, originally obtained from the National Institute of Radiological Sciences (NIRS-QST), weighed less (below 40 g, age- and sex-matched) than the published background data for C57BL/6J by Jackson Laboratories (*58*). This difference could be attributed to genetic drift (*59*).

Survival Curves, Mean Lifespans and Causes of Death

The mean lifespans for all generations of mice (F0 to F2), male and female, in this study were comparable to published data on C57BL/6J mice (*60*). The mean lifespan of the non-irradiated control male F0 C57BL/6J was shorter [866.8 days (839.7–893.9)] compared to the non-irradiated controls in the lifespan study (*35*) using male B6C3F1 mice [912.7 days (895.8–928.2)]. At 20 mGy/day, the survival curve of the C57BL/6J F0 sires exposed to 20 mGy/day showed a similar shift towards the left as the B6C3F1 males but with less life shortening (average of 58.2 days, 6.71%) than the similarly exposed B6C3F1 males (average of 100.7 days, 11.0%) (*35*). These differences in lifespan and degree of life shortening are attributed to strain differences in tumor spectra and neoplasm incidence rates (e.g. higher incidence rates for liver tumors in B6C3F1 than C57BL/6J).

Overall, the mean lifespans of C57BL/6J F0 sires were significantly shorter than the male F1 or F2 generations, regardless of radiation exposure and radiation dose, and could also, in part, be attributed to stress from cohabitation with different females from the multiple matings initiated during the 400-day period of irradiation to maintain reproductive behavior.

The decrease in the mean survival in the F0 sires exposed to 20 mGy/day (808.6 ± 13.9 days) is due to early deaths, overall, from neoplasms (all fatal, Table 4A, 887.3 ± 14.5 days), mostly from malignant lymphoma (32.2%, 969.8 ± 16.7 days). The significant decrease in the mean survival of male F1 progeny (855.5 ± 14.3 days) born from F0 mice exposed to 20 mGy/day (compared to the non-irradiated F1 males) is also attributed to early death

TABLE 6B

Frequency of Multiple Primary Neoplasms in C57BL6 Female Mice (F1 & F2) Born from Sires (F0) Continuously Exposed to Very Low-Dose-Rate Gamma Rays Pre-conception

	F1				F2			
	Non-irradiated 0 mGy n = 274 (%)	0.05 mGy/day 20 mGy n = 239 (%)	1 mGy/day 400 mGy n = 237 (%)	20 mGy/day 8000 mGy n = 215 (%)	Non-irradiated 0 mGy n = 389 (%)	0.05 mGy/day 20 mGy n = 345 (%)	1 mGy/day 400 mGy n = 373 (%)	20 mGy/day 8,000 mGy n = 292 (%)
Average number/mouse	1.32	1.21	1.27	1.23	1.25	1.29	1.23	1.17
Number of neoplams								
One or more	216 (78.8)	177 (74.1)	189 (79.7)	168 (78.1)	303 (77.9)	274 (79.4)	287 (76.9)	231 (79.1)
0	58 (21.2)	62 (25.9)	48 (20.3)	47 (21.9)	86 (22.1)	71 (20.6)	86 (23.1)	61 (20.9)
1	107 (39.1)	96 (40.2)	101 (42.6)	102 (47.4)	171 (44.0)	146 (42.3)	162 (43.4)	144 (49.3)
2	79 (28.8)	57 (23.8)	64 (27.0)	44 (20.5)	90 (23.1)	91 (26.4)	86 (23.1)	65 (22.3)
3	24 (8.8)	19 (7.9)	23 (9.7)	15 (7.0)	34 (8.7)	33 (9.6)	35 (9.4)	20 (6.8)
4	4 (1.5)	3 (1.3)	1 (0.4)	6 (2.8)	7 (1.8)	3 (0.9)	2 (0.5)	2 (0.7)
5	2 (0.7)	2 (0.8)		1 (0.5)	1 (0.3)	1 (0.3)	2 (0.5)	

from fatal neoplasms (956.2 ± 12.1 days) but could not be associated to a specific neoplasm. Except for the male F2 progeny exposed to 0.05 mGy/day (864.1 ± 11.5 days), the F2 males (879.9–888.1 days) had longer lifespans overall compared to either the F0 (808.6–866.8 days) or F1 (855.5–893.3 days) generations. It is interesting to note that while there was no significant life shortening in the F0 males exposed to 1 mGy/day, their F1 male progeny had significantly shorter lifespans compared to the non-irradiated F1 males.

Although the mean survival of the female F1 and F2 progeny born from non-irradiated and irradiated F0 were not significantly different, it is notable that the mean survival of female F1 (n = 239, 794.8 days) and F2 (n = 345, 797.8 days) progeny born from F0 sires exposed to 0.05 mGy/day (Table 1) were shorter (but not statistically significant) than those born from F0 sires that were non-irradiated or exposed to 1 and 20 mGy/day. The shorter mean survival times in the F1 and F2 females are due to decreased overall mean survival from fatal neoplasms (both F1 and F2) (Table 4B) as well as due to early deaths from malignant lymphomas in the F2 generation. Further investigation with a larger number of animals may confirm the cause of this trend towards shorter mean survival times in female progeny. We also did not observe any significant difference in the lifespans among F1 females that were not bred, bred but not pregnant or bred with pups (data not shown) regardless of radiation exposure.

Regardless of sex, generation and radiation exposure, neoplasms remain the major COD determinant, mostly lymphoma and hematopoietic neoplasms with incidence rates comparable to that reported in aging studies in C57BL/6N or C57BL/6J mice (*61*). Other significant contributors to COD in males include vascular neoplasms (hemangiomas and hemangiosarcomas) and inflammatory conditions. In females, other significant contributors to COD were pituitary neoplasms as well as inflammatory conditions, with no significant differences among the non-irradiated and irradiated progeny or between generations.

Neoplasm Incidence and Multiple Primary Neoplasms

Neoplasm spectra were similar for all generations within sexes regardless of radiation exposure. While not statistically significant within each generation of males, incidence rates for malignant lymphomas were comparatively higher in the F0 generation than the F1 or F2 generations regardless of radiation exposure. However, for histiocytic sarcomas, incidence rates in the F1 (12.7–19.4%) and F2 (17.6–20.5) generations were higher than the F0 (5.6–11.7%) generation with no lifespan shortening, suggesting that this increase is due to competing risks (decreased mortality and incidence rates for malignant lymphomas, thereby resulting in more animals surviving to develop histiocytic sarcoma at a later age). While the total incidence rates (Tables 4A and 4B) for histiocytic sarcoma (F0 to F2 males = 5.6–20.5%; F1 and F2 females = 6.8–11.8%) were lower in the present study compared to that reported by Blackwell et al. (*62*) (male = 54.5–58.0%; female 32.4–50%), the total incidence rates for malignant lymphoma (F0 to F2 males = 21.6–37.8%; F1 and F2 females = 37.7– 45.5%) were comparatively higher (males = 4.0– 9.3%, females = 6.7–9.3%) for C57BL6 mice (unspecified substrain) (*62*).

In the F0 generation, the incidence rate for Harderian gland tumors was significantly increased (10.6%, $P < 0.001$) only in the group exposed to 20 mGy/day (compared to the non-irradiated controls) but was comparatively lower than that observed for B6C3F1 males (27.3%) (*36*) similarly exposed in the lifespan study. The non-neoplastic lesions observed in this study were those associated with aging (*43*) as in the lifespan study using B6C3F1 mice (*36*).

Compared to the B6C3F1 lifespan study (*36*), C57BL/6J males (F0) exposed to low-dose-rate gamma rays developed fewer primary neoplasms averaging 1.49 neoplasms/mouse at 20 mGy/day ($P < 0.01$). Male F1 and F2 progeny developed fewer primary neoplasms (0.99–1.10/mouse) than the female F1 and F2 progeny (1.17–1.32/mouse) with no significant difference between progenies from non-irradiated and irradiated sires. Overall, tumor multiplicity in the current study was lower than that reported by Blackwell et al. (*62*) for C57BL6 mice regardless of diet (ad libitum or diet restricted).

Brinkworth (*63*) and more recently Dubrova and Sarapultseva (*64*) reviewed evidence from animal studies (mammals and invertebrates) that show paternal exposure to radiation results in impaired viability, fertility and genome stability in offspring, depending on the radiation quality, dose, dose-rate, exposure conditions and test system. Liu

et al. (*65*) reported the highest increase in apoptosis in both spermatogonia and spermatocytes for 75 mGy in Kunming mice exposed to X rays at chronic dose-rate (12.5 mGy/min, 25–200 mGy total dose). Barber et al. (*29*) showed that the extent of transgenerational increase in ESTR mutation rates in F1 and F2 progeny of sires exposed to 2 Gy of X rays, or 0.4 Gy fission neutrons depended on the radiosensitivity of the mouse strain with the C57BL6 mice being the least radiosensitive compared to BALBc and CBA mice strains. Paternal exposures of BALB/c mice to acute lower doses (100–250 mGy) and at chronic low-dose-rate (0.05 mGy/min) exposure to 1000 mGy has not been shown to affect the stability of genomes in F1 offspring (*33*).

Although it is well established that spermatogenesis is extremely sensitive to radiation exposure, epidemiological evidence on the effect of low-dose radiation exposures on the male reproductive system is lacking and the underlying molecular mechanisms for radiosensitivity remain unclear (*50*).

Kong et al. (*66*) reported that in humans, almost 80% of de novo mutations transmitted to the offspring arise from the paternal germline. Paternal mutation bias had been attributed to the greater number of cell divisions in the male germline (*49*) compared to the female germ line, until it was recently demonstrated by de Manuel et al. (*67*) in 42 species of amniotes that the paternal biases are due to sex differences in the balance of DNA damage vs. DNA repair.

Ogura et al. (*68*) analyzed 25 randomly selected families each from the non-irradiated and the 20 mGy/day groups from the current study and found an increase in the copy number variations (CNVs) (deletions) in the F1 offspring of the irradiated group and suggested that this may be associated with shorter lifespans. Kovalchuk and Baulch (*69*) suggested that the transgenerational effects of low-dose radiation, such as increased prevalence of genomic instability and non-Mendelian mode-of inheritance mechanisms, are epigenetic in nature. Dubrova et al. (*70*) showed that elevated ESTR mutation rates in F1 offspring are inherited equally from the unexposed F0 dams and irradiated F0 sires, indicating that these are not inherited from a damaged allele of the sire and suggests that the radiation-exposure signal is inherited through the sperm epigenetically. Although the mechanisms underlying epigenetic memory are not clear, data suggest that abnormal RNA production in sperm due to disruption of a genomic locus may be transmitted transgenerationally for at least 2 generations (*71*).

Despite extensive evidence of germ-cell (chemical) mutagens in rodent studies, a workshop in 2004 (*72*) concluded that the lack of evidence in humans is mainly due to technical issues, i.e. the lack of suitable analytical methods (*73*). While several studies on paternal exposures to ionizing radiation have reported increased mutation rates in children (*14*, *74*), concerns over proper control populations used, unidentified environmental factors other than radiation and the dosimetry hinder their acceptance (*73*). De Marini (*73*) also reviewed the possible reasons for the negative results from the atomic bomb survivors and Chernobyl clean-up workers. Nakamura (*75*, *76*) suggests that humans may lack genes that are sensitive to radiation that can be used for screening purposes, hence making it difficult to detect germline mutations as compared to animal models such as mice. Other human biological factors (*76*) also make it difficult for irradiated germ cells to produce abnormal offspring such as low mutation induction rates in spermatogonia, low-oxygen microenvironment in the ovary, and the inclination of human pregnancies for miscarriage, 50–80% of which occur in the first trimester and are attributed to "sporadic" chromosomal mutation (*77*). A recent review by Stephens et al. (*78*) concluded that there is a lack of evidence which makes the assessment of radiation-related intergenerational effects difficult.

Studies investigating parent-of-origin, sex-specific effects expected to provide clues towards further understanding transgenerational inheritance produce variable results in the male or female offspring (*79*, *80*) depending on the factor(s) investigated such as parental nutrition (overnutrition, starvation, dietary composition) or stress (trauma) as well as on the exposed parent (sire or dam). Brinkworth (*63*) illustrated the hypothetical routes by which heritable mutations may be induced in the male germ line based on dose and exposure conditions and suggests further research focusing on mechanisms involved in genomic instability and apoptosis suppression to gain insights on maintaining genomic integrity of the male germline. Tharmalingam et al. (*81*) illustrated the proposed mechanism for low dose ionizing radiation-induced cellular effects, citing free radical production resulting in oxidative stress that targets epigenetic regulators altering gene regulation patterns.

Although beyond the scope of the current work, other factors to consider in multi- and transgenerational effects of pre-conception radiation exposure also include the restoration of oxidative damage and the roles of DNA damage repair in pre-implantation zygotes (*82*) and epigenetic mechanisms (*83*). Low-dose exposures to radiation may also elicit an adaptive response where irradiated cells may be able to repair the damage; this slight increase in repair and regulatory proteins may be protective (*81*).

The current study shows that male C56BL/6J mice (F0) chronically exposed for 400 days pre-conceptionally to a low-dose rate of 20 mGy/day had shorter lifespans due to early death from malignant lymphomas. While the cause of life shortening in male F1 progeny of mice exposed to 1 mGy/day and 20 mGy/day could not be verified, an increase in the sample size in future studies may be helpful for clarification. The female F1 and F2 progeny did not show any multi- or transgenerational effects in any of the parameters examined. No significant increase in neoplasm incidence was observed in all generations except for the Harderian gland tumors in the male F0 exposed to 20 mGy/day. There was also no change in

tumor spectra in all generations regardless of sex or radiation exposure.

Future research and scientific strategies for clarifying the effects of parental (pre-conception) and in utero exposures to low doses and low-dose-rate radiation was previously reviewed by Grison et al. (*2*). Investigating multi- and transgenerational effects of pre-conceptional exposure to low doses and low dose rates of radiation is complicated, time-consuming and expensive (*2*). Cross-sectional studies on oxidative stress response at various phases of the spermatogenic cycle, as well as further investigation into the DNA damage response pathways and DNA repair machinery, including those that occur in the preimplantation zygote, are necessary to find ways to preserve the genetic integrity of spermatogenic cells after exposure to ionizing radiation. Although many studies have shown that ancestral exposures influence phenotypes for several generations, the possible mode(s) of transmission remain unclear and the prospect of genetic diseases (transgenerational and heritable) and intergenerational effects continue to be of concern in exposed populations such as Japanese atomic bomb survivors, survivors of childhood and adolescent cancers, radiation-exposed workers (occupational exposures such as astronauts, airline pilots and cabin crew), and environmentally exposed groups. Nevertheless, societal concerns on the effect of radiation to their immediate offspring and succeeding generations must be addressed with care (*84*).

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