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Authors: Tanaka, Ignacia B., Nakahira, Rei, Komura, Jun-ichiro, and Tanaka, Satoshi

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Life Span, Cause of Death and Neoplasia in B6C3F1 Mice Exposed In Utero to Low- and Medium-Dose-Rate Gamma Rays

Ignacia B. Tanaka III,[1] Rei Nakahira, Jun-ichiro Komura, Satoshi Tanaka

Department of Radiobiology, Institute for Environmental Sciences. 1-7, Ienomae, Obuchi, Rokkasho-mura, Kamikita-gun, Aomori 039-3212, Japan

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Previously, we reported that while low-dose-rate (LDR) gamma-ray exposure to 20 mGy/day for the entire gestation period (gestation days 0–18) did not result in any significant effect in B6C3F1 pups up to 10 weeks of age when compared to the non-irradiated controls, exposure to medium-dose-rates (MDR, 200 and 400 mGy/day) resulted in growth retardation and gonadal hypoplasia, in addition to delayed ossification (only at 400 mGy/day). In the present work, we investigated the late effects of continuous in utero exposure to gamma rays at LDRs (0.05, 1.0 and 20 mGy/day) and at an MDR of 400 mGy/day, on life span, causes of death, neoplastic and non-neoplastic disease incidences in B6C3F1 mice. Reproductive parameters such as litter size and weaning rates was not significantly different among the LDR groups, but was significantly decreased in the MDR group, when compared to the non-irradiated controls. Mean life spans were not significantly different among the LDR exposed groups compared to the non-irradiated controls, whereas the life spans of those exposed to the MDR were significantly shorter than the non-irradiated controls. There was no significant difference in tumor spectra between the non-irradiated and LDR nor MDR irradiated groups. In mice exposed to MDR in utero, the over-all incidence rates shifted with increased incidences in the number of neoplasms of liver (both sexes) and endocrine (adrenals, pituitary and ovaries in females) origin with corresponding decreases in the incidence of malignant lymphomas (both sexes) and lung neoplasms (males). Multiple primary neoplasms were significantly increased only in females exposed to MDR. Results show that B6C3F1 mice exposed to gamma-rays in utero at LDRs of 0.05, 1 and 20 mGy/day for the entire gestation period (18 days) does not significantly alter lifespan, cause of death, neoplasm incidence rates and tumor spectra.

INTRODUCTION

Radiation exposure during pregnancy is a health concern for both the mother and the unborn child and is a source of anxiety in pregnant women. Except for radiation therapy, fluoroscopy-guided interventional procedures and some computed tomography (CT) scans, diagnostic medical imaging procedures such as CT, conventional fluoroscopy and nuclear medicine expose the embryo or fetus to absorbed doses of 0.1 Gy or less (*1, 2*). X-ray exposures of $\leq$5 rad ($\leq$5 mGy) at any stage of pregnancy show no measurable risk (*3*), neither birth defects or miscarriage (*1*).The maximum permissible dose to the fetus is 0.5 mSv (0.05 rem)/month and a dose of 0.1 Gy (10 rad) to the embryo during the sensitive period of gestation (10 days to 25 weeks) is frequently considered as the cutoff point above which therapeutic abortion should be considered to avoid the possibility of an abnormal child (*4*).

The biological effects of in utero radiation exposure depend on many factors such as radiation quality, dose and dose-rate, gestation age at the time of exposure, and are further confounded by maternal factors (age, health status) and genetic predilection (*5*), all of which need to be taken into consideration when assessing risk.

The effects of ionizing radiation exposure on the fetus are broadly classified as either deterministic effects (tissue reactions), such as death (pregnancy loss = miscarriage/stillbirth), congenital malformations, growth/developmental disturbances, microcephaly and intellectual disability and increased cancer risks, or as stochastic effects, such as cancer induction as in childhood cancers (*6*). In humans, doses to the fetus above 0.10 Gy (100 mGy, 10 rads) increases the risk of tissue reactions (deterministic effects) (*6*) in addition to neurobehavioral dysfunction, growth retardation, fetal death and increased cancer risk. Evidence on the detrimental effects of radiation on the fetus have been obtained primarily from animal studies, nuclear accidents (e.g., Chernobyl disaster) and from the atomic bomb survivors of Hiroshima and Nagasaki (*6*).

Surviving animal fetuses exposed in utero to acute high doses of radiation result in growth retardation, congenital anomalies, including those of the central nervous system, cognitive and behavioral abnormalities (*7–9*), and increased

[1] Corresponding author: Ignacia B. Tanaka III, D.V.M., Ph.D., Institute for Environmental Sciences, 1-7, Ienomae, Obuchi, Rokkasho-mura, Kamikita-gun, Aomori 039-3212, Japan; e-mail: tanakaib@ies.or.jp.

cancer incidences (*10*) depending on the gestation age at the time of exposure as well as on the mouse strain.

Animal studies on in utero radiation exposures at low (LDR) (*11*) or medium (MDR) dose-rates (*12*) and at fractionated doses (*13*) has been briefly reviewed previously (*14*). Depending on the gestation age at the time of exposure, acute doses from 0.5–5.7 Gy result in pre-implantation loss, embryonic death, decreased litter sizes (*8, 15*), increased fetal mortality, increased incidences of congenital malformations and growth retardation, and decreased body size and brain weight (*8*). Growth retardation, delay in the appearance of physiological markers such as pinna detachment, fur development, eye opening, vaginal opening, and testicular descent have also been reported (*16*). Studies in both rats (*17*) and mice (*12, 18*) on in utero exposures for the entire gestation period (days 1 to 18) using gamma rays from 25 mGy/day to 124 mGy/day reported no effect. In rats, the no observed adverse effect level (NOAEL) for lethal effects of radiation is approximately 0.15–0.20 Gy (15–20 rad) at 0–8 days post conception (equivalent to 0–16 days post conception in humans) (*19*) with no increased risk of growth retardation in surviving embryos receiving 0.20 Gy or less. Sasaki (*10*) reported that mice exposed to γ rays at acute doses of 1.9 to 5.7 Gy during the late-fetal period shortened the average lifespan and increased solid tumor incidence in the lungs, liver, pituitary, ovaries, and uterus in adulthood. Radiation exposure during organogenesis and fetal development have been reported to result in behavioral changes, impairments in learning and memory, effects on the central nervous system, delay in the appearance of physiological markers of development, low birth weight and growth reduction (*9*), but it is not clear however, whether these behavioral changes, attributed to radiation exposure during organogenesis, persist later in life (*9*).

Previously, we (*14*) showed that in utero exposure to a LDR of 20 mGy/day for the entire gestation period did not cause any significant effect in pups up to 10 weeks of age when compared to age-matched non-irradiated controls, whereas, increased post implantation loss, due to early embryonic deaths (early resorption), dose-related growth retardation with delayed ossification (400 mGy/day) and gonadal hypoplasia/atrophy in both sexes were observed at MDRs of 200 or 400 mGy/day. Further investigation by Nakahira et al. (*20*) verified the absence of germ cells in gestation day (GD) 18 fetuses exposed to MDRs of 200 and 400 mGy/day, confirming gonadal hypoplasia. Exposure to acute high dose-rate (HDR) of 2 Gy at GD11 increased the incidence rates for external abnormalities at birth and failed to survive to 10 weeks of age (*14*).

The need to investigate the long-term/late effects on offspring exposed in utero at low dose and low-dose-rate exposures leads to the present work on the effects continuous in utero to gamma-ray exposure, for the entire gestation period (gestation day 0–18) at LDR (0.05, 1 and 20 mGy/day) and MDR (400 mGy/day) in B6C3F1 mice on lifespan, neoplasm and non-neoplastic disease incidences exposed.

MATERIALS AND METHODS

Mice and Animal Husbandry

Six-week-old specific pathogen free (SPF) C57BL/6JJcl females and C3H/HeNJcl males purchased from CLEA Japan were used as parent stock. C3H/HeNJcl males were housed individually in plastic cages (218 × 320 × 133 mm). Virgin C57BL/6JJcl females, housed in groups (up to 20 to a cage, 267 × 426 × 150 mm), were transferred into the cages housing the males (1:1) in the afternoon and allowed to mate overnight. Females with confirmed vaginal plugs the following morning were considered pregnant at gestation day (GD) 0 and were transferred to individual cages and randomly assigned to non-irradiated control (n = 34) or irradiated dose groups (n = 140). The number of pregnant females required per dose group was estimated (based on previous breeding experience) to produce at least 100 pups per sex per dose group.

The entire study was conducted under SPF environmental conditions as described in the life span study (*21*). Husbandry (cage changes, feed and water and health monitoring or clinical inspection), monitoring of SPF status and irradiation has been described previously (*21*).

Irradiation

Pregnant C57BL/6JJcl dams assigned to irradiated groups were continuously exposed to whole-body gamma-ray radiation for 22 h/day from GD 0 to GD 18 to LDRs of 20 mGy/day (n = 33), 1 mGy/day (n = 32) and 0.05 mGy/day (n = 31), and to an MDR of 400 mGy/day (n = 44), to total doses of 360, 18, 0.9 and 7,200 mGy, respectively. After completion of the radiation exposure at GD 18, the pregnant females were moved back to the animal rooms with the non-irradiated pregnant females and allowed to give birth.

Justification for selected radiation dose and dose rates. Based on previous work (*14, 21–22*), LDRs of 0.05, 1 and 20 mGy/day were selected with an MDR of 400 mGy/day as positive control (*14, 20*)

Dosimetry. The absorbed doses by the pregnant dam are based on measurements made using thermoluminesence dosimeters (TLDs) inserted into the abdomen of mice as described by Shiragai et al. (*23*).

Monitoring and Pathological Examination

Pups were carefully counted (total n = 1,184; 614 males and 570 females) as soon as possible after birth and were allowed to stay with their dams until weaning at day 21 (3 weeks of age), at which time they were individually identified with ear notches, weighed, separated by sex and group caged (4 mice/cage). The pups were allowed to die a natural death upon which they were subjected to necropsy (gross examination) and organs collected, weighed and fixed in 10% neutral buffered formalin for histopathological examination based on a standard protocol (*22*). 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 WHO/IARC (*24*) and the NTP (*25*) as described previously (*22*).

A cause of death (COD) was assigned, as described by Tanaka IB et al. (*22*) in the life span study, to all animals in the study.

Multiple primary neoplasms and pathologies were treated as in the previous life span study (*22*) wherein multiple (including multiple or metastatic foci) neoplasms of the same type were counted only once. All neoplasms were counted into the overall incidence.

TABLE 1
Reproductive Parameters (95% Confidence Interval) in B6C3F1 Mice Continuously Exposed to Low- and Medium-Dose-Rate Gamma Rays for 18 Days In Utero

Dose rate	Non-irradiated		0.05 mGy/day		1 mGy/day		20 mGy/day		400 mGy/day	
Total dose	0 mGy		0.9 mGy		18 mGy		360 mGy		7,200 mGy	
Number of pregnant dams	34		31		32		33		44	
Total no. of implantation sites	311		286		286		298		406	
Average no. of implantation sites/dam	9.1	(8.8–9.5)	9.2	(8.8–9.6)	8.9	(8.5–9.4)	9.0	(8.5–9.5)	9.2	(8.6–9.8)
Total no. of early resorbed fetuses	43		40		34		51		118	
Average no. of early resorbed fetuses/litter	1.3	(0.9–1.6)	1.3	(0.9–1.6)	1.1	(0.5–1.6)	1.5	(1.1–2.0)	2.7	(2.1–3.3)[a]
Litter size, average no. of pups born/litter	7.9	(7.2–8.5)	7.9	(7.3–8.5)	7.5	(6.7–8.3)	7.5	(6.8–8.3)	5.8	(4.8–6.7)[a]
Litter size, average no. of male pups born/litter	3.9	(3.3–4.5)	4.3	(3.5–5.0)	3.7	(3.1–4.3)	4.2	(3.6–4.8)	3.1	(2.4–3.8)
Litter size, average no. of female pups born/litter	3.9	(3.4–4.5)	3.6	(3.0–4.3)	3.8	(3.1–4.5)	3.4	(2.8–4.0)	2.7	(2.1–3.2)[a]
Average no. of weaned pups/litter	7.1	(6.1–8.1)	7.4	(6.4–8.4)	7.2	(6.2–8.1)	7.2	(6.2–8.1)	5.6	(4.7–6.6)[a]
Average no. of weaned male pups/litter	3.5	(2.8–4.2)	3.9	(3.1–4.7)	3.5	(2.8–4.1)	3.9	(3.2–4.6)	3.0	(2.3–3.7)
Average no. of weaned female pups/litter	3.6	(3.0–4.3)	3.5	(2.8–4.3)	3.7	(3.0–4.4)	3.3	(2.6–3.9)	2.6	(2.0–3.2)
Average no. of pre-weaning loss/litter	0.7	(–0.1–1.5)	0.5	(–0.2–1.2)	0.3	(–0.2–0.9)	0.4	(–0.2–0.9)	0.1	(–0.1–0.3)
Average no. of pre-weaning loss/litter, male	0.4	(–0.1–0.8)	0.4	(–0.2–0.9)	0.2	(–0.2–0.6)	0.3	(–0.1–0.6)	0.1	(–0.0–0.2)
Average no. of pre-weaning loss/litter, female	0.3	(0.0–0.36)	0.1	(–0.1–0.3)	0.1	(–0.1–0.3)	0.1	(–0.1–0.3)	0	(–0.0–0.1)
Average weaning rate (%)	90.2	(79.9–100.6)	93.3	(83.9–102.8)	95.9	(89.1–102.8)	93.7	(85.1–102.2)	96.7	(91.1–102.3)
Average weaning rate, male (%)	90.9	(80.6–101.3)	93.3	(83.9–102.8)	95.6	(84.7–102.0)	93.3	(84.7–102.0)	96.5	(90.8–102.3)
Average weaning rate, female (%)	89.8	(79.5–100.1)	93.3	(83.9–102.8)	96.2	(89.3–103.0)	96.9	(90.5–103.2)	97.2	(91.6–102.9)

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

After the pups were weaned at 3 weeks of age, all the dams were sacrificed in a similar manner, necropsied, examined for gross pathological changes and the uteri collected. The uteri were clarified (*26*), and the number of implantation sites counted and recorded accordingly.

All experiments were conducted according to legal regulations in Japan and following the Guidelines for Animal Experiments of the Institute for Environmental Sciences.

Statistical Analyses

Fischer's exact tests were used to analyze the crude mortality rates, causes of death, non-neoplastic lesions and neoplasm incidence. Neoplasm multiplicity was analyzed using the Wilcoxon test. Analyses of mean life span and body weights were done using 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 including the number of implantation sites, litter sizes and weaning rates, are summarized in Table 1. The number of implantation sites/dam was not significantly different between the non-irradiated and irradiated groups. Litter size was significantly (<0.05) decreased in those exposed to 400 mGy/day, with a significant (<0.05) decrease in the number of female pups, compared to the non-irradiated controls. Although there was no significant difference in the average number of pre-weaning losses/litter and weaning rates among the LDR- and non-irradiated pups, the average number of weaned pups/litter in those exposed to 400 mGy/day was significantly decreased as a secondary consequence to the significantly small litter size.

Pathological changes were not observed in any of the dams at the time of necropsy and collection of uteri.

Body Weights

Body weights were monitored by weighing a representative number ($n = 60–69$) of animals/group every 4 weeks from weaning (3 weeks of age), up to 179 weeks as shown in Fig. 1. There was no significant difference in body weights and rate of body weight gain between the non-irradiated control and LDR irradiated groups. Mean bodyweights of male mice exposed to 400 mGy/day in utero were significantly ($P < 0.01$) lower compared to the non-irradiated controls, weighing less from 7 to 147 weeks of age (heaviest between 51–67 weeks of age), whereas females exposed to the same dose weighed significantly ($P < 0.01$) heavier from 15–87 weeks of age (heaviest between 67–87 weeks of age), subsequently declining significantly ($P < 0.01$, from 111 to 143 weeks of age) as the mice aged.

Survival Curves

Lifespans of mice exposed to LDRs 20 mGy/day and below were not significantly different from the non-irradiated controls. Survival curves (Fig. 2) of males and females exposed to 400 mGy/day show a significant shift towards the left. Mean lifespans of both males (862.6 ± 209 days, $P = 0.0462$) and females (794 ± 136.2 days, $P < 0.0001$) in the 400 mGy/day group showed a significant lifespan shortening (Steel test) (Table 2).

Causes of Death (COD)

The causes of death with their corresponding incidence rates, classified according to tissue/organ of origin (in alphabetical order) are shown in Table 3. Majority (79.6–93.8%) of the mice died from neoplasms regardless of radiation exposure.

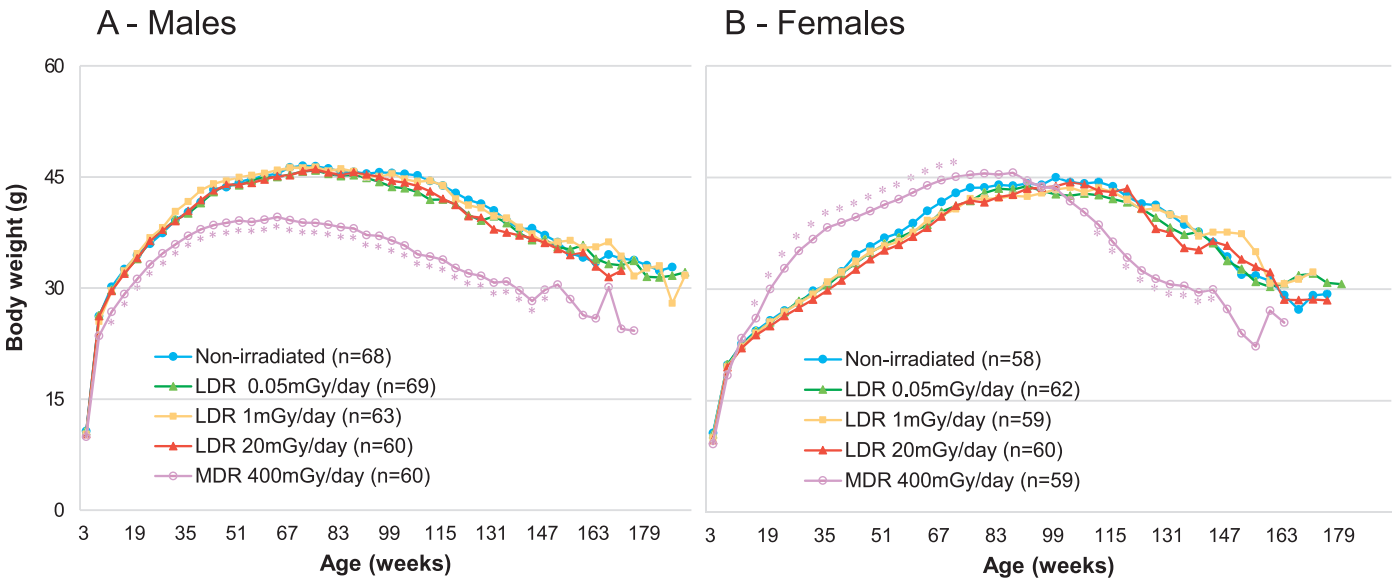

FIG. 1. Average body weights of B6C3F1 mice exposed to low- (LDR) and medium-(MDR) dose-rate gamma rays for 18 days in utero. *P < 0.01

Male mice died mostly from malignant lymphomas (25.6–33.3%) and liver tumors (23.1–31.0%) with no significant difference in the incidence rates among the non-irradiated and low dose-rate (0.5, 1 and 20 mGy/day) groups. At 400 mGy/day exposure however, COD from liver neoplasms (42.9%, P < 0.01) was significantly increased with a corresponding decrease in malignant lymphomas (15.0%, P < 0.01) as COD.

Females also died mostly from malignant lymphomas (44.4–48.8%) and from soft tissue neoplasms (9.3–11.4%), with no significant differences in incidence rates among the non-irradiated and irradiated groups (0.5 to 20 mGy/day). At 400 mGy/day, there was a significant decrease in the number of female mice dying from malignant lymphoma (24.8%, P < 0.01), and significantly increased numbers of mice dying from neoplasms originating from the pituitary gland (pars distalis) (15.9%, P < 0.01) and the ovaries (14.2%, P < 0.01), mainly malignant granulosa cell tumors (9.7%, P < 0.01).

Non-neoplastic causes of death, including interstitial pneumonia, dental dysplasia, malocclusion, hyaline glomerulopathy, systemic arteritis and amyloidosis, etc., accounted for 2.7–15.7% of all CODs and were not significantly different between non-irradiated controls and irradiated groups. Undetermined/unknown CODs comprised 2.4–6.0% of all deaths in both sexes.

For other causes of death, no significant differences were observed in the incidence rates between the non-irradiated and irradiated groups in neither sex.

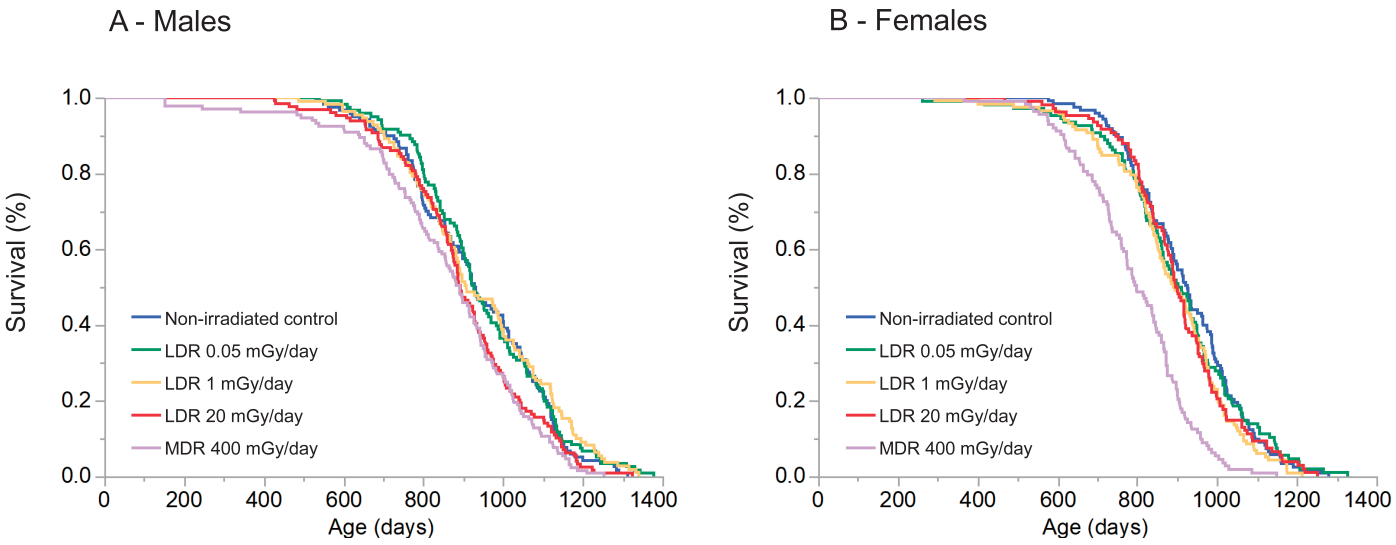

FIG. 2. Survival curves of B6C3F1 mice exposed to low- (LDR) and medium-(MDR) dose-rate gamma rays for 18 days in utero.

TABLE 2
Mean Life Spans of B6C3F1 Mice Continuously Exposed to Low- and Medium-Dose-Rate Gamma Rays for 18 Days In Utero

	Dose rate (mGy/day)	Irradiation period (days)	Total dose (mGy)	Number of mice	Average lifespan ±95 CI (days)	
Males						
	0	18	0	120	935.3	(903.4, 967.1)
	0.05	18	0.9	121	948.9	(918.4, 979.4)
	1	18	18	111	941.9	(906.3, 977.5)
	20	18	360	129	900.7	(870.8, 930.6)
	400	18	7200	133	862.6	(826.7, 898.5)[a]
Females						
	0	18	0	123	923.8	(898.8, 948.8)
	0.05	18	0.9	108	902.3	(868.9, 935.6)
	1	18	18	118	885.0	(856.0, 913.9)
	20	18	360	108	904.8	(877.6, 931.9)
	400	18	7200	113	794.9	(769.5, 820.3)[a]

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

Mean Survival of Causes of Death

Kaplan-Meier estimates of mean survival of the major causes of death with results of a log-rank test are shown in Table 4. There was no significant life shortening for malignant lymphoma as COD in all groups. In males exposed to 400 mGy/day, significant life shortening ($P < 0.01$) was observed only for liver neoplasms. For females exposed to 400 mGy/day, significant life shortening ($P < 0.01$) was observed for liver, lung, soft tissue, and pituitary gland neoplasms.

Neoplasm Incidence

All the neoplasms, fatal (caused death) and incidental, are listed in Table 5, classified according to organ/tissue (in alphabetical order) of origin. Overall, there was no significant differences in the incidence rates of neoplasms between the non-irradiated and low-dose irradiated groups.

Digestive system. There was a significant increase ($P < 0.001$) in the total incidence of neoplasms originating from the digestive tract in both sexes (35.4% in females, 67.7% in males) exposed to 400 mGy/day, mainly due to the significant ($P < 0.001$) increase in the number of neoplasms arising from the liver (34.5% in females and 66.9% in males). Hepatocellular adenoma incidence in females (21.2%, $P < 0.01$), but not in males (21.1%, n.s.), was significantly increased in the 400 mGy/day group. Incidence rates of hepatocellular carcinomas in both sexes (11.5% in females and 44.4% in males) exposed to 400 mGy/day were significantly increased ($P < 0.01$). Incidence rates for other neoplasms originating from the digestive system were low (<2%).

Endocrine system. A significant over-all increase ($P < 0.001$) in the incidence of endocrine neoplasms was observed in both sexes (85.0% in females and 30.1% in males) exposed to 400 mGy/day. The increase in female mice is attributed to neoplasms originating from the adrenal (15.9%, $P < 0.01$), pituitary [55.8%, $P < 0.01$, mostly adenomas of the pars distalis (54.0%, $P < 0.01$)] and the thyroid (12.4%, not statistically significant) glands. In males, the overall increase is attributed to the increases in the numbers of adrenal (9.0%, $P = 0.0737$) and thyroid (16.5%, $P = 0.0942$) neoplasms compared to the non-irradiated controls.

Reproductive system. An overall increase in the incidence rate of neoplasms originating from the reproductive tract (112.4 %, $P < 0.01$) in females exposed to 400 mGy/day due to significant increases in the numbers ovarian neoplasms mainly, tubulostromal adenomas (61.9%, $P < 0.01$) and malignant granulosa cell tumors (14.2%, $P < 0.01$). Solitary primary ovarian neoplasms were found in 75.22 % ($n = 85$) of the animals while 14.15% ($n = 16$) had 2 primary ovarian neoplasms. The remaining ovaries in 12 females with no neoplasms were either hypoplastic or cystic.

Testes of males in 400 mGy/day group were all hypoplastic. There was no significant increase in the number of neoplasms originating from the male reproductive tract was observed.

Harderian gland. Incidence rates for Harderian gland neoplasms were low (maximum of 9%) and were not significantly different between the non-irradiated controls and irradiated groups.

Hematopoietic System. A large majority of the neoplasms originated from the hematopoietic system, mostly malignant lymphomas and was highest in the non-irradiated controls (females = 54.5%; males = 39.2%), decreasing as the radiation dose increased. Incidence rates for malignant lymphoma were significantly decreased in both sexes (females = 29.2%, $P < 0.01$; males = 22.6%, $P < 0.01$) exposed to 400 mGy/day.

Respiratory system. Although overall incidence rates for respiratory neoplasms were not significantly different between LDR and nonirradiated controls. Incidence rate for males were higher than the females but significantly decreased as the radiation dose increased only in males

TABLE 3
Causes of Death in B6C3F1 Mice Continuously Exposed to Low- and Medium-Dose-Rate Gamma Rays for 18 Days In Utero

	Female									
	Non-irradiated 0 mGy[a] n = 123 (%)		0.05 mGy/day 0.9 mGy n = 108 (%)		1 mGy/day 18 mGy n = 118 (%)		20 mGy/day 360 mGy n = 108 (%)		400 mGy/day 7200 mGy n = 113 (%)	
Neoplasms	111	(90.2)	94	(87.0)	104	(88.1)	86	(79.6)	103	(91.2)
Digestive System										
Stomach										
Carcinoma, squamous cell										
Esophagus										
Carcinoma, squamous cell										
Liver	2	(1.6)	2	(1.9)	4	(3.4)	7	(6.5)	10	(8.8)
Adenoma, hepatocellular			1	(0.9)	2	(1.7)	2	(1.9)	2	(1.8)
Carcinoma, hepatocellular	1	(0.8)	1	(0.9)	1	(0.8)	3	(2.8)	7	(6.2)
Carcinoma, hepatocholangiocellular										
Cholangiocarcinoma					1	(0.8)			1	(0.9)
Hepatoblastoma	1	(0.8)								
Tumor, Ito cell, benign							2	(1.9)		
Endocrine system	8	(6.5)	7	(6.5)	10	(8.5)	2	(1.9)	20	(17.7)
Adrenal gland									2	(1.8)
Carcinoma, cortical									1	(0.9)
Tumor, medullary, benign									1	(0.9)
Tumor, medullary, malignant										
Pancreas	1	(0.8)			1	(0.8)				
Carcinoma, islet cell	1	(0.8)			1	(0.8)				
Pituitary Gland	7	(5.7)	7	(6.5)	9	(7.6)	1	(0.9)	18	(15.9) [b]
Adenoma, pars distalis	3	(2.4)	6	(5.6)	6	(5.1)			16	(14.2) [b]
Carcinoma, pars distalis	4	(3.3)	1	(0.9)	3	(2.5)	1	(0.9)	2	(1.8)
Carcinoma, pars intermedia										
Thyroid gland							1	(0.9)		
Adenoma, follicular cell							1	(0.9)		
Harderian Gland	1	(0.8)			1	(0.8)			1	(0.9)
Adenocarcinoma, harderian					1	(0.8)			1	(0.9)
Adenoma, harderian	1	(0.8)								
Hematopoietic system	64	(52.0)	57	(52.8)	59	(50.0)	51	(47.2)	28	(24.8) [b]
Leukemia, myeloid	1	(0.8)					1	(0.9)		
Lymphoma, malignant	60	(48.8)	52	(48.1)	56	(47.5)	48	(44.4)	28	(24.8) [b]
Sarcoma, histiocytic	3	(2.4)	5	(4.6)	3	(2.5)	2	(1.9)		
Thymoma										
Tumor, mast cell, malignant										
Integumentary system	3	(2.4)	1	(0.9)			2	(1.9)	1	(0.9)
Carcinoma, basal cell	2	(1.6)	1	(0.9)			1	(0.9)	1	(0.9)
Carcinoma, squamous cell	1	(0.8)					1	(0.9)		
Keratoacanthoma										
Melanoma										
Mammary gland	7	(5.7)	5	(4.6)	6	(5.1)	3	(2.8)	1	(0.9)
Adenocarcinoma, mammary	3	(2.4)	4	(3.7)	3	(2.5)	3	(2.8)	1	(0.9)
Adenoma, mammary	3	(2.4)	1	(0.9)	3	(2.5)				
Fibroadenoma, mammary	1	(0.8)								
Mesothelium									1	(0.9)
Mesothelioma, malignant									1	(0.9)
Nervous system					1	(0.8)	1	(0.9)		
Ependymoma, malignant										
Meningioma, malignant					1	(0.8)	1	(0.9)		
Reproductive system	2	(1.6)	3	(2.8)	1	(0.8)			16	(14.2) [b]
Uterus	2	(1.9)								
Adenocarcinoma, uterus	1	(0.8)								
Sarcoma, endometrial stromall, uterus	1	(0.8)								
Ovary			3	(2.8)	1	(0.8)			16	(14.2) [b]
Adenocarcinoma, tubulostromal									1	(0.9)
Carcinoma, yolk sac			2	(1.9)						
Choriocarcinoma									2	(1.8)
Tumor, granulosa cell, benign					1	(0.8)			2	(1.8)
Tumor, granulosa cell, malignant			1	(0.9)					11	(9.7) [b]

Continued on next page

TABLE 3
Extended.

Male									
Non-irradiated 0 mGy n = 120 (%)		0.05 mGy/day 0.9 mGy n = 121 (%)		1 mGy/day 18 mGy n = 111 (%)		20 mGy/day 360 mGy n = 129 (%)		400 mGy/day 7,200 mGy n = 133 (%)	
109	(90.8)	107	(88.4)	100	(90.1)	121	(93.8)	119	(89.5)
						1	(0.8)	1	(0.8)
						1	(0.8)		
						1	(0.8)		
								1	(0.8)
								1	(0.8)
32	(26.7)	28	(23.1)	26	(23.4)	40	(31.0)	57	(42.9) [b]
5	(4.2)	7	(5.8)	7	(6.3)	11	(8.5)	10	(7.5)
27	(22.5)	21	(17.4)	18	(16.2)	28	(21.7)	45	(33.8)
				1	(0.9)			2	(1.5)
						1	(0.8)		
		1	(0.8)	1	(0.9)			5	(3.8)
								2	(1.5)
								1	(0.8)
								1	(0.8)
								1	(0.8)
								1	(0.8)
		1	(0.8)	1	(0.9)			2	(1.5)
				1	(0.9)			2	(1.5)
		1	(0.8)						
				2	(1.8)				
				2	(1.8)				
44	(36.7)	46	(38.0)	40	(36.0)	41	(31.8)	27	(20.3) [b]
				1	(0.9)				
40	(33.3)	36	(29.8)	32	(28.8)	33	(25.6)	20	(15.0) [b]
3	(2.5)	10	(8.3)	7	(6.3)	7	(5.4)	7	(5.3)
						1	(0.8)		
1	(0.8)								
		2	(1.7)	2	(1.8)			2	(1.5)
		1	(0.8)	1	(0.9)				
								2	(1.5)
		1	(0.8)						
				1	(0.9)				
1	(0.8)					1	(0.8)	1	(0.8)
1	(0.8)					1	(0.8)	1	(0.8)
				1	(0.9)				
				1	(0.9)				

Continued on next page

TABLE 3
Continued.

	Female									
	Non-irradiated 0 mGy[a] n = 123 (%)		0.05 mGy/day 0.9 mGy n = 108 (%)		1 mGy/day 18 mGy n = 118 (%)		20 mGy/day 360 mGy n = 108 (%)		400 mGy/day 7200 mGy n = 113 (%)	
Respiratory system	2	(1.6)	1	(0.9)	3	(2.5)	3	(2.8)	6	(5.3)
Adenoma, bronchiolo-alveolar										
Carcinoma, bronchiolo-alveolar	2	(1.6)	1	(0.9)	3	(2.5)	3	(2.8)	6	(5.3)
Skeletal system and teeth	2	(1.6)	1	(0.9)	2	(1.7)			1	(0.9)
Osteoma	1	(0.8)								
Osteosarcoma	1	(0.8)	1	(0.9)	2	(1.7)			1	(0.9)
Soft tissue	14	(11.4)	12	(11.1)	12	(10.2)	10	(9.3)	12	(10.6)
Fibroma										
Fibrosarcoma	11	(8.9)	9	(8.3)	5	(4.2)	9	(8.3)	5	(4.4)
Leiomyosarcoma					1	(0.8)			1	(0.9)
Myxosarcoma	1	(0.8)			1	(0.8)			1	(0.9)
Rhabdomyosarcoma	1	(0.8)	2	(1.9)	3	(2.5)			1	(0.9)
Sarcoma, NOS									1	(0.9)
Schwannoma, benign									1	(0.9)
Schwannoma, malignant	1	(0.8)	1	(0.9)	2	(1.7)	1	(0.9)	2	(1.8)
Special sense organs										
Adenoma, Zymbal's gland										
Urinary system									1	(0.9)
Carcinoma, renal tubule									1	(0.9)
Vascular	6	(4.9)	5	(4.6)	5	(4.2)	7	(6.5)	5	(4.4)
Hemangioma, vascular			1	(0.9)			4	(3.7)	2	(1.8)
Hemangiosarcoma, vascular	6	(4.9)	4	(3.7)	5	(4.2)	3	(2.8)	3	(2.7)
Inflammation	7	(5.7)	5	(4.6)	5	(4.2)	9	(8.3)		
Digestive system										
Heart			2	(1.9)	2	(1.7)	1	(0.9)		
Liver	1	(0.8)					1	(0.9)		
Mesothelium							1	(0.9)		
Nervous system	1	(0.8)			1	(0.8)				
Respiratory system	3	(2.4)	2	(1.9)	2	(1.7)	4	(3.7)		
Teeth	1	(0.8)								
Systemic	1	(0.8)	1	(0.9)			2	(1.9)		
Urinary system										
Vascular										
Others	2	(1.6)	3	(2.8)	5	(4.2)	8	(7.4)	3	(2.7)
Digestive system					1	(0.8)	3	(2.8)		
Heart										
Liver							1	(0.9)	1	(0.9)
Reproductive system	1	(0.8)	2	(1.9)	2	(1.7)	2	(1.9)	1	(0.9)
Respiratory system					1	(0.8)			1	(0.9)
Skeletal system and teeth										
Dental dysplasia (incisor teeth)										
Malocculsion										
Systemic	1	(0.8)	1	(0.9)						
Urinary system		(0.0)			1	(0.8)	2	(1.9)		
Unknown	3	(2.4)	6	(5.6)	4	(3.4)	5	(4.6)	6	(5.3)

(19.5%, $P < 0.01$) exposed to 400 mGy/day and is due to decreases in the incidences of both bronchiolo-alveolar adenoma (11.3%, $P < 0.01$) and carcinoma (8.3%, n.s.).

Other organs/tissues. Incidence rates for neoplasms originating from the mesothelium, skin, soft tissues, urinary system, vascular system and the Zymbal gland were low and were not significantly different between the non-irradiated controls and irradiated groups.

Multiple primary neoplasms. The frequencies of multiple primary neoplasms are shown in Table 6. Over 91.7% of the animals died with more than 1 primary neoplasm with a few animals having as many as 6 neoplasms. A significant increase in the average number of primary neoplasms was observed only in female mice (3.11, $P < 0.01$) exposed to MDR of 400 mGy/day in utero with almost half (42.5 %) of the animals having 3 neoplasms each. The maximum number of 6 primary neoplasms were observed in female mice exposed to 1 mGy/day (n = 1, 0.8%) and 400 mGy/day (n = 5, 4.4%), and in 1 (0.8%) male mouse exposed to 400 mGy/day.

TABLE 3
Extended. Continued.

Male									
Non-irradiated 0 mGy n = 120 (%)		0.05 mGy/day 0.9 mGy n = 121 (%)		1 mGy/day 18 mGy n = 111 (%)		20 mGy/day 360 mGy n = 129 (%)		400 mGy/day 7,200 mGy n = 133 (%)	
8	(6.7)	9	(7.4)	10	(9.0)	15	(11.6)	5	(3.8)
								1	(0.8)
8	(6.7)	9	(7.4)	10	(9.0)	15	(11.6)	4	(3.0)
1	(0.8)	1	(0.8)	1	(0.9)	1	(0.8)	1	(0.8)
1	(0.8)	1	(0.8)	1	(0.9)	1	(0.8)	1	(0.8)
9	(7.5)	5	(4.1)	6	(5.4)	5	(3.9)	4	(3.0)
1	(0.8)								
4	(3.3)	3	(2.5)	5	(4.5)	5	(3.9)		
1	(0.8)			1	(0.9)				
								1	(0.8)
								2	(1.5)
3	(2.5)	2	(1.7)					1	(0.8)
								1	(0.8)
								1	(0.8)
14	(11.7)	15	(12.4)	11	(9.9)	17	(13.2)	15	(11.3)
6	(5.0)	4	(3.3)	3	(2.7)	2	(1.6)	3	(2.3)
8	(6.7)	11	(9.1)	8	(7.2)	15	(11.6)	12	(9.0)
2	(1.7)	6	(5.0)	3	(2.7)	2	(1.6)	5	(3.8)
		1	(0.8)	1	(0.9)			1	(0.8)
		1	(0.8)						
		1	(0.8)						
1	(0.8)	1	(0.8)						
		1	(0.8)	1	(0.9)	2	(1.6)	1	(0.8)
				1	(0.9)			1	(0.8)
1	(0.8)							2	(1.5)
		1	(0.8)					1	(0.8)
3	(2.5)	4	(3.3)	3	(2.7)	4	(3.1)	2	(1.5)
		1	(0.8)						
		1	(0.8)						
						2	(1.6)		
2	(1.7)			1	(0.9)			1	(0.8)
2	(1.7)								
				1	(0.9)				
1	(0.8)	2	(1.7)	2	(1.8)	1	(0.8)	1	(0.8)
						1	(0.8)		
6	(5.0)	4	(3.3)	5	(4.5)	2	(1.6)	8	(6.0)

[a] Total dose (for 18 days).
[b] $P < 0.01$ (Fisher's exact test).

DISCUSSION

Reproductive parameters. The results of the breeding parameters in the present study are similar to that previously reported, such as average number of implantation sites/dam (*14, 20*), and average number of pups born/litter (*14*), and are comparable across all matching dose groups, non-irradiated control and irradiated (20 mGy/day and 400 mGy/day). As in a previous study (*14*), the significant reduction in mean litter size of dams (average number of pups born/litter) alongside the significant increase in the number of resorbed fetuses exposed to MDR of 400 mGy/day, indicates significant fetal loss between post implantation (from GD 6) and birth. Overall, the average weaning rates in the present study were higher in all dose groups compared to a previous report (*14*) wherein the group exposed to MDR of 400 mGy/day had an exceptionally low-average

TABLE 4
Kaplan-Meier Estimates of Mean Survival of Major Causes of Death in B6C3F1 Mice

	Dose rate (total dose)	Non-irradiated (0 mGy)	0.05 mGy/day (0.9 mGy)	1 mGy/day (18 mGy)	20 mGy/day (360 mGy)	400 mGy/day (7,200 mGy)
Male						
All causes of death						
Number of mice		120	121	111	129	133
Mean lifespan (days ± SE)		935.3 ± 16.1	948.9 ± 15.4	941.9 ± 18.0	900.7 ± 15.1	862.6 ± 18.1[b]
All fatal neoplasms	Incidence (%)	109 (90.8)	107 (88.4)	100 (90.1)	121 (93.8)	119 (89.5)
	Mean survival	947.6 ± 17.0	966.7 ± 16.6	960.2 ± 18.8	911.0 ± 15.4	892.7 ± 16.6
Lymphoma, malignant	Incidence (%)	40 (33.3)	36 (29.8)	32 (28.8)	33 (25.6)	20 (15.0)[a]
	Mean survival	1105.1 ± 20.9	1107.6 ± 18.3	1152.1 ± 25.8	1088.2 ± 19.4	1118.5 ± 17.9
Liver neoplasms	Incidence (%)	32 (26.7)	28 (23.1)	26 (23.4)	40 (31.0)	57 (42.9)[a]
	Mean survival	1086.0 ± 17.4	1202.7 ± 29.3	1168.0 ± 23.6	1042.3 ± 18.6	988.0 ± 18.8[b]
Lung neoplasms	Incidence (%)	8 (6.7)	9 (7.4)	10 (9.0)	15 (11.6)	5 (3.8)
	Mean survival	1262.3 ± 19.3	1197.6 ± 12.5	1204.3 ± 14.8	1128.3 ± 14.4	1120.1 ± 8.7
Soft tissue neoplasms	Incidence (%)	9 (7.5)	5 (4.1)	6 (5.4)	5 (3.9)	4 (3.0)
	Mean survival	1127.4 ± 11.7	1309.1 ± 19.8	1152.3 ± 10.0	1128.1 ± 7.6	886.7 ± 3.9
Vascular neoplasms	Incidence (%)	14 (11.7)	15 (12.4)	11 (9.9)	17 (13.2)	15 (11.3)
	Mean survival	1112.31 ± 11.0	1178.3 ± 16.0	1027.8 ± 7.5	1209.8 ± 27.4	1109.8 ± 13.8
Non-neoplastic lesions	Incidence (%)	5 (4.2)	10 (8.3)	6 (5.4)	6 (4.7)	7 (5.3)
	Mean survival	1115.8 ± 6.7	1272.2 ± 19.8	1130.8 ± 8.6	1157.7 ± 9.4	1060.3 ± 7.2
Female						
All causes of death						
Number of mice		123	108	118	108	113
Mean survival (days)		923.8 ± 12.6	902.3 ± 16.8	885.0 ± 14.6	904.8 ± 13.7	794.9 ± 12.8[b]
All fatal neoplasms	Incidence (%)	111 (90.2)	94 (87.0)	104 (88.1)	86 (79.6)	103 (91.2)
	Mean survival	935.3 ± 13.1	922.7 ± 17.9	906.9 ± 13.8	935.8 ± 14.2	806.7 ± 13.1[b]
Lymphoma, malignant	Incidence (%)	60 (48.8)	52 (48.1)	56 (47.5)	48 (44.4)	28 (24.8)[a]
	Mean survival	993.9 ± 15.5	1021.6 ± 22.0	979.0 ± 18.1	1013.7 ± 19.3	933.9 ± 13.4
Liver neoplasms	Incidence (%)	2 (1.6)	2 (1.9)	4 (3.4)	7 (6.5)	10 (8.8)
	Mean survival	1088.5 ± 3.5	1105.1 ± 2.7	1155.8 ± 12.8	972.3 ± 4.2	903.1 ± 6.3[b]
Lung neoplasms	Incidence (%)	2 (1.6)	1 (0.9)	3 (2.5)	3 (2.8)	6 (5.3)
	Mean survival	1184.6 ± 8.8	946.0 ± 0	1050.8 ± 5.4	945.4 ± 2.3	1060.0 ± 13.9[b]
Soft tissue neoplasms	Incidence (%)	14 (11.4)	12 (11.1)	12 (10.2)	10 (9.3)	12 (10.6)
	Mean survival	1171.3 ± 21.2	1143.2 ± 17.6	1021.0 ± 6.6	1116.2 ± 14.9	909.5 ± 7.5[b]
Pituitary Gld. neoplasms	Incidence (%)	7 (5.7)	7 (6.5)	9 (7.6)	1 (0.9)	18 (15.9)[a]
	Mean survival	1087.7 ± 7.3	1135.7 ± 9.8	990.8 ± 3.9	870.0 ± 0	965.8 ± 14.0[b]
Ovary Neoplasms	Incidence (%)	0	3 (2.8)	1 (0.8)	0	16 (14.2)[a]
	Mean survival	-	1011.8 ± 7.5	890.0 ± 0	-	1048.7 ± 24.4[b]
Vascular neoplasms	Incidence (%)	6 (4.9)	5 (4.6)	5 (4.2)	7 (6.5)	5 (4.4)
	Mean survival	920.9 ± 3.2	1049.5 ± 6.3	974.6 ± 4.3	1066.7 ± 8.7	903.0 ± 5.8
Non-neoplastic lesions	Incidence (%)	9 (7.3)	8 (7.4)	10 (8.5)	17 (15.7)	3 (2.7)
	Mean survival	1214.6 ± 22.5	1164.8 ± 15.1	1155.4 ± 19.6	1143.6 ± 24.1	952.6 ± 5.5

[a] $P < 0.01$ (Fisher's exact test).
[b] $P < 0.01$ (log-rank test).

weaning rate. This difference in weaning parameters may be attributed to batch differences and the larger number of litters examined in the present study (44 vs. 23 litters). Analyses of the reproductive parameters in the present study show that in utero LDR γ-ray exposures of 0.05, 1.0 and 20 mGy/day from GD 0-18 were not significantly different from the non-irradiated controls.

Body weight and growth retardation. The difference in the pattern of body weight gain between sexes was striking in pups exposed to 400 mGy/day in utero where male pups appeared to ''catch-up'' from 3 to 7 weeks of age but showed slower and smaller gains in weight thereafter (persistent growth retardation). Female pups similarly exposed however, continued to gain weight at a rate faster than the non-irradiated controls and the LDR groups until 87 weeks of age, after which they started to lose weight progressively, until body weight monitoring was discontinued at 187 weeks of age. It is interesting to note that this pattern of weight loss in females exposed to 400 mGy/day is similar to the females in the lifespan study (*22*) exposed to 20 mGy/day.

Our previous reports (*14, 20*) on in utero exposures showed similar results where GD18 pups exposed to MDRs of 200 and 400 mGy/day had significantly lower body weights than the non-irradiated controls that prevailed (persistent growth retardation) only in male pups until the end point at 10 weeks of age (*14*). The same report (*14*) also showed that while the pups (both sexes) exposed to MDRs appeared to ''catch-up'' in growth, observed as an increase

in body weight, the organ weights (absolute and relative) at 10 weeks of age showed that this increase was due to increased fat deposition. Sreetharan et al. (*27*) reported similar reductions in body weights of male C57Bl/6J mice exposed to a total of 1,000 mGy at HDRs in utero. Jauhari et al. (*28*) exposed pregnant Swiss mice to 800 mGy (at both high- and low-dose rates) of gamma rays on day 18 post conception resulted in a biphasic weight loss in male offspring but a monophasic weight loss in female offspring. Coppenger et al. (*29*) reported similar results in rats exposed to 50 r daily during the entire gestation period, wherein males were sterile and gained weight slower, whereas females demonstrated higher weight gains, than the non-irradiated controls and attributed this to the ''castrated'' condition in females. Rugh and Wohlfromn (*30*) attributed the decrease in body weight to radiation-induced cell death, resulting in growth retardation as a permanent consequence of prenatal radiation exposure (*31*). In our previous studies (*14, 20*), small GD18 fetuses exposed to MDRs of 200 and 400 mGy/day for the entire gestation period also had placentas that were smaller in size and weight (unpublished data) but preliminary histopathological examination did not reveal any abnormalities.

In human infants, fetal growth restriction (FGR)/ in utero growth restriction/retardation (IUGR) and short/small for gestational age (SGA) are often used interchangeably in clinical practice even though they are not synonymous (*32–35*), the differences, however, are beyond the scope of this work (and these terms will be used interchangeably in this paper for convenience). Regardless of etiology (*32, 36*), FGR/IUGR or SGA cause a spectrum of both short- (*37*) and long-term complications (*38–40*), many of which are amplified by postnatal weight gain (*41, 42*) or spontaneous catch-up growth (*35*) requiring different interventions or treatment (*34*) and monitoring/surveillance duration (*33*). Associated morbidities and severity also depend on the onset of placental dysfunction and gestation age at birth and with the degree of severity dependent on numerous factors (such as dose, dose-rate and gestation age at the time of exposure in the case of in utero exposure.

It is also possible that the vascular damage (*43*), caused by chronic MDR exposure, may lead to placental insufficiency that ultimately results in FGR. At high-dose exposures to the human uterus, such as those used in cancer therapy, increased risks of unfavorable pregnancy and neonatal outcomes [e.g. premature birth, low-birth weights, small for gestational age (SGA) and in some cases perinatal mortality] has been attributed to radiation-induced uterine damage (*44, 45*). Most studies in humans show that obesity is associated with elevated estradiol both in men (*46*) and in women after menopause (*47*). Similar findings were reported by Gulay et al. (*14*) in 10-week-old males and females exposed MDR of 400 mGy/day and in females exposed to MDR of 200 mGy/day where significant increases in fat deposits (based on relative organ weights) were observed. Although we did not observe FGR/IUGR in mice exposed to a LDR of 20 mGy/day, hypogonadism, associated with IUGR in both humans and animals regardless of etiology, with significantly greater prevalence in obese human males (*46*), may need to be considered as a late effect. Evaluation and long-term management of FGR/IUGR and/or SGA that result from in utero exposure must take into consideration these long-term consequences, in addition to any secondary effects, that may result from hypogonadism and other hormone imbalances that affect the growth of the fetus, including cancer. Further investigation is needed to determine whether in utero radiation exposure is additive or synergistic to the effects of FGR/IUGR or SGA.

Nakahira et al. (*20*) confirmed that the gonadal hypoplasia observed at 10 weeks of age by Gulay et al. (*14*) in pups exposed in utero to 400 mGy/day was present at GD 18 and that the lesion was irreversible/permanent (no recovery observed in adults). In the present study, we also confirmed (data not shown) gonadal hypoplasia histopathologically in all male pups exposed in utero to 400 mGy/day.

The disparity in the weight gain between sexes in the pups exposed in utero to 400 mGy/day suggest that there are other contributing factors and/or mechanisms that affect growth, or as in this study, weight gain, other than gonadal hypoplasia, particularly in males. Factors, or a combination of several (possibly additive and/or synergistic), include disruptions (of the normal hormonal feedback system) in the hypothalamic-pituitary-gonadotropin axis in both the irradiated dam and pups exposed in utero, transgenerational effects as a phenotypic outcome of developmental programming (also known as Barker's hypothesis) resulting from maternal stress (in this case, radiation exposure), which are fetal sex and temporal specific (*48*), during pregnancy (*27*), cell killing in embryonic/fetal organs and tissues as a direct result of radiation exposure and radiation-induced uterine damage (*44, 45*). Gender differences in response to chronic low dose exposure have not been investigated in detail, but it has been suggested that the differences may be due to intrinsic differences in gametogenesis such as differences in the duration of meiosis and age at exposure (*49*). Nakamura et al. (*50*) showed that female B6C3F1 mice exposed continuously to a low-dose rate of 20 mGy/day for 400 days had significantly increased body weights due to adiposity accompanied by increased serum leptin and liver lipid content, but with no increase in feed consumption.

Life span. No life span shortening was observed in pups exposed to low-dose rates of 0.05,1 and 20 mGy/day, and this may be attributed to the very low-dose rates and very low-total-accumulated doses over the short irradiation period of 18 days. At 400 mGy/day however, the life span was significantly shorter in both males and females since the total accumulated dose over the 18-day in utero exposure was much higher at 7,200 mGy (7.2 Gy). It is interesting to note that the mean lifespans (862.6 days in

TABLE 5
Incidence of Neoplasms in B6C3F1 Mice Continuously Exposed to Low- and Medium-Dose-Rate Gamma Rays for 18 Days In Utero

	Female									
	Non-irradiated 0 mGy[a] n = 123 (%)		0.05 mGy/day 0.9 mGy n = 108 (%)		1 mGy/day 18 mGy n = 118 (%)		20 mGy/day 360 mGy n = 108 (%)		400 mGy/day 7,200 mGy n = 113 (%)	
Bone and tooth	2	(1.6)	1	(0.9)	2	(1.7)			2	(1.8)
Ossifying fibroma										
Osteoma	1	(0.8)								
Osteosarcoma	1	(0.8)	1	(0.9)	2	(1.7)			2	(1.8)
Central nervous system					1	(0.8)	2	(1.9)		
Ependymoma, malignant										
Meningioma, malignant					1	(0.8)	2	(1.9)		
Digestive system	12	(9.8)	11	(10.2)	12	(10.2)	16	(14.8)	40	(35.4)[b]
Intestine	1	(0.8)			2	(1.7)			1	(0.9)
Adenoma, duodenum	1	(0.8)			2	(1.7)				
Adenocarcinoma, ileum									1	(0.9)
Liver	11	(8.9)	11	(10.2)	10	(8.5)	16	(14.8)	39	(34.5)[b]
Adenoma, hepatocellular	8	(6.5)	7	(6.5)	8	(6.8)	8	(7.4)	24	(21.2)[b]
Adenoma, hepatochlangiocellular										
Carcinoma, hepatocellular	2	(1.6)	4	(3.7)	1	(0.8)	5	(4.6)	13	(11.5)[b]
Carcinoma, hepatocholangiocellular										
Cholangiocarcinoma					1	(0.8)			1	(0.9)
Hepatoblastoma	1	(0.8)					1	(0.9)	1	(0.9)
Tumor, Ito cell, benign							2	(1.9)		
Stomach										
Carcinoma, squamous cell										
Tumor, neuroendocrine cell, malignant										
Endocrine system	57	(46.3)	47	(43.5)	60	(50.8)	37	(34.3)	96	(85.0)[b]
Adrenal Gld.	3	(2.4)	4	(3.7)	1	(0.8)	2	(1.9)	18	(15.9)[b]
Adenoma, cortical							1	(0.9)	4	(3.5)
Adenoma, subcapsular cell			2	(1.9)	1	(0.8)			5	(4.4)
Carcinoma, cortical									1	(0.9)
Tumor, medullary, benign	3	(2.4)	2	(1.9)			1	(0.9)	7	(6.2)
Tumor, medullary, malignant									1	(0.9)
Pancreas	1	(0.8)			1	(0.8)	1	(0.9)	1	(0.9)
Adenoma, islet cell							1	(0.9)	1	(0.9)
Carcinoma, islet cell	1	(0.8)			1	(0.8)				
Parathyroid Gld.							1	(0.9)		
Adenoma							1	(0.9)		
Pituitary Gld.	37	(30.1)	26	(24.1)	35	(29.7)	22	(20.4)	63	(55.8)[b]
Adenoma, pars distalis	32	(26.0)	25	(23.1)	31	(26.3)	18	(16.7)	61	(54.0)[b]
Adenoma, pars intermedia	1	(0.8)			1	(0.8)	2	(1.9)		
Carcinoma, pars distalis	4	(3.3)	1	(0.9)	3	(2.5)	2	(1.9)	2	(1.8)
Carcinoma, pars intermedia										
Thyroid Gld.	16	(13.0)	17	(15.7)	23	(19.5)	11	(10.2)	14	(12.4)
Adenoma, C-cell			1	(0.9)						
Adenoma, follicular cell	16	(13.0)	16	(14.8)	23	(19.5)	11	(10.2)	14	(12.4)
Carcinoma, follicular cell										
Female Reproductive System	28	(22.8)	18	(16.7)	20	(16.9)	18	(16.7)	127	(112.4)[b]
Mammary Gld.	16	(13.0)	10	(9.3)	8	(6.8)	6	(5.6)	9	(8.0)
Adenoacanthoma, malignant					1	(0.8)				
Adenoma	9	(7.3)	5	(4.6)	4	(3.4)	2	(1.9)	2	(1.8)
Adenocarcinoma	6	(4.9)	5	(4.6)	3	(2.5)	4	(3.7)	7	(6.2)
Fibroadenoma	1	(0.8)								
Ovary	6	(4.9)	7	(6.5)	9	(7.6)	10	(9.3)	117	(103.5)[b]
Adenoma, tubulostromal	2	(1.6)			4	(3.4)	3	(2.8)	78	(69.0) [b]
Adenocarcinoma, tubulostromal									5	(4.4)
Carcinoma, yolk sac			2	(1.9)						
Choriocarcinoma									3	(2.7)
Cyst adenoma	2	(1.6)	2	(1.9)	3	(2.5)	4	(3.7)		
Luteoma, benign			1	(0.9)					2	(1.8)
Teratoma, benign			1	(0.9)			1	(0.9)	1	(0.9)
Thecoma, benign									2	(1.8)

Continued on next page

TABLE 5
Extended.

Male									
Non-irradiated 0 mGy n = 120 (%)		0.05 mGy/day 0.9 mGy n = 121 (%)		1 mGy/day 18 mGy n = 111 (%)		20 mGy/day 360 mGy n = 129 (%)		400 mGy/day 7,200 mGy n = 133 (%)	
2	(1.7)	2	(1.7)	1	(0.9)	1	(0.8)	1	(0.8)
1	(0.8)	1	(0.8)						
1	(0.8)	1	(0.8)	1	(0.9)	1	(0.8)	1	(0.8)
				1	(0.9)				
				1	(0.9)				
58	(48.3)	51	(42.1)	47	(42.3)	60	(46.5)	90	(67.7) [b]
				2	(1.8)	1	(0.8)		
				1	(0.9)	1	(0.8)		
				1	(0.9)				
58	(48.3)	50	(41.3)	45	(40.5)	58	(45.0)	89	(66.9) [b]
27	(22.5)	22	(18.2)	20	(18.0)	22	(17.1)	28	(21.1)
		1	(0.8)						
31	(25.8)	26	(21.5)	24	(21.6)	35	(27.1)	59	(44.4) [b]
				1	(0.9)			2	(1.5)
						1	(0.8)		
		1	(0.8)						
		1	(0.8)			1	(0.8)	1	(0.8)
						1	(0.8)	1	(0.8)
		1	(0.8)						
16	(13.3)	14	(11.6)	13	(11.7)	13	(10.1)	40	(30.1) [b]
4	(3.3)	3	(2.5)	3	(2.7)	3	(2.3)	12	(9.0)
1	(0.8)	1	(0.8)						
2	(1.7)					2	(1.6)	8	(6.0)
								1	(0.8)
1	(0.8)	2	(1.7)	3	(2.7)	1	(0.8)	2	(1.5)
								1	(0.8)
1	(0.8)							1	(0.8)
1	(0.8)								
								1	(0.8)
		2	(1.7)	3	(2.7)	1	(0.8)	5	(3.8)
				3	(2.7)			5	(3.8)
		1	(0.8)			1	(0.8)		
		1	(0.8)						
11	(9.2)	9	(7.4)	7	(6.3)	9	(7.0)	22	(16.5)
						1	(0.8)	1	(0.8)
11	(9.2)	9	(7.4)	6	(5.4)	8	(6.2)	21	(15.8)
				1	(0.9)				

Continued on next page

TABLE 5
Continued.

	Female									
	Non-irradiated 0 mGy[a] n = 123 (%)		0.05 mGy/day 0.9 mGy n = 108 (%)		1 mGy/day 18 mGy n = 118 (%)		20 mGy/day 360 mGy n = 108 (%)		400 mGy/day 7,200 mGy n = 113 (%)	
Tumor, granulosa cell, benign	1	(0.8)			2	(1.7)	2	(1.9)	6	(5.3)
Tumor, granulosa cell, malignant	1	(0.8)	1	(0.9)					18	(15.9) [b]
Tumor, sertoli cell, malignant									1	(0.9)
Tumor, sex cord stromal, mixed, benign									1	(0.9)
Uterus	6	(4.9)	1	(0.9)	3	(2.5)	2	(1.9)	1	(0.9)
Adenoma, uterus	1	(0.8)							1	(0.9)
Adenocarcinoma, uterus	1	(0.8)			1	(0.8)	1	(0.9)		
Papilloma, squamous cell, uterus	1	(0.8)								
Polyp, endometrial stromal	1	(0.8)			1	(0.8)	1	(0.9)		
Polyp, glandular	1	(0.8)	1	(0.9)	1	(0.8)				
Sarcoma, endometrial stromal, uterus	1	(0.8)								
Harderian Gld.	7	(5.7)	7	(6.5)	8	(6.8)	5	(4.6)	4	(3.5)
Adenoma	7	(5.7)	7	(6.5)	8	(6.8)	5	(4.6)	4	(3.5)
Adenocarcinoma	1	(0.8)			1	(0.8)			1	(0.9)
Hematopoietic system	71	(57.7)	61	(56.5)	70	(59.3)	55	(50.9)	33	(29.2)[b]
Leukemia, myeloid	1	(0.8)					1	(0.9)		
Lymphoma, malignant	67	(54.5)	55	(50.9)	67	(56.8)	52	(48.1)	33	(29.2)[b]
Sarcoma, histiocytic	3	(2.4)	6	(5.6)	3	(2.5)	2	(1.9)		
Thymoma										
Tumor, mast cell, malignant										
Male reproductive system										
Adenocarcinoma, prostate gld										
Adenoma, Leydig cell, testis										
Mesothelium									1	(0.9)
Mesothelioma, malignant									1	(0.9)
Respiratory system	20	(16.3)	14	(13.0)	16	(13.6)	24	(22.2)	20	(17.7)
Adenoma, bronchiolo-alveolar	11	(8.9)	11	(10.2)	10	(8.5)	16	(14.8)	10	(8.8)
Carcinoma, bronchiolo-alveolar	9	(7.3)	3	(2.8)	6	(5.1)	8	(7.4)	10	(8.8)
Skin	3	(2.4)	2	(1.9)			3	(2.8)	1	(0.9)
Carcinoma, basal cell	2	(1.6)	1	(0.9)			1	(0.9)	1	(0.9)
Carcinoma, squamous cell	1	(0.8)					1	(0.9)		
Keratoacanthoma			1	(0.9)			1	(0.9)		
Melanoma										
Soft tissue	18	(14.6)	17	(15.7)	13	(11.0)	12	(11.1)	15	(13.3)
Fibroma			3	(2.8)						
Fibrosarcoma	13	(10.6)	10	(9.3)	6	(5.1)	9	(8.3)	7	(6.2)
Leiomyoma			1	(0.9)			1	(0.9)		
Leiomyosarcoma	2	(1.6)			1	(0.8)			1	(0.9)
Liposarcoma										
Myxosarcoma	1	(0.8)			1	(0.8)			2	(1.8)
Rhabdomyosarcoma	1	(0.8)	2	(1.9)	3	(2.5)			1	(0.9)
Sarcoma, NOS									1	(0.9)
Schwannoma, benign									1	(0.9)
Schwannoma, malignant	1	(0.8)	1	(0.9)	2	(1.7)	1	(0.9)	2	(1.8)
Tumor, granular cell, benign							1	(0.9)		
Urinary system			1	(0.9)					1	(0.9)
Adenoma, renal tubule										
Carcinoma, renal tubule			1	(0.9)					1	(0.9)
Vascular	12	(9.8)	7	(6.5)	14	(11.9)	18	(16.7)	10	(8.8)
Hemangioma	5	(4.1)	3	(2.8)	7	(5.9)	11	(10.2)	7	(6.2)
Hemangiosarcoma	7	(5.7)	4	(3.7)	7	(5.9)	7	(6.5)	3	(2.7)
Zymbal's Gld.										
Adenoma										

TABLE 5
Extended. Continued.

Male									
Non-irradiated 0 mGy n = 120 (%)		0.05 mGy/day 0.9 mGy n = 121 (%)		1 mGy/day 18 mGy n = 111 (%)		20 mGy/day 360 mGy n = 129 (%)		400 mGy/day 7,200 mGy n = 133 (%)	
5	(4.2)	14	(11.6)	9	(8.1)	7	(5.4)	12	(9.0)
5	(4.2)	14	(11.6)	9	(8.1)	7	(5.4)	12	(9.0)
				3	(2.7)	1	(0.8)	1	(0.8)
51	(42.5)	51	(42.1)	46	(41.4)	42	(32.6)	37	(27.8)
				1	(0.9)				
47	(39.2)	39	(32.2)	38	(34.2)	34	(26.4)	30	(22.6)[b]
3	(2.5)	10	(8.3)	7	(6.3)	7	(5.4)	7	(5.3)
		1	(0.8)			1	(0.8)		
1	(0.8)	1	(0.8)						
1	(0.8)	3	(2.5)	4	(3.6)	5	(3.9)	4	(3.0)
						1	(0.8)		
1	(0.8)	3	(2.5)	4	(3.6)	4	(3.1)	4	(3.0)
1	(0.8)					1	(0.8)	1	(0.8)
1	(0.8)					1	(0.8)	1	(0.8)
60	(50.0)	62	(51.2)	56	(50.5)	73	(56.6)	26	(19.5) [b]
32	(26.7)	31	(25.6)	24	(21.6)	39	(30.2)	15	(11.3) [b]
28	(23.3)	31	(25.6)	32	(28.8)	34	(26.4)	11	(8.3)
2	(1.7)	3	(2.5)	2	(1.8)			2	(1.5)
		1	(0.8)	1	(0.9)				
								2	(1.5)
2	(1.7)	1	(0.8)						
		1	(0.8)	1	(0.9)				
13	(10.8)	9	(7.4)	8	(7.2)	7	(5.4)	7	(5.3)
2	(1.7)			1	(0.9)	1	(0.8)	2	(1.5)
5	(4.2)	5	(4.1)	6	(5.4)	5	(3.9)		
1	(0.8)			1	(0.9)				
								1	(0.8)
		1	(0.8)					2	(1.5)
1	(0.8)							1	(0.8)
1	(0.8)								
3	(2.5)	3	(2.5)			1	(0.8)	1	(0.8)
3	(2.5)	1	(0.8)	2	(1.8)	2	(1.6)	3	(2.3)
3	(2.5)			2	(1.8)	1	(0.8)	3	(2.3)
		1	(0.8)			1	(0.8)		
21	(17.5)	18	(14.9)	24	(21.6)	28	(21.7)	21	(15.8)
13	(10.8)	6	(5.0)	9	(8.1)	6	(4.7)	7	(5.3)
8	(6.7)	12	(9.9)	15	(13.5)	22	(17.1)	14	(10.5)
								1	(0.8)
								1	(0.8)

[a] Total dose (for 18 days).
[b] $P < 0.01$ (Fisher's exact test, two-sided).

TABLE 6
Frequencies of Multiple Primary Neoplasms in B6C3F1 Mice Continuously Exposed to Low- and Medium-Dose-Rate Gamma Rays for 18 Days In Utero

	Female									
	Non-irradiated 0 mGy	(%)	0.05 mGy/day 0.9 mGy	(%)	1 mGy/day 18 mGy	(%)	20 mGy/day 360 mGy	(%)	400 mGy/day 7200 mGy	(%)
Number of mice (n)	123		108		118		108		113	
Number of neoplasms										
Average number/mouse	1.88		1.72		1.84		1.76		3.11[a]	
One or more	120	(97.6)	103	(95.4)	113	(95.8)	99	(91.7)	111	(98.2)
1	47	(38.2)	45	(41.7)	47	(39.8)	40	(37.0)	6	(5.3)
2	46	(37.4)	38	(35.2)	37	(31.4)	36	(33.3)	22	(19.5)
3	20	(16.3)	15	(13.9)	22	(18.6)	15	(13.9)	48	(42.5)
4	3	(2.4)	5	(4.6)	6	(5.1)	7	(6.5)	23	(20.4)
5	4	(3.3)					1	(0.9)	7	(6.2)
6					1	(0.8)			5	(4.4)

males; 794.9 days in females) and rates of lifespan shortening (7.8% in males and 14.0% in females) of mice exposed in utero to 400 mGy/day, were comparable to the results of life span study (*21*) in B6C3F1 mice exposed to 20 mGy/day for 400 days [total dose 8 Gy; (mean life spans = 812.0 days in males; 740.9 days in females) and (rates of lifespan shortening = 11.03% in males; 13.9% in females)] despite the large difference in the number of mice examined as well as a shift in the causes of death and neoplasm incidence rates as discussed below.

Neoplasm incidence. Carcinogenic effects are often seen in animal studies when radiation exposure occurs during the late stages of fetal development (*6*). Our results show no significant differences in the incidence rates of neoplasms that caused death among the non-irradiated controls and those exposed in utero to LDRs of 0.05, 1 and 20 mGy/day in either sex.

At MDR exposure, a shift, based on incidence rates, in the major COD was observed in males, from malignant lymphomas at LDRs to liver neoplasms, with significant life span shortening (Table 2). This shift towards liver neoplasms is likely due to the genetic predilection of male B6C3F1 mice for developing liver neoplasms, that is inherited as a dominant trait from its C3H sire (*25*), in combination with in utero exposure to 400 mGy/day of gamma rays resulting in both initiation (increased incidence) and progression (shorter life spans). Although not statistically significant, Sasaki et al. (*51*) showed a similar increase in liver neoplasms in $B6WF_1$ males exposed to 200R of X rays at 16–18 days post coitus (dpc) compared to the non-irradiated controls whereas those exposed at 12 dpc did not develop any liver neoplasms at all. Nitta et al. (*52*) reported similar an overall increase in liver neoplasms incidence in both male and female B6C3F1 mice acutely exposed in utero on GD 16.5 to neutrons (Cf: 1 Gy), but not in mice similarly exposed to gamma rays (Co: 1 and 2.7 Gy).

In females exposed to 400 mGy/day, there was a huge reduction (50.8%) in the rate at which malignant lymphomas caused death, and shifted to an increase in the incidence of neoplasms with endocrine function originating from the pituitary (with significant lifespan shortening) and the ovaries. Using B6C3F1 females exposed in utero to 3.8 Gy of gamma rays, Sasaki (*10*) reported similar increases in the neoplasm incidence rates originating from the liver, pituitary and the ovaries when exposed during the late fetal stage (day 17 post coitus) but did speculate on possible pathogenetic mechanisms. Acute in utero exposure to neutrons (Cf: 1Gy) and gamma-rays (Co: 2.7 Gy) on GD 16.5 has also been shown to increase the incidence of pituitary and mammary gland tumors (*52*) in female B6C3F1 mice. In the current study these shift in neoplasm incidence rates could also be related to the some of the same factors that contribute to the differences in growth (weight gain) such as disruptions in the normal hormonal feedback system of the hypothalamic-pituitary-gonadotropin axis in both the irradiated dam and pups exposed in utero. The hormonal insufficiency/imbalance resulting from ovarian hypoplasia is also a huge factor that contributes to the increase in the incidence of ovarian neoplasms.

It is also of interest to note the absence of any significant change in the incidence rates for Harderian gland neoplasms among the non-irradiated, LDR- and MDR-in-utero-irradiated groups in the present study. Although it might be reasonable to assume that at LDRs the total accumulated dose is too low to induce Harderian gland tumors, there was no increase incidence in the MDR group despite the relatively high total accumulated dose at 7.2 Gy suggesting other factors may influence its development and sensitivity to radiation exposure.

The over-all incidence rates of endocrine neoplasms in both sexes exposed to 400 mGy in utero were significantly increased compared to the non-irradiated controls. When classified based on organ of origin, only neoplasms originating from the adrenals, pituitary and ovaries were significantly increased in females and this may be partly related to the hormone imbalance brought about by gonadal hypoplasia and disruptions (of the normal hormonal

TABLE 6
Extended.

Male									
Non-irradiated 0 mGy	(%)	0.05 mGy/day 0.9 mGy	(%)	1 mGy/day 18 mGy	(%)	20 mGy/day 360 mGy	(%)	400 mGy/day 7200 mGy	(%)
120		121		111		129		133	
1.94		1.88		1.95		1.86		1.86	
119	(99.2)	119	(98.3)	107	(96.4)	128	(99.2)	126	(94.7)
40	(33.3)	44	(36.4)	40	(36.0)	51	(39.5)	42	(31.6)
50	(41.7)	45	(37.2)	35	(31.5)	48	(37.2)	56	(42.1)
24	(20.0)	26	(21.5)	23	(20.7)	23	(17.8)	22	(16.5)
4	(3.3)	4	(3.3)	8	(7.2)	6	(4.7)	4	(3.0)
1	(0.8)			1	(0.9)			1	(0.8)
								1	(0.8)

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

feedback system) in the hypothalamic-pituitary-gonadotropin axis. Intrinsic differences in the hormone regulation in the endocrine system between sexes may account for some of the differences in neoplastic incidence rates.

In the present study, no change in tumor spectra was observed in mice exposed to both LDRs and MDRs in utero. The average tumor burden, reflected as the number of multiple primary tumors, however, was significantly increased only in females exposed in utero to MDR of 400 mGy/day despite the shorter lifespan due to increased incidence rates of neoplasms originating from liver, pituitary and the ovaries.

None of the neoplasms observed in the present study correspond to what may be considered as childhood cancer (often affecting children at ages 0 to 14 years). Stewart et al. (*53*) was first to report on childhood cancers resulting from prenatal diagnostic and assessment X-ray exposure in 1956, but a follow-up of the Hiroshima and Nagasaki atomic bomb survivors exposed in utero (n = 1,630) reported only 2 childhood cancers without a single case of leukemia (*54*). In the present study, we found only 3 animals with myeloid leukemia, 2 females (non-irradiated and 20 mGy/day) and 1 male (1 mGy/day). While ICRP (*55*) concluded that evidence for solid tumors, particularly childhood brain cancer, was not strong due to lack of evidence of increased risk in cohort studies (atomic bomb survivors) as well as the unusual homogeneity of the relative risk of all childhood cancers in the Oxford survey of childhood cancers, NCRP (*56*) stated that in utero exposure maybe of concern. Although an analysis of epidemiological studies on prenatal x-ray exposure to about 10 mGy show a consistent relative risk of 1.4 for childhood cancer, the individual probability for childhood cancer would be very low since the background incidence is so low (about 0.2-0.3%) (*5*). Based on absolute risks for childhood cancer deaths exposed to 1,000 mGy in utero has been estimated to be around 0.06% per 10 mGy or equivalent to 1 cancer death per 1,700 children exposed to 10 mGy in utero (*5*). Uncertainties remain regarding the contribution of in utero diagnostic radiology studies to leukemia induction until its mechanism is understood (*3*).

A number of studies (*57, 58*) report neurocognitive deficits and behavioral changes (*58*) such as aggressive behavior (*57*) in several strains of mice as well as in rats (*59–61*) exposed acutely to high doses of radiation at various gestation ages. Structural changes in the cortical layer of juvenile rats with behavioral changes suggest that long-term effects of fetal exposure are not only due to cell loss during development but also due to an overall perturbation of regulatory systems responsible for growth and development (*61*). The standard twice daily cage-side observations in the resent study failed to detect neither abnormal neurobehavioral symptoms (data not shown) nor histo-morphological changes (histopathology) in the brains of non-irradiated nor irradiated mice. Behavioral testing is beyond the scope and objectives of present study design as this would require a separate group of animals, space and manpower. Studies on the effect(s) of in utero exposures to chronic low dose rates of radiation on behavior and neurocognitive changes are needed.

Biological response(s) to radiation exposure, particularly its chronic and late effects, should be examined with a holistic approach and at whole organism level (*62*) as it is more complex than the LNT relationship between health risk and dose where radiation always induces gene mutations in direct proportion to radiation dose (*63*). The possibility that the same low-dose radiation that induces epigenetic mechanisms that contribute to cancer induction, also induces a hormetic response (*63*). In vitro studies show that low doses may not induce efficient repair of double-strand breaks (*64–65*). While the current report focuses on the effect of in utero radiation exposure on life span, cause of death and neoplasm incidences in mice, other responses to radiation exposure such as DNA damage repair, bystander effects, and, non-neoplastic diseases, and their

contribution to life span shortening and cause of death require further investigation.

It is also necessary to differentiate the effects of in utero radiation exposure from the heritable effects that result from radiation exposure of germ cells (oocytes or spermatocytes) that cause chromosomal aberrations and/or mutations in genes themselves (*66*). Our previous reports (*14, 20*) on in utero exposures at MDRs of 200 and 400 mGy/day have shown severe hypoplasia of both the ovaries and testes in GD 18 fetuses and at 10 weeks of age (no recovery = irreversible), indicating that these mice are sterile and therefore it is reasonable to assume that any radiation injury incurred at these doses will not be passed on to future progeny/generations. As mentioned in the results, all males exposed to 400 mGy/day in utero had testicular hypoplasia and a large majority of females had ovarian neoplasms with a few having more than one primary ovarian neoplasm. Nakahira et al. (*20*) reported a slight, but not statistically significant, decrease in germ cell counts in mice exposed in utero to a LDR of 20 mGy/day. Further investigation is required to determine whether the surviving germ cells in these mice exposed in utero to LDRs (20 mGy/day and below) carry heritable defects that could be passed on to future progeny/generations.

The results of the present work show that in utero exposure of mice to LDRs (0.05, 1 and 20 mGy/day) for the entire gestation period (GD 0 to 18) did not significantly alter any of the parameters (life span, cause of death, neoplasm incidence) examined when compared to the non-irradiated controls suggest that the effect on the irradiated embryo/fetus and the pregnant dam (uterine physiology) may be too small to be detected statistically. As mentioned, our previous life span study (*22*), the absence of an observable effect in the present in utero exposure study, particularly at LDR exposures of 20 mGy/day, does not mean that increased risk does not exist (*67*), since the probability of observing any effect as statistically significant would be limited by the magnitude of the effect and the sample size. In the previous in utero exposure study (LDR = 20 mGy/d; MDR = 200 and 400 mGy/d; and an acute high dose rate 0.77/Gy/min) (*14*), several parameters showed significant trends (e.g., increasing post-implantation loss; decreasing body sizes, weaning rates, body and organ weights, etc.) as the dose-rate increased. In the present study, there was no significant trend in the mean life spans within the LDR group (data not shown).

Extrapolation of the current results to in utero exposures in humans should take into consideration the dose rates and the total accumulated dose the mice were exposed to over the entire gestation period of 18 days as compared to the length of the human gestation period of 280 days. Total doses at LDRs of 0.05 and 1 mGy/day were still very low at 0.9 and 18 mGy, respectively. At the LDR of 20 mGy/day however, the total dose of 360 mGy for the entire gestation period exceeds 200 mGy, the maximum dose considered by UNSCEAR (1986, 1993) (*67*) as low dose.

Further studies however are necessary to determine whether or not chronic in utero radiation exposure to low dose and low dose rates will affect future reproductive capabilities of in utero exposed mice and their exposed dams (effect on succeeding pregnancies, life span, neoplasia and non-neoplastic diseases).

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