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Life-Span Carcinogenicity Studies on Sprague–Dawley Rats Exposed to γ-Radiation: Design of the Project and Report on the Tumor Occurrence After Post-Natal Radiation Exposure (6 Weeks of Age) Delivered in a Single Acute Exposure

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Background *Experimental long-term carcinogenicity bioassays conducted on rats and mice proved that ionizing radiation can induce a variety of tumor types. However few studies have been conducted on rats.*

Methods *This report deals with the effects of γ-radiation in groups of 416–1,051 6-weeks old Sprague–Dawley rats exposed to 0, 0.1, 1, or 3 Gy of γ-radiation delivered in a single acute exposure. The experiment lasted for the animals' lifespan and all were necropsied and underwent full histopathological evaluation.*

Results *The results confirm the dose-related carcinogenic effects of γ-radiation for several organs and tissues. Moreover they indicate that exposure to 0.1 Gy induces a statistically significant increased incidence in Zymbal gland carcinomas and pancreas islet cell carcinomas in females.*

Conclusions *Our data show that exposure to γ-radiation induces carcinogenic effects at all tested doses.* Am. J. Ind. Med. 58:46–60, 2015. © 2014 Wiley Periodicals, Inc.

KEY WORDS: life-span bioassay; rat; carcinogenicity; γ-radiation; low-dose

INTRODUCTION

Most of the recently reported epidemiological research on the relationship between ionizing radiation and cancer has concerned moderate-high-dose (0.5–1 Gy) whole-body exposure. However because low dose (defined as $\leq$0.1 Gy) or protracted exposure are of major interest when it comes to protecting people from ionizing radiation, the research has started to be more focused on occupational cohorts, in particular workers at nuclear plants, or persons/patients exposed to multiple diagnostic radiological examination.

Epidemiological studies have shown that exposure to doses (>0.1 Gy) increased the risk of developing both solid cancers and leukemias among survivors of Hiroshima and Nagasaki [Preston et al., 2007], as well as among radiologists and physicians specialized in interventive medicine [Linet et al., 2010], workers in the nuclear industry [Cardis et al., 2007].

Some unresolved questions include: (1) differences in risk between acute and chronic exposure [Jacob et al., 2009]; (2) effects at low doses and the appropriateness of the linear no threshold model [Mullenders et al., 2009; Averbeck, 2010]; (3) what cancer effects, if any, may be the result of

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preconception exposure or in utero exposure [Barber and Dubrova, 2006; Abouzeid Ali et al., 2012]; (4) identification of possible radio-sensitive subgroups in the population and how these subgroups can be identified [Jeggo, 2010]; (5) the possible interaction between ionizing radiation and other well-known carcinogenic agents or potential diffuse carcinogenic risks (e.g., chemical agents, extremely low frequency magnetic fields of power energy, or radiofrequency electromagnetic fields from cell phones).

To answer these questions, related in particular to the effects of low doses, more adequate data is still needed. To face this challenge, researchers use both epidemiological and experimental studies. However, it is quite understandable that when dealing with low-exposure scenarios (such as those of 0.1 Gy or less) with multiple confounding factors, epidemiological studies may lack sufficient statistical power to detect cancer risks. In these cases mechanistic and in vivo animal studies can help epidemiology to better evaluate the carcinogenic risk of low dose exposure.

Experimental studies conducted on mice and rats have proved that ionizing radiation can cause a variety of tumor types in both species. In a series of mouse studies [IARC, 2000, 2012] conducted using a large number of animals per group and sex exposed to either X-rays or to γ-rays with a range of doses and dose rates, it was observed an increased incidence of several types of neoplasms, including in particular myeloid leukemia and thymic lymphoma, ovarian, pituitary and Harderian gland tumors, lung and mammary carcinomas.

Despite the interest in the effects of ionizing radiation in mice, few studies were conducted on rats and, among them, the majority were focused on the study of mammary carcinogenesis. Rats are the most suitable animal species for studying mammary carcinogenesis by radiation since they are responsive and develop very similar tumors to their human counterpart. The responsiveness in developing mammary tumors depends on the strain and Sprague–Dawley rats are the most susceptible [Russo and Russo, 1996].

Studies conducted on female Sprague–Dawley rats showed an increased incidence of mammary cancers after exposure to X-ray dose over the range 0.25–6 Gy [Shellabarger et al., 1957, 1966; Bond et al., 1960]. It has been reported that in female Sprague–Dawley rats neutrons at a single dose as low as 0.1 Gy produced a significant increase in mammary tumors [Shellabarger et al., 1980]. Subsequent studies conducted on Sprague–Dawley rats and as well as other strains confirmed the strong carcinogenic effects of ionizing radiation on the mammary gland [Shellabarger et al., 1982; Broerse et al., 1986, 1987; Bartstra et al., 1998].

The experimental carcinogenic bioassays on rats conducted to date has their own limitations: the small size of animal groups, the duration of the study, which is all too often arbitrarily truncated, not to mention the lack of details of pathology. Indeed long-term carcinogenicity bioassays on rodents, when well-planned and conducted using adequate animal models (as close as possible to the human equivalent tumor type incidences may provide better indications as to the carcinogenic effects which can be extrapolated to humans for risk assessment [Huff, 1999; Maltoni et al., 1999]).

Based on previous experience in conducting large-scale carcinogenicity bioassays, a project of long-term experiments on rats exposed to γ-radiation in different situations and at various dose levels have been conducted at the laboratories of the Cesare Maltoni Cancer Research Center of the Ramazzini Institute (CMCRC/RI) located in the province of Bologna, Italy, where this type of research has been performed since the early 1970s [Soffritti et al., 1999, 2002].

Overall, the project comprises four large scale experiments:

(1) the main experiment which studies the effects at three dose levels (0.1, 1, and 3 Gy) plus controls, delivered in a single acute dose or in 10 doses (once every 4 weeks) to 4,016 six-week-old (starting age) male and female rats;
(2) a second experiment, which studies the effects on male and female offspring of three doses at 0.1, 0.5, and 1 Gy delivered in one acute dose to pregnant dams, irradiated on the 12th day of pregnancy (2,799 rats);
(3) a third experiment, which studies the effects on male and female offspring of three doses 0.1, 1, and 3 Gy delivered in a single acute dose to male breeders before mating (2,557 rats);
(4) and finally a fourth experiment, which studies the effects of standard feed, administered after irradiation by 1 or 4 kGy, to pregnant dams from the 12th day of pregnancy, and then to their male and female offspring until spontaneous death (1,139 animals).

These four experiments started concurrently and the experimental animals were those born during the breeding of 4,000 rats (2,000 males plus 2,000 females).

The main experiment includes one control group which is common to all four experiments.

THE CARCINOGENIC EFFECTS OF EXPOSURE OF 6-WEEK-OLD SPRAGUE-DAWLEY RATS TO γ-RADIATION DELIVERED IN A SINGLE ACUTE EXPOSURE (MAIN EXPERIMENT)

This report deals with results in terms of overall carcinogenic effects in three groups of 416–1,046 male and female rats from the main experiment which exposed Sprague–Dawley rats at 6 weeks of age to three different dose levels of γ-radiation (0.1, 1, 3 Gy) delivered in a single acute exposure. One group of 1,051 males and females served as control. The highest dose level was selected on the basis of

data available in the literature. The lowest dose was chosen as representative of human scenarios of high exposure to ionizing radiation in various workplaces or during radiation therapy. Also 0.1 Gy is the lowest experimental dose in the mouse studies [Ullrich and Storer, 1979a,b].

MATERIALS AND METHODS

γ-Radiation Exposure Conditions and Facility

The irradiation facility consisted of a telecobalt therapy unit of the "Theratron 780" type (Fig. 1), that utilized Co60 as its radiation source, with an activity of about 56 TBq (1,500 Ci). The irradiation procedure was carried out inside a properly shielded irradiation room (bunker), constructed of reinforced concrete and was 5 m × 4 m × 3 m in size. The control board and exposure monitoring facilities were located in a room next to the bunker.

The "Theratron 780" unit was equipped with a turntable made of a 1 cm thick perspex sheet (size 48 cm × 48 cm). The turntable has a pneumatic system for 180° rotation on its axis and is positioned 120 cm away from the radiation source. The system is controlled from outside the bunker and allows animals to be irradiated ventrally and dorsally at two different times. In order to obtain an evaluation of the irradiation beam effect on the whole body, it proved necessary to administer a uniform radiation dose inside the animal. This result was achieved by placing the animals in different positions relative to the source. To keep them from moving during exposure, animals were locked in special perspex holders, with 1 cm thick walls, 20.8 cm long × 6.5 cm broad, placed on the turntable. The area used was 46 cm × 46 cm in size and made it possible to radiate up to 10 animals at the same time, with an absorbed dose rate of about 0.21 Gy/min.

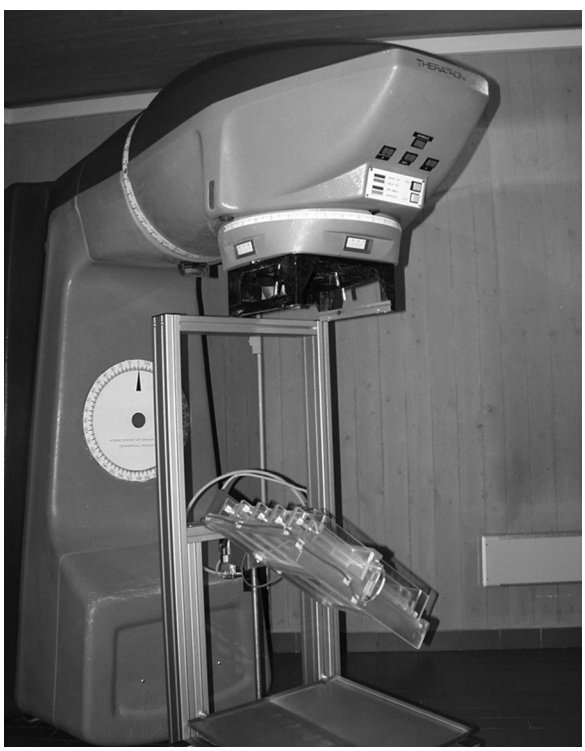

FIGURE 1. Exposure system.

To measure the dose administered, a Nuclear Enterprise dosimeter type 2571A was used, with a 0.6 cc graphite ionization chamber, calibrated in terms of dose absorbed to water with 4% uncertainty. The dosimeter, irradiated together with the animals, was permanently fitted to the under-side of the turntable, located along the axis of the beam. The dose readings in the two positions (ventrally and dorsally) showed uniformity in the absorbed dose with the maximum differences between the various parts of the body in the order of a few percent (3–5%).

Possible sources of operator error in carrying out treatment may be incorrect selection of irradiation times, or non-rotation of the turntable after the first part of treatment. To ensure correct execution of the treatment, two checks were devised: (1) the dosimeter, irradiated together with the animals, was permanently fixed to the turntable; (2) this dosimeter was located along the axis of the beam, on the under-side of the turntable about 1 cm from its rotation axis, so that dose readings in the two positions must differ by more than 2%.

Animals

Strain

The animals used were Sprague–Dawley rats from the same colony as used for more than 40 years in the laboratories of the CMCRC/RI. The basic expected tumorigenesis and its fluctuations were based upon data deriving from more than 18,000 historical controls.

Generation of experimental animals

The generation of experimental animals took place as follows: (a) inbred breeders were distributed in four groups and the offspring of these were assigned to the respective groups; (b) randomization by body weight of the inbred brother and sister among the four breeder groups was as homogenous as possible; (c) mating of the breeders that generated the off-spring for the experiment was strictly outbred (made possible by pedigree identification number of each animal) in each breeder group and lasted 72 hr; (d) the size of breeder groups was dictated by the number of offspring required; (e) in order to evaluate the family effect in the carcinogenic process, the experimental groups included

all the offspring of each litter; (f) all the male and female breeders were sacrificed 6 weeks after birth and 1 week after the weaning of the offspring, respectively.

The animals of all experiments were weaned, weighted, separated by sex and identified by ear punch at 5 weeks of age.

Conduct of the experiment

The animals were kept in highly standardized environmental conditions. They were housed 5 per cage, in makrolon cages with a solid top in stainless steel. A shallow layer of white wood fir-tree shavings served as bedding. The animals were kept in a temperature-controlled laboratory at 21–24°C, with relative humidity at 40–60% and 12-hr light/dark alternation. The experiment was conducted in accordance with Italian law regulating the use and human treatment of animals for scientific purposes [Decreto Legislativo, 1992].

After weaning, animals received food and water ad libitum. The food was purchased from "Mangimificio COMER" (Bologna), which is the same as used in the CMCRC/RI for more than 40 years, while the water came from the local water supply. Water and feed were periodically analyzed to monitor for the presence of possible contaminants.

Animals were kept under observation until natural death (life-span). The daily consumption of feed and water were measured in a sample of 100 animals (50 males and 50 females) from each group and each experiment from the age of 6 weeks every 4 weeks until 110 weeks of age. Body weight was recorded from the age of 6 weeks every 4 weeks until 110 weeks of age, then every 8 weeks until the end of the experiment. The health and behavior of the animals were controlled three times daily throughout the experiment. Checks for pathological lesions, including mammary tumors (the most frequent lesion in females), were performed every 2 weeks throughout the experiment. Animals with lumps greater than 3 cm in diameter were isolated from other animals and individually caged to avoid cannibalization. The development of the lumps/masses was monitored until spontaneous death. A systematic gross examination was performed and all pathological changes recorded.

For histopathological examination, in addition to macroscopically observed pathological lesions (with a margin of surrounding normal tissue), the following tissues and organs were taken: skin, subcutaneous tissue, mammary gland, brain, pituitary gland, Zymbal gland, salivary glands, Harderian glands, cranium, tongue, thyroid and parathyroid, pharynx, larynx, thymus, trachea, lung, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands, oesophagus, stomach (fore and glandular), intestine (four levels), bladder, prostate, uterus, ovaries, testes, interscapular fat pad and subcutaneous, mediastinal and mesenteric lymph nodes.

All specimens were fixed in 70% alcohol, except for bones and other tissues of a bone-like consistency which were fixed in 10% formalin. The trimming was performed according to the CMCRC/RI Standard Operating Procedures (SOP). All pathological tissues were trimmed in order to maintain a border of normal tissue around the lesions. With regard to normal tissue and organs: (1) the decalcified cranium was trimmed at five levels from nose to occipital bone, including oral and nasal cavities and internal ear ducts; (2) parenchymal organs were dissected through the hilus to expose the widest surface; and (3) hollow organs were sectioned across the greatest diameter(s). Trimmed specimens were processed as paraffin blocks, and 3–5 μm sections of every specimen were obtained. Sections were routinely stained with hematoxylin and eosin (H&E). Histopathological evaluation of the lesions, in particular benign and malignant tumors, was performed according to the National Toxicology Program criteria [Boorman et al., 1990]. The evaluation was performed by the same group of pathologists on all tissues and organs. The same supervisor reviewed all lesions of oncological interest. All pathologists used the same evaluation and classification criteria.

Statistical Analysis

Cox proportional hazard models were used to estimate the relative risk of malignant tumors as a function of dose. For benign tumors logistic regression was used with age as a covariate. The risk model used was:

$$\lambda(t, D) = \lambda(t, 0)\exp\{f(D)\}$$

where λ (t, D) is the hazard rate, t is the age of the animal in days at death, D is the dose and $\exp\{f(D)\}$ is the relative risk. For estimating the relative risk of the individual dose group D_i ($i = 1$, 2, or 3) we used $f(D) = \sum a_i D_i$ with D_i equal to 1 for group i and 0 for the other two. For modeling the relative risk as a function of dose the model used was:

$$\lambda(t, D) = \lambda(t, 0)\{1 + aD + bD^2\}$$

with the estimates of a and b restricted to being non-negative. The computer package used for the data analysis was Egret. In using the standard Cox proportional hazard analysis competing risks and differential survival were thus taken into account. A dose group with reduced survival may have a greater estimated hazard rate than controls yet a smaller incidence of cases. Details can be found in the biostatistical textbook of Hosmer and Lemeshow [1999].

RESULTS

During the experiment, no noticeable alteration of the behavior or health of the animals in the various groups was observed. Evaluation of the probability of survival in males

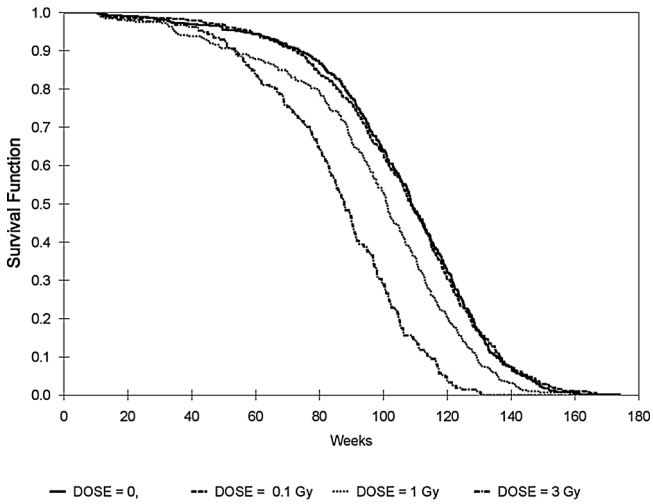

FIGURE 2. Survival male Sprague–Dawley rats.

and females exposed to γ-radiations compared to controls is shown as Kaplan–Meier curves (Figs. 2 and 3). The hazard ratios and median survival times in males and females are presented in Table I. The data showed that no change in survival for either sex was observed among animals exposed to 0.1 Gy compared to controls.

Multiple tumors of differing type and site, of differing type at the same site, of the same type in bilateral organs, of the same type in the skin, in subcutaneous tissue, in mammary glands, or at distant sites of diffuse tissue (e.g., bones and skeletal muscles) were counted as single/independent tumors. Multiple tumors of the same type in the same tissue and organ, apart from those listed above, were counted only once.

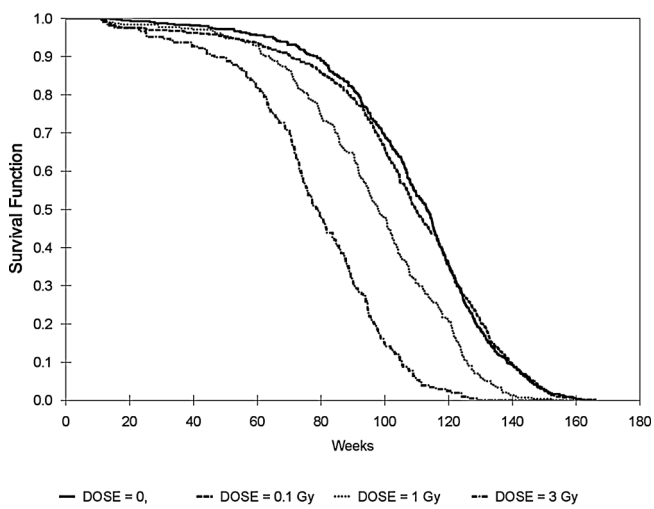

FIGURE 3. Survival female Sprague–Dawley rats.

TABLE I. Hazard Ratios and Median Survival Time in Male (M) and Female (F) Sprague–Dawley Rats

Group no. (Gy)	Animals[a] sex, no		Hazard ratio		Median survival (days)	
	M	F	M	F	M	F
I (0)	514	537	Reference		760	792
II (0.1)	524	522	0.99	1.01	760	767
III (1)	318	301	1.39**	1.74**	707	685
IV (3)	211	205	2.73**	4.38**	613	551

*Statistically significantly different from 1.00 at $P \leq 0.01$ by Cox Proportional Hazard analysis.
[a]Six weeks old at the time of exposure.

Significant oncological results are reported in Tables II–VII. Because there were differences in survival between the control group and the 1 and 3 Gy exposure groups of males and females, statistical tests adjusted for differences in survival were applied to the pairwise and trend analysis. Historical control data on specific spontaneous tumor incidences regarding 2,180 and 2,189 male and female offspring recorded during the period 1984–1997 were also considered. Moreover, the large number of animals per sex and per group allowed us to express more sharply the variation effects and indeed to raise the accuracy and statistical sensitivity of the study, reducing the likelihood of getting false positive results.

Benign and Malignant Tumors

Overall benign and malignant tumor incidence is reported in Table II. A significant dose-related increased incidence of animals bearing benign tumors occurred both in males and females ($P \leq 0.01$). Significant increased incidences ($P \leq 0.01$) were observed in males and females exposed to 1 and 3 Gy compared to controls. An increased number of total benign tumors per 100 animals occurred in males and females exposed to 1 and 3 Gy compared to the controls and to the lowest treated group. A significant dose-related increased incidence ($P \leq 0.01$) of animals bearing malignant tumors occurred in males and females. Significant increased incidences were observed in males and females exposed to 3 Gy ($P \leq 0.01$) compared to the controls. A sharp increase in the total number of malignant tumors per 100 animals occurred in males and females treated at 3 Gy as compared to controls. The statistically significant differences in males and females bearing benign or malignant tumors exposed to 1 and 3 Gy, as compared to controls, are due to the reduced survival in the two treated groups. This explains the still statistically significant increased tumor incidences and the greater estimated cumulative hazard rate (Figs. 4 and 5)

TABLE II. Benign and Malignant Tumors in Male (M) and Female (F) Sprague–Dawley Rats

			Benign tumors				Malignant tumors			
	Animals[a]		Tumor-bearing animals		Tumors[b]		Tumor-bearing animals		Tumors[b]	
Group no. (Gy)	Sex	No.	No.	%	No.	Per 100 animals	No.	%	No.	Per 100 animals
I (0)	M	514	320	62.3##	612	119.1	241	46.9##	294	57.2
	F	537	439	81.7##	1264	235.4	280	52.1##	404	75.2
	M + F	1051	759	72.2##	1876	178.5	521	49.6##	698	66.4
II (0.1)	M	524	331	63.2	685	130.7	253	48.3	339	64.7
	F	522	420	80.5	1320	252.9	280	53.6	431	82.6
	M + F	1046	751	71.8	2005	191.7	533	51.0	770	73.6
III (1)	M	318	202	63.5**	416	130.8	157	49.4**	208	65.4
	F	301	253	84.0**	851	282.7	142	47.2**	229	76.1
	M + F	619	455	73.5**	1267	204.7	299	48.3**	437	70.6
IV (3)	M	211	142	67.3**	366	173.5	156	73.9**	225	106.6
	F	205	173	84.4**	628	306.3	136	66.3**	223	108.8
	M + F	416	315	75.7**	994	238.9	292	70.2**	448	107.7

**Significantly greater than control ($P \leq 0.01$).
##Near the control incidence are the P values ($P \leq 0.01$) associated with Cox Proportional Hazard Model for the analysis of the trend.
[a]Six weeks old at the time of exposure.
[b]One animal can bear more than one tumor.

in the two exposed groups in spite of their incidences being reduced.

Cancers of the Skin, Subcutis Liposarcomas, and Breast

The occurrence of malignant tumors of the skin, subcutis liposarcomas and breast are reported in Table III. A dose-related increased incidence ($P \leq 0.01$) of malignant tumors of the skin was observed in males and females compared to controls. In males exposed to 3 Gy, the incidences of basocellular and squamous cell carcinomas were both significantly increased ($P \leq 0.01$) compared to male controls. A dose-related increased incidence of animals bearing subcutis liposarcomas was observed in males ($P \leq 0.01$) and in females ($P \leq 0.05$). Among the males exposed to 3 Gy the incidence was significantly increased ($P \leq 0.01$) compared to male controls.

The incidences of animals bearing breast adenocarcinomas and sarcomas (encompassing fibrosarcomas, liposarcomas, hemangiosarcomas, and osteosarcomas) show a significant dose-related increase ($P \leq 0.01$) in males and females for both adenocarcinomas and sarcomas. When compared to the untreated controls, the incidence of adenocarcinomas in males and females exposed to 1 or 3 Gy were significantly increased ($P \leq 0.01$) in both sexes. When compared to controls, the incidence of sarcomas of all types were significantly increased in males and females ($P \leq 0.01$) exposed to 3 Gy. At 1 Gy, the incidence of sarcomas was significantly increased in males ($P \leq 0.01$) and also in females ($P \leq 0.05$). When females bearing atypical lesions of mammary glands were aggregated with females bearing mammary adenocarcinomas, a significant increased incidence ($P < 0.05$) was also observed at 0.1 Gy, compared to controls (Table IV). The increase in mammary carcinomas plus their atypical precursor lesions was extremely marked and confirms our first results [Soffritti et al., 1999].

It is, of course, well known that intraductal atypical hyperplasia of the mammary gland is a precursor of carcinomas

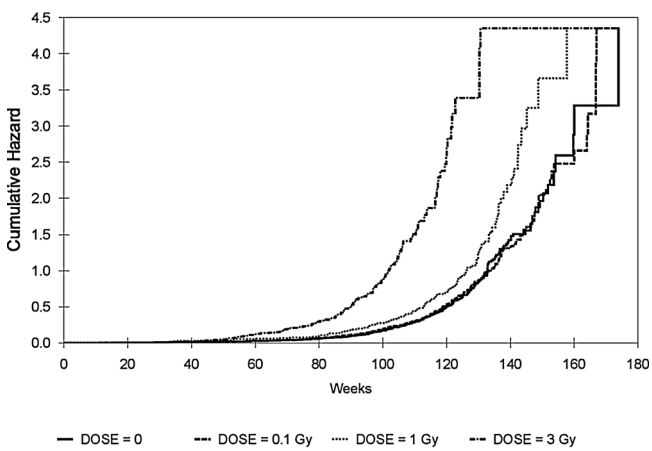

FIGURE 4. Cumulative hazard for total malignant tumors male Sprague–Dawley rats.

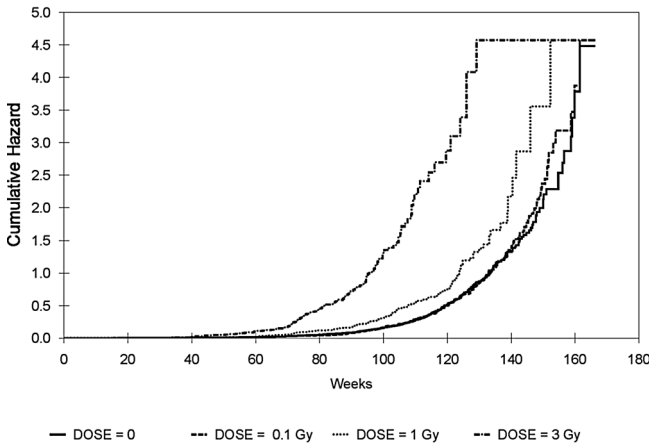

FIGURE 5. Cumulative hazard for total malignant tumors female Sprague–Dawley rats.

in rats [Russo and Russo, 2004]. As described by the WHO, this lesion is characterized by morphological changes that include altered architecture and abnormalities in cytology and differentiation [WHO—OMS—IARC, 2003]. The relative risk of developing a mammary cancer among patients with atypical ductal hyperplasia is 4–5 times higher, as reported by the Cancer Committee of the College of American Pathologists [Fitzgibbons et al., 1998]. For these reasons the aggregation of animals bearing atypical mammary lesions with animals bearing carcinomas is justified to show the significant increased risk of mammary neoplasms in female rats exposed to 0.1 Gy.

Head and Neck, Thyroid Gland, and Parathyroid Tumors

The incidence of tumors of head and neck, including thyroid and parathyroid, are reported in Table V. In females a significant dose-related increased incidence ($P \leq 0.05$) of Zymbal gland carcinomas was observed; the incidence of carcinomas was significantly increased in females exposed to 3 Gy and in males exposed to 0.1 Gy ($P \leq 0.01$), the lowest exposure dose. When males and females were aggregated, the incidence of carcinomas increased significantly at 0.1 Gy ($P \leq 0.05$). Zymbal glad carcinomas are not frequent in Sprague–Dawley rats of our colony. Out of 2,180 untreated male historical controls, the overall incidence was 1.7% (range: 0.0–3.0%).

Significant increased incidence of ear duct carcinomas was observed in females exposed to 3 Gy ($P \leq 0.05$) compared to the controls.

A dose-related increased incidence of nasal cavities carcinomas was observed in males ($P \leq 0.01$) and specifically

TABLE III. Cancers of the Skin, Subcutis, and Breast in Male (M) and Female (F) Sprague–Dawley Rats

			Tumor-bearing animals							
							Breast cancers			
	Animals[a]		Skin cancers		Subcutis liposarcomas		Adenocarcinomas		Sarcomas[b]	
Group no. (Gy)	Sex	No.	No.	%	No.	%	No.	%	No.	%
I (0)	M	514	6	1.2##	5	1.0##	3	0.6##	2	0.4##
	F	537	2	0.4##	0	—#	76	14.2##	7	1.3##
	M + F	1051	8	0.8##	5	0.5##	79	7.5##	9	0.9##
II (0.1)	M	524	6	1.1	9	1.7	3	0.6	8	1.5
	F	522	3	0.6	0	—	78	14.9	14	2.7
	M + F	1046	9	0.9	9	0.9	81	7.7	22	2.1*
III (1)	M	318	5	1.6	3	0.9	7	2.2**	14	4.4**
	F	301	4	1.3	1	0.3	86	28.6**	3	1.0*
	M + F	619	9	1.5	4	0.6**	93	15.0**	17	2.7**
IV (3)	M	211	9	4.3**	15	7.1**	7	3.3**	9	4.3**
	F	205	3	1.5**	2	1.0	91	44.4**	4	2.0**
	M + F	416	12	2.9**	17	4.1**	98	23.6**	13	3.1**

*Significantly greater than controls ($P \leq 0.05$), **($P \leq 0.01$). #Near the control incidence are the P values ($P \leq 0.05$) or ##($P \leq 0.01$) associated with Cox Proportional Hazard Model for the analysis of the trend.

[a]Six weeks old at the time of exposure.

[b]Fibrosarcomas, liposarcomas, hemangiosarcomas, and osteosarcomas.

TABLE IV. Animals Bearing Mammary Adenocarcinomas or Their Atypical Precursors in Female (F) Sprague–Dawley Rats

			Animals bearing mammary atypical precursors[b] or adenocarcinoma					
	Animals[a]		Atypical precursors		Adenocarcinomas		Total number of animals bearing atypical precursors or adenocarcinomas[c]	
Group no. (Gy)	Sex	No.	No.	%	No.	%	No.	%
I (0)	F	537	40	7.4	76	14.2$^{\#\#}$	116	21.6$^{\blacklozenge\blacklozenge}$
II (0.1)	F	522	68	13.0	78	14.9	146	28.0$^{\blacklozenge}$
III (1)	F	301	27	9.0	86	28.6**	113	37.5$^{\blacklozenge\blacklozenge}$
IV (3)	F	205	11	5.4	91	44.4**	102	49.8$^{\blacklozenge\blacklozenge}$

**Significantly greater than control ($P \leq 0.01$). $^{\#\#}$Near the control incidence is the P-value ($P \leq 0.01$) associated with Cox Proportional Hazard Model for the analysis of the trend. $^{\blacklozenge}P$ values ($P \leq 0.05$) and $^{\blacklozenge\blacklozenge}P$ values ($P \leq 0.01$) using Mantel–Haenszel Model for the analysis *of combined lesions*.
[a]Six weeks old at the time of exposure.
[b]Atypical hyperplasia in mammary gland or in fibroadenoma.
[c]Animals bearing more than one type of lesion are plotted only one according to the most severe lesion.

a significant increase ($P \leq 0.01$) was observed in males exposed to the highest dose.

The incidence of thyroid malignant tumors indicate a significant dose-related increase of follicular cell adenocarcinomas in females ($P \leq 0.01$). When compared to untreated females, the increased incidence of follicular cell adenocarcinomas was significant ($P \leq 0.01$) in females exposed to 3 Gy. A significant dose-related increased incidence of C-cell carcinomas was observed in females ($P \leq 0.01$). When compared to untreated male and female controls, a significant increased incidence of C-cell carcinomas was observed in males and females exposed to 3 Gy ($P \leq 0.05$ and $P \leq 0.01$, respectively).

A dose-related increased incidence of parathyroid glands adenomas was observed both in males and females ($P \leq 0.01$). The incidence of adenomas was significantly increased ($P \leq 0.01$) in both males and females exposed to 1 and 3 Gy. No malignant tumors were observed among the treated and untreated males and females. Parathyroid glands adenomas are rare tumors in our rat colony. Among the

TABLE V. Tumors of Head, Neck, Thyroid, and Parathyroid Glands in Male (M) and Female (F) Sprague–Dawley Rats

			Tumor-bearing animals											
	Animals[a]		Zymbal gland carcinomas		Ear ducts carcinomas		Nasal cavities carcinomas		Thyroid follicular cell carcinomas		Thyroid C-cell carcinomas		Parathyroid adenomas	
Group no. (Gy)	Sex	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I (0)	M	514	9	1.8	32	6.2	2	0.4$^{\#\#}$	3	0.6	6	1.2	8	1.6$^{\#\#}$
	F	537	15	2.8$^{\#}$	46	8.6	1	0.2	1	0.2$^{\#\#}$	7	1.3$^{\#\#}$	4	0.7$^{\#\#}$
	M + F	1051	24	2.3$^{\#}$	78	7.4	3	0.3$^{\#\#}$	4	0.4$^{\#\#}$	13	1.2$^{\#\#}$	12	1.1$^{\#\#}$
II (0.1)	M	524	25	4.8**	45	8.6	1	0.2	3	0.6	13	2.5	13	2.5
	F	522	16	3.1	50	9.6	4	0.8	5	1.0	4	0.8	7	1.3
	M + F	1046	41	3.9*	95	9.1	5	0.5	8	0.8	17	1.6	20	1.9
III (1)	M	318	6	1.9	12	3.8	2	0.6	1	0.3	3	0.9	14	4.4**
	F	301	3	1.0	11	3.7	1	0.3	1	0.3	2	0.7	10	3.3**
	M + F	619	9	1.5	23	3.7	3	0.5	2	0.3	5	0.8	24	3.9**
IV (3)	M	211	3	1.4	4	1.9	4	1.9**	0	—	2	0.9*	8	3.8**
	F	205	4	2.0**	6	2.9*	0	—	2	1.0**	4	2.0**	3	1.5**
	M + F	416	7	1.7**	10	2.4*	4	1.0**	2	0.5**	6	1.4**	11	2.6**

*Significantly greater than controls ($P \leq 0.05$), **($P \leq 0.01$). $^{\#}$Near the control incidence are the P values ($P \leq 0.05$) or $^{\#\#}$($P \leq 0.01$) associated with Cox Proportional Hazard Model for the analysis of the trend.
[a]Six weeks old at the time of exposure.

historical controls, during the period 1984–1997 (when the biophase of this study was conducted), the overall incidence of parathyroid glands adenomas was 0.5% (range: 0.0–1.6%) out of 2,180 males and 0.4% (range: 0.0–2.0%) out of 2,189 females. No malignant tumor was recorded.

Carcinomas of the Liver, Lung, Endocrine Pancreas, Kidneys, and Adrenal Glands

The incidence of malignant tumors of the liver, lung, endocrine pancreas, kidneys and adrenal glands are reported in Table VI. The data indicate a dose-related increased incidence ($P \leq 0.01$) of hepatocellular carcinoma (HCC) in males and females. When compared to untreated males and females, the incidence of HCC was significantly increased both in males and females exposed to 1 Gy ($P \leq 0.01$ in males and $P \leq 0.05$ in females), and to 3 Gy ($P \leq 0.01$).

Although not significant, a numerical increase in the incidence of animals bearing lung adenocarcinomas was observed in males exposed at 1 and 3 Gy (0.6% and 1.9%, respectively), as compared to 0.2% in male controls. Adenocarcinoma of the lung is a very uncommon malignant tumor among male Sprague–Dawley rats from our colony. Out of 2,180 males the overall incidence of lung adenocarcinomas was 0.10% (range: 0.0–1.1%).

Regarding the endocrine pancreas, the data indicate a significant dose-related increased incidence ($P \leq 0.01$) of islet-cell carcinomas both in males and females. The incidence of islet-cell carcinomas in males exposed to 1 and 3 Gy were significantly increased ($P \leq 0.05$ and $P \leq 0.01$ respectively) compared to male controls. The incidence of islet-cell carcinomas in females exposed to 0.1, 1 and 3 Gy were significantly higher ($P \leq 0.01$) than in female controls. Islet-cell carcinoma of the pancreas is a very rare type of tumor among the Sprague–Dawley rats from our colony. Moreover, it is interesting to note that 80 weeks after X-ray irradiation, Watanabe and Kamiya [2008] observed a significant increased incidence of pancreas islet cell adenomas in rats. The overall incidence of islet-cell carcinomas in our historical controls was 0.2% (range: 0.0–0.8%) out of 2,180 males and 0.05% (range: 0.0–0.1%) out of 2,189 females.

The incidence of kidney adenocarcinoma-bearing animals occurred with a significant dose-related increase in males ($P \leq 0.01$) and females ($P \leq 0.05$). The incidence of malignant tumors was significantly increased in males exposed to 3 Gy ($P \leq 0.01$). Adenocarcinomas of the kidneys are very uncommon malignant tumors among Sprague–Dawley rats from our colony. Indeed, the overall incidence of kidney adenocarcinomas was 0.2% (range: 0.0–0.3%).

A significant dose-related increased incidence of cortical adenocarcinomas was observed in females ($P \leq 0.01$), in particular in females exposed to 1 Gy ($P \leq 0.05$) and 3 Gy ($P \leq 0.01$) compared to controls. The overall incidence of cortical adenocarcinomas of adrenal glands in our historical controls was 1.2% (range: 0.0–4.0%).

TABLE VI. Malignant Tumors of the Liver, Lung, Endocrine Pancreas, Kidneys, and Adrenal Glands in Male (M) and Female (F) Sprague–Dawley Rats

			Tumor-bearing animals									
			Hepatocellular carcinomas		Adenocarcinoma of the lung		Islet cell carcinoma of the pancreas		Adenocarcinoma of the kidneys		Cortical adenocarcinoma of the adrenal glands	
Group	Animals[a]											
no. (Gy)	Sex	No.	No.	%	No.	%	No.	%	No.	%	No.	%
I (0)	M	514	4	0.8$^{\#\#}$	1	0.2	4	0.8$^{\#\#}$	1	0.2$^{\#\#}$	2	0.4
	F	537	1	0.2$^{\#\#}$	0	—	0	—$^{\blacklozenge\blacklozenge}$	2	0.4$^{\blacklozenge}$	6	1.1$^{\#\#}$
	M + F	1051	5	0.5$^{\#\#}$	1	0.1	4	0.4$^{\#\#}$	3	0.3$^{\#\#}$	8	0.8$^{\#\#}$
II (0.1)	M	524	4	0.8	0	—	8	1.5	1	0.2	2	0.4
	F	522	4	0.8	1	0.2	11	2.1$^{\blacklozenge\blacklozenge}$	1	0.2	7	1.3
	M + F	1046	8	0.8	1	0.1	19	1.8**	2	0.2	9	0.9
III (1)	M	318	8	2.5**	2	0.6	5	1.6*	2	0.6	3	0.9
	F	301	3	1.0*	0	—	7	2.3$^{\blacklozenge\blacklozenge}$	0	—	5	1.7*
	M + F	619	11	1.8**	2	0.3	12	1.9**	2	0.3	8	1.3**
IV (3)	M	211	6	2.8**	4	1.9	25	11.8**	7	3.3**	0	—
	F	205	3	1.5**	0	—	4	2.0$^{\blacklozenge\blacklozenge}$	3	1.5	5	2.4**
	M + F	416	9	2.2**	4	1.0	29	7.0**	10	2.4**	5	1.2**

*Significantly greater than controls ($P \leq 0.05$), **($P \leq 0.01$). $^{\#\#}$Near the control incidence are the P values ($P \leq 0.01$) associated with Cox Proportional Hazard Model for the analysis of the trend. $^{\blacklozenge}P$ values ($P \leq 0.05$) and $^{\blacklozenge\blacklozenge}P$ values ($P \leq 0.01$) using Mantel–Haenszel Model for the analysis (insufficient data to use Cox model).
[a]Six weeks old at the time of exposure.

Hemangiosarcomas of Circulatory System (All Sites) and Hemolymphoreticular Neoplasias

The incidences of hemangiosarcoma of all sites of the circulatory system and hemolymphoreticular neoplasias (HLRN) are reported in Table VII. The data indicate a significant dose-related increased incidence of hemangiosarcomas in males and females ($P \leq 0.01$). When compared to controls, the incidence of hemangiosarcoma was increased in males exposed to 0.1 Gy ($P = 0.06$), significantly increased at 1 Gy ($P \leq 0.01$), 3 Gy ($P < 0.01$) and in females exposed to 3 Gy ($P \leq 0.01$) and significantly increased at all tested doses when males and females are combined. In historical controls the overall incidence of hemangiosarcomas (all sites) in males was 0.5% (range 0.0–1.3%).

For HLRN the data show a significant dose-related increased incidence ($P \leq 0.01$) in males. When compared to male and female controls, the incidence of HLRN was significantly increased in males ($P \leq 0.01$) and females ($P \leq 0.05$) exposed to 3 Gy. The most frequent histotypes observed among the hemolymphoreticular neoplasias were lymphomas and leukemias. Both lymphomas and leukemias are neoplasias arising from hemolymphoreticular tissues and their aggregation is used because solid and circulating phases are present in many lymphoid neoplasms, and distinction between them is artificial [Harris et al., 2001].

TABLE VII. Hemangiosarcomas (All Sites) and Hemolymphoreticular Tumors in Male (M) and Female (F) Sprague–Dawley Rats

			Tumor-bearing animals			
Group	Animals[a]		Hemangiosarcomas		Hemolymphoreticular tumors	
no. (Gy)	Sex	No.	No.	%	No.	%
I (0)	M	514	0	—◆◆	120	23.3##
	F	537	4	0.7##	96	17.9
	M + F	1051	4	0.4##	216	20.6##
II (0.1)	M	524	5	1.0◆	118	22.5
	F	522	8	1.5	70	13.4
	M + F	1046	13	1.2*	188	18.0
III (1)	M	318	6	1.9◆◆	59	18.6
	F	301	3	1.0	22	7.3
	M + F	619	9	1.5**	81	13.1
IV (3)	M	211	16	7.6◆◆	55	26.1**
	F	205	11	5.4**	17	8.3*
	M + F	416	27	6.5**	72	17.3**

*Significantly greater than controls ($P \leq 0.05$), **($P \leq 0.01$). ##Near the control incidence are the P values ($P \leq 0.01$) associated with Cox Proportional Hazard Model for the analysis of the trend; ◆P values ($P \leq 0.05$) and ◆◆P values ($P \leq 0.01$) using Mantel–Haenszel Model for the analysis (insufficient details to use Cox model).
[a]Six weeks old at the time of exposure.

Cancer Risk Estimation

The Cox proportional hazard model used in the statistical testing of the various tumor types and sites also provides estimates of hazard ratios or relative risk estimates.

Hazard ratios were calculated for animal bearing malignant solid tumors, and for lymphomas and leukemias combined. For the solid tumors it is shown in Table VIII that statistically significant increases occur at 1 and 3 Gy. The increase rises rapidly and the data fit to a standard linear-quadratic dose response model. For lymphomas/leukemias, effects are not seen until 3 Gy and the dose–response is purely quadratic.

Finally for several of the cancer sites namely, mammary adenocarcinomas, Zymbal gland carcinomas, thyroid C-cell carcinomas, pancreas islet cell carcinomas and hemangiosarcomas (Table IX), the hazard ratios were estimated with the sexes combined but stratified in the analysis. Although the data are limited, it is useful to observe the risk ratios since the life shortening at the higher doses will underestimated the risk from simply observing the incidences of the tumors as is reported in the main tables. Interestingly the increase in pancreatic carcinomas has not been observed in the A-bomb survivors although it has been suggested that there is a possible association with pancreas cancer in Thorotrast patients [Travis et al., 2003].

DISCUSSION

The capacity of ionizing radiation to induce solid cancers and leukemias in humans at high exposure doses and also at low and very low doses (≤0.1 Gy) has been shown among the survivors of Nagasaki and Hiroshima atomic bombing [Preston et al., 2007].

The effects and mechanism of interaction between ionizing radiation and living matter is based on the biophysical theory of how ionizing radiation interacts with DNA, which is considered the major biological target. Thus, the extension of DNA damage to cells is related to the radiation dose delivered, with clear evidence of the relationship between DNA damage, mutation and cancer induction [Hanahan and Weinberg, 2000].

However in the last few decades, low doses and very low doses of ionizing radiation have been demonstrated to elicit effects on biological tissues that are independent of their direct interaction with DNA, namely the so-called non-targeted effects which include bystander effects, genomic instability, hormesis, adaptive and transgenerational radiation response [Tubiana et al., 2006]. These findings, as reviewed by Averbeck [2010] and Mullenders et al. [2009], suggest some differences in the biological responses to higher and low doses of ionizing radiation which may affect the evaluation of the risk at low exposures. Although the extent

TABLE VIII. Hazard Ratios for Male (M) and Female (F) Sprague–Dawley Rats Bearing Malignant Solid Tumors and Lymphomas/Leukemias

				Malignant solid tumors				Lymphomas/leukemias			
	Animals[a]			Observed hazard ratio		Linear-quadratic		Observed hazard ratio		Linear-quadratic[b]	
Group (Gy)	M	F		M	F	M	F	M	F	M	F
I (0)	514	537	Reference								
II (0.1)	524	522		1.11	1.12	1.01	1.09	0.97	0.76	1.00	1.00
III (1)	318	301		1.98**	2.10**	1.71	1.92	1.10	0.76	1.22	1.11
IV (3)	211	205		7.53**	9.59**	7.35	9.34	2.91**	1.86*	2.94	1.98

*Statistically significantly different from 1.00 at $P \leq 0.05$ and ** at $P \leq 0.001$.
[a]Six weeks old at the time of exposure.
[b]Only a simple quadratic dose–response model fits the data.

to which these phenomena reflect different molecular mechanisms is not clear, experimental evidence may contribute to better defining the carcinogenic dose-response relationship at low exposure levels.

The capacity of ionizing radiation to induce various types of tumors in experimental animals has been well recognized for many years. In animals the carcinogenic effects can may be affected by the total dose delivered (acute or fractioned), by the dose rate and type of radiation, by the responsiveness of the animal species and strain used, by the age at the time of irradiation, by hormonal factors and by the exposure to co-carcinogenic agents [Upton, 1968; IARC, 2012].

Over the years the interest of animal research in ionizing radiation has focused on quantitative aspects of the carcinogenic effects, in particular from low doses. We here summarize the results of some major studies on rats and mice.

Apart from the studies on the induction of mammary cancer in Sprague–Dawley rats by Shellabarger et al., few other studies have been performed on this species. Bartel-Friedrich et al. reported a significant increase in malignant tumors of the head and neck in Wistar rats following exposure of the head region to X-radiation at a total dose of 60 Gy fractionated in 2 Gy per day [Bartel-Friedrich et al., 1999]. Watanabe and Kamiya [2008] reported a significant increase in islet cell adenomas of the pancreas in Long-Evans rats which are very susceptible to the induction of pancreas islet tumors. The rats were exposed to two doses of 10 Gy each to the gastric region with a 3-day interval, and sacrificed after 80 weeks (not sufficient time to develop carcinomas). In a 2-year study conducted by Lafuma et al. [1989] to compare the induction of lung carcinomas in male Sprague–Dawley rats by exposure at low doses to radon daughters, fission neutrons or γ-rays, it was shown that 3.6% out of 505 rats exposed to 1 Gy γ-radiation developed lung carcinomas. This incidence (0.6%) is little higher than we found in our experiment on males exposed to 1 Gy of γ-radiation. In rats generally, apart from the carcinogenic effects observed in the mammary gland, there are very few qualitative and quantitative data on dose–response carcinogenic effects for use in risk assessment.

Upton et al. [1970] examined the development of neoplasms in large groups of male and female mice (more

TABLE IX. Hazard Ratios for Several Cancer Sites in Sexes Combined (M + F) Sprague–Dawley Rats

	Animals[a]		Cancer sites[b]				
	Sex combined, no		Mammary	Zymbal	Thyroid C-cell	Pancreas islet	
Group (Gy)	M + F		adenocarcinoma	glands	carcinomas	cell carcinomas	Hemangiosarcomas
I (0)	1051	Reference					
II (0.1)	1046		1.05	1.71*	1.30	4.33**	3.18*
III (1)	619		3.94**	1.11	1.17	11.34**	6.51*
IV (3)	416		19.4**	4.04**	7.93***	205**	73.5**

*$P \leq 0.05$.
**$P \leq 0.01$.
***$P \leq 0.001$.
[a]Six weeks old at the time of exposure.
[b]Cox regression with stratification on gender.

than 4,000 in all). Whole-body irradiation started at 10 weeks of age, after which the animals were observed for the lifespan. All animals were fully necropsied, but only selected lesions were examined histopathologically, as and when needed to confirm diagnoses. The dose ranged from 0.25 Gy to 4.5 Gy for acute X-radiation and from ∼1 to 98.75 Gy for chronic Co60 γ-irradiation. An increased incidence of various neoplasms was observed even at the lowest dose. Specifically, an increased incidence of myeloid leukemia and thymic lymphoma was observed in males and females, and an increased incidence of ovarian tumors in females.

A highly comprehensive series of studies on the induction of tumors by γ radiation in mice was performed by Ullrich and Storer [1979a,b,c]. A total of more than 19,000 male and female RFM mice (plus almost 5,200 controls) were exposed to doses from 0.1 to 3 Gy. More than 5,600 female BALB/C mice (plus 865 controls) were exposed to a range of doses from 0.5 to 2 Gy. An increased dose-related incidence of myeloid leukemia and thymic lymphoma was observed in male and female RFM mice. A dose-dependent increased incidence in ovarian, pituitary and Harderian gland tumors was observed in female RFM mice. At 0.25 Gy the incidence of ovarian cancer was increased by almost three times. An increased incidence of ovarian tumors and lung and mammary carcinomas in BALB/C mice was observed even at the lowest dose. These studies, with its large groups of animals and dose range (0.1–3 Gy), afforded a more complete analysis of the dose–response in mice.

More recently Di Majo et al. [2003] published a summary of several studies conducted over several years on the carcinogenic effects of ionizing radiation in mice. The aim of this summary was to evaluate the effects of the dose–response at low doses (4, 8, 16, and 32 cGy) of 250 KVpX-rays. The small sample size (n = 52–97) provided limited evidence for an increase in cancer risks, apart from a significant increase in ovarian tumors at 8, 16, and 32 cGy.

A large study on the effects of low-dose γ rays was conducted by Tanaka et al. [2007] using 4,000 mice exposed over 400 days to a total dose of 2, 40, and 800 cGy. Compared to the controls, the incidence of myeloid leukemias in males, the incidence of soft tissue neoplasms and malignant granulosa cell tumors in females, and the incidence of hemangiosarcomas in both sexes, were significantly increased at the highest dose.

Taken together, these major experiments on rats and mice and others available in the literature suffer from limitations that could lead one to query the capacity of lifespan rodent bioassays to show the carcinogenic effects of ionizing radiation at low doses.

For this reason we planned a project with experimental groups of adequate size to evaluate the potential carcinogenic effects in rats at low doses of exposure, as well as the dose–response relationship.

In the present study, conducted on large groups of male and female Sprague–Dawley rats, exposed at 6 weeks of age to three different dose levels (0.1, 1, or 3 Gy) delivered in a single acute exposure, we found: (a) a significant dose-related increased incidence of males and females bearing malignant tumors in the groups exposed to 1 and 3 Gy; (b) in animals exposed to 3 Gy, we observed a specific significant increase in the incidences of skin cancers (males and females), subcutis liposarcomas (males), breast adenocarcinomas and sarcomas (males and females), Zymbal gland carcinomas (females), ear duct carcinomas (females), nasal cavity carcinomas (males), thyroid follicular cell carcinomas (males and females), hepatocellular carcinomas (males and females), pancreas islet cell carcinomas (males and females); kidney adenocarcinomas (males), cortical adrenal gland adenocarcinomas (females), hemangiosarcomas (all sites) (males and females), hemolymphoreticular neoplasias (males and females); (c) a significant increased incidence of breast adenocarcinomas and breast sarcomas (in males and females), hepatocellular carcinomas (males and females), pancreas islet cell carcinomas (males and females), cortical adrenal gland adenocarcinomas (females), and hemangiosarcomas (all sites) (in males) was also observed at the exposure of 1 Gy; (d) a significant increased incidence of Zymbal gland carcinomas (males), pancreas islet cell carcinomas (females) was also observed at 0.1 Gy. Concerning the significant increased incidence of carcinomas of the Zymbal glands and of the pancreas islet cell at 0.1 Gy, it is unlikely that these findings are due to chance if we consider that: (1) these malignant tumors are very rare among the historical control of our Sprague–Dawley rat colony; (2) the differences in absolute number between treated versus control groups is remarkable; (3) except for Zymbal gland carcinomas, which are significantly increased in males only at 0.1 Gy

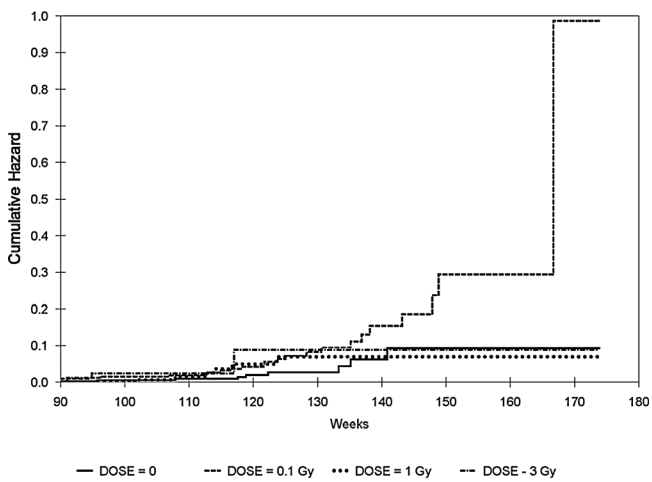

FIGURE 6. Cumulative hazard for Kaplan–Meier estimation: Zymbal glands carcinoma in male Sprague–Dawley rats.

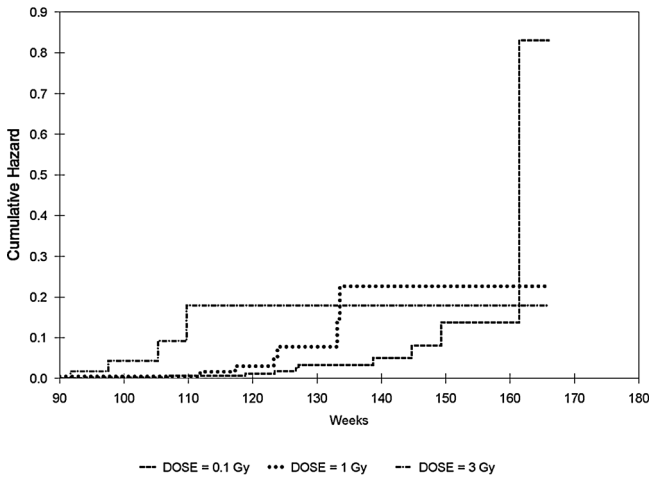

FIGURE 7. Cumulative hazard for Kaplan–Meier estimation: pancreatic islet cell carcinoma in female Sprague–Dawley rats.

(but with a significant dose-related relationship), islet cell carcinomas of the pancreas in females was in turn significantly increased at 1 and 3 Gy, with a significant dose-related relationship, as shown also by the cumulative hazard for Kaplan–Meier Estimation (Figs. 6 and 7).

Concerning the carcinogenic effects of exposures to 1 Gy, the results from the studies on A-bomb survivors showed that the relative risk for solid cancer mortality is about 1.4 [Ozasa et al., 2012] instead of 2 which is observed in Table VIII of this study. This relative risk of 1.4 is for an individual exposed at age 30 years. If the exposure were for an individual aged 10–15 years, then the relative risk would also be about 2, as we found for young exposed Sprague–Dawley rats. It is also interesting to note that in this study lymphoma and leukemia dose-response is resulted to be purely quadratic. The same has been observed for acute myeloid leukemia (the most common) among A-bomb survivors [Hsu et al., 2013].

CONCLUSIONS

Taken together, the experiments on rats available in the literature suffer from limitations depending on the dose levels, number of animals per group and duration of the studies.

For this reason we planned a project with experimental groups of adequate size to evaluate the potential carcinogenic effects in rats at low doses of exposure, as well as the dose-response relationship.

With this experiment we have shown that acute exposure to γ radiation causes a significant increase in the incidence of a few types of tumors in males and females at the dose of 0.1 Gy. These results confirm that our Sprague–Dawley rat colony is suitable to identify and quantify carcinogenic risks. They also show that large-scale experiments produce important new quantitative results, particularly in the area of low exposure. However, since the animals were randomized by breeders and since all the experimental groups include all the offspring of each litter, it may be possible to evaluate the synergism between familiar susceptibility and γ radiation exposure at the lowest dose.

The results call for attention to various human scenarios of low radiation exposure. Thus, one can hardly underestimate the fact that the dose to tissues absorbed from a whole body CT scan typically in the range of 0.05–0.1 Gy and this must be weighed all the more seriously if those examined are children or adolescents [Brenner, 2010].

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