RADIATION RESEARCH 138, 252–259 (1994)

Neutron-Induced Tumors in BC3F$_1$ Mice: Effects of Dose Fractionation

Vincenzo Di Majo,* Mario Coppola,* Simonetta Rebessi,* Anna Saran,* Simonetta Pazzaglia,* Lorraine Pariset† and Vincenzo Covelli*

*ENEA-Casaccia, AMB-EFF Section, and †AIRP Fellow, ENEA-Casaccia, AMB-EFF Section, Via Anguillarese 301, 00060 Roma, Italy

Di Majo, V., Coppola, M., Rebessi, S., Saran, A., Pazzaglia, S., Pariset, L. and Covelli, V. Neutron-Induced Tumors in BC3F$_1$ Mice: Effects of Dose Fractionation. *Radiat. Res.* 138, 252–259 (1994).

An experimental study of the biological effectiveness of multi-fractionated low doses of high-LET radiation was carried out using BC3F$_1$ male mice. They were treated with whole-body irradiation with five equal daily fractions of fission neutrons to yield cumulative doses of 0.025, 0.05, 0.10, 0.17, 0.25, 0.36, 0.535 and 0.71 Gy at the RSV-TAPIRO reactor (mean neutron energy 0.4 MeV, in terms of kerma, $\bar{y}_D$ = 51.5 keV/μm, dose rate 0.004 Gy/min) and were followed for their entire life span. The statistical method described by Peto *et al.* (IARC Monograph, Suppl. 2, 1980) to establish the existence of a carcinogenic effect in long-term animal experiments was applied to the data sets. This analysis was done for myeloid leukemia and for the presence of selected solid tumors. Myeloid leukemia was absent in the control group and was rarely found in irradiated animals. However, a positive significant trend was found in the dose ranges 0–0.17 Gy and higher. Epithelial tumors were induced at doses from 0.17 Gy on. Tumor occurrence was evaluated further as final incidences with age adjustment for the differences in mortality rates. Survival and incidence data for selected classes of tumors after 0.17, 0.36 and 0.71 Gy were compared with those from a previous experiment at corresponding doses given acutely (dose rate between 0.05 and 0.25 Gy/min). This indicated no marked overall influence of the time regimen of neutron irradiation on survival and tumor induction.

INTRODUCTION

In recent years the biological effectiveness of low radiation doses and dose rates has caused growing concern, as proven by the new lower dose limit recommended for radiological protection (*1*). Risk estimates should rest mainly on knowledge of the *in vivo* effects of radiation at low doses in humans. However, the absence of data for humans exposed to low doses and at low dose rates of either low- or high-LET radiation necessitates the use of mathematical models for extrapolation to the initial part of the dose–response relationships and the use of experimental data. To verify the predictions based on the models it is not easy to find substitutes for studies of tumor induction and life shortening in experimental animals.

Fractionation or protraction of the dose of high-LET radiation has resulted in an enhancement of tumor induction in several *in vivo* experiments in comparison with the results obtained after the corresponding cumulative doses given acutely. In particular, Vogel and Dickson (*2*) observed an increase in the percentage of Sprague-Dawley rats with mammary tumors, during 10 months of observation, after exposure to fission-spectrum neutrons, with a significant difference between protracted and acute exposures at a dose of 0.5 Gy. The induction of lung cancer after single or repeated irradiation was studied by Lundgren *et al.* (*3*) in C57Bl/6J female mice with α particles from inhaled $^{239}PuO_2$, showing that the incidence of pulmonary tumors was around four times greater after repeated exposures than after single exposures at doses to the lungs between approximately 3 and 18 Gy. Incidence data for lung adenocarcinoma induced by fission neutrons in female BALB/c mice (*4*) have shown that a separation of 24 h between two dose fractions increased the induction by a maximum of a factor of 2 at 0.5 Gy. No effect of dose fractionation was evident in the induction of mammary adenocarcinoma in the same mouse strain. However, a dose protraction at a dose rate lower than 8×10^{-5} Gy/min was reported to increase the low-dose response for mammary tumors by a factor of 2 (*4*).

Life-span shortening was reported by Maisin *et al.* (*5*) to be enhanced slightly after fractionated exposure to d(50)+Be neutrons at cumulative doses between 0.18 and 1.65 Gy in C57Bl/Cnb male mice, and was observed in (C57Bl/6 × BALB/c)F$_1$ male mice by Thomson *et al.* (*6*) at a cumulative dose of fission neutrons of 2.4 Gy.

Evidence that dose fractionation may increase the overall effectiveness of neutrons in mice is also provided for other end points, such as the induction of lens opacities (*7, 8*) and of dominant lethal mutations (*9*).

Epidemiological studies of lung cancer in U.S. uranium miners (*10*) and Chinese tin miners (*11*) have shown a dose-rate effect with low exposure rates being more harmful per unit of cumulative exposure.

0033-7587/94 $5.00

Enhanced neoplastic transformation frequency after multiple doses of high-LET radiation has been observed in several *in vitro* experiments at doses well below 0.5 Gy. In particular, Hill *et al.* (*12*) reported a strong enhancement (up to eight times) of the neoplastic transforming potential of fractionated doses or protracted exposures to fission neutrons at low dose rate, in comparison with single exposures at high dose rate, using the C3H 10T1/2 cell assay. In addition, enhanced rates of neoplastic transformation were reported for Syrian hamster embryo cells (*13*) and human cell hybrids (HeLa × skin fibroblasts) (*14*) after exposure to low-dose-rate fission neutrons from the JANUS reactor.

With this in mind, we carried out an experiment with fractionated doses of fission-spectrum neutrons from the TAPIRO reactor to study life-span shortening and to observe tumor induction in mice as a function of the dose. The experimental design was such that it allowed a comparison of the results with those of a previous study of acute exposures to test the presence of an inverse dose-rate effect in whole-body-irradiated BC3F$_1$ male mice at doses that were thought to be critical for this effect. For this, we adopted a five-fraction irradiation protocol, with equal doses delivered at 24-h intervals, reproducing the protocol of Hill *et al.* (*12*) which had proven to be very effective in producing neoplastic transformation of cells *in vitro*. Moreover, each fraction was delivered at a low dose rate of 0.004 Gy/min, on the basis of a paper by Hill *et al.* (*15*) which suggested that a dose of 0.21 Gy of fission neutrons was maximally effective at a dose rate of 0.0043 Gy/min or somewhat less. Cumulative doses of 0.025, 0.05, 0.10, 0.17, 0.25, 0.36, 0.535 and 0.71 Gy were tested. For comparison of the effect of single and fractionated exposures, data at the doses of 0.17, 0.36 and 0.71 Gy from a previous experiment on BC3F$_1$ male mice (*16*) carried out in the same neutron field with single doses were considered in the present paper.

MATERIALS AND METHODS

Mice. The animals were young adult (C57Bl/Cne × C3H/HeCne)F$_1$ (BC3F$_1$) male mice bred and kept in our animal house. They were treated with whole-body irradiation at 3 months of age. Animal husbandry and care complied with Italian law, and every effort was made to reduce animal stress and discomfort to a minimum.

Irradiation conditions. Fission-neutron irradiation was carried out at the experimental fast nuclear reactor RSV-TAPIRO of CRE-Casaccia (Rome). The facility has been described elsewhere (*17*). The exposure was mostly unilateral (ventrodorsal), since the cage floor was facing the incident neutrons; differences between doses to individual animals, measured with the help of a phantom, were well within 4%. The calculated energy spectrum showed an average energy of about 0.4 MeV, in terms of kerma. The dose-averaged lineal energy measured at $d = 2$ μm was $\bar{y}_D = 51$ keV/μm for the total radiation field (*18*). The γ-ray contamination of the field, due mainly to the inelastic scattering of neutrons in the surrounding materials and to the fission product component, was estimated to be about 12% of the total dose. Biological effects due to the γ-ray component of the total dose were expected to add negligibly to those of neutrons, and therefore no attempt was made to subtract the γ-ray contribution from the total effects. Thus neutron dose refers to the total dose. Mice were irradiated whole-body with five daily doses of 0.005, 0.01, 0.02, 0.034, 0.05, 0.072, 0.107 or 0.142 Gy, at a dose rate of 0.004 Gy/min. A different set of data from a previous experiment (*16*), in which BC3F$_1$ male mice were acutely irradiated in the same neutron field, was also considered in the present paper for comparison. In this series, animals received 0.17, 0.36, 0.71, 1.07, 1.43, 1.79 or 2.14 Gy of neutrons (dose rate between 0.05 and 0.25 Gy/min).

Follow-up and pathology. All mice were housed five or fewer to a cage and were followed for their entire life span with daily inspection (six per week). Soon after spontaneous death of the mice, autopsies were performed on 1264 (98%) of the 1295 mice under observation which survived 30 days after treatment. The necropsy included external and internal gross examination. Tissue masses as well as sections of the major organs were taken and processed for histological analysis.

Data analysis. A unique set of longevity and pathology data for the control group was used for data analysis. The statistical method described by Peto *et al.* (*19*) to establish the existence of a carcinogenic effect in long-term animal experiments was applied to the data sets. This method tests for positive trend with dose and involves a basic comparison between the number of tumors observed at death in a particular treatment group and the number that would have been expected had the onset of age-specific tumor rates been similar in all groups after correction for differences in longevity. The application of this method requires that each neoplastic lesion be classified according to its "observation context." To this end, the myeloid leukemia cases and all malignant solid tumors have been classified as fatal. The method was applied to progressively reduced dose ranges, i.e. each time the highest dose was excluded, to ascertain whether a positive trend still existed in the lower-dose region (*20*). Tumor occurrence was evaluated further in terms of percentage (hereafter referred to as incidence) of tumor-bearing animals, with age adjustment for the differences in mortality rates of the treated groups (*20, 21*). Weighted least-squares regressions were used to fit mean survival data and epithelial tumor incidences as a function of the dose.

RESULTS

Longevity. The analysis of the life span of neutron-irradiated animals revealed first that the mean survival after 0.025 Gy of fractionated neutrons was higher than in the controls (mean survival ± SE: 847 ± 13 days at 0.025 Gy vs 816 ± 12 days in the controls; Student's t test = 1.748, df = 393, $P = 0.041$). A measurable decrease was seen at neutron doses from 0.17 Gy on, and it was reasonably well correlated with increasing dose in the range 0.17–0.71 Gy (Table I). Mean survival data for acute exposures are reported in Table II. Linear dose–effect relationships yielded satisfactory fits to the two sets of data (Fig. 1, panel A) in the dose range 0–0.71 Gy. The regression coefficients corresponded to a life loss of 160 ± 36 ($R = 0.89$) and 158 ± 20 ($R = 0.99$) days/Gy for fractionated and acute doses of neutrons, respectively.

Pathology. The pathology of the neoplasms and non-neoplastic lesions is reported in detail in Tables I and II for fractionated and acute exposures, respectively, with each table being further divided per site and histotype. From the data in these tables, it is evident that no lesion could be associated preferentially with a certain irradiation protocol. Systemic neoplasms observed were malignant lymphomas and myeloid leukemia. The incidence of malignant lym-

TABLE I
Survival and Pathology Data for $BC3F_1$ Male Mice Treated with Five Daily Doses of Whole-Body Fission Neutrons

Total dose (Gy)	0	0.025	0.05	0.10	0.17	0.25	0.36	0.535	0.71
Survivors at 30 days	193	202	148	105	74	53	54	54	52
Mean survival	816	847[a]	828	831	771[a]	788	740[b]	745[b]	737[b]
(±SD, days)	(±163)	(±188)	(±171)	(±170)	(±154)	(±169)	(±166)	(±203)	(±164)
No. of autopsied mice	189	199	145	104	73	52	53	53	52
Malignant lymphoma	109	94	74	56	35	21	26	20	16
Myeloid leukemia					2	2	1		1
Solid tumors: Site and type									
Lung									
Alveolar adenoma	4	2		2		5		1	
Alveolar adenocarcinoma	3	4	7	2	5	2	6	2	3
Liver									
Hepatocellular adenoma		2	3		1	1	2		2
Hepatocellular adenocarcinoma	15	24	3	15	12	9	10	3	6
G.I. tract									
Adenocarcinoma			1		1		1		
Adrenal gland									
Cortical adenoma								5	2
Cortical adenocarcinoma	2			1		1	1		1
Kidney									
Adenoma			1					2	
Carcinoma		1		1			1	1	3
Soft tissues									
Fibrosarcoma	7	3	9	1	3	3	1	4	5
Mammary gland									
Adenocarcinoma					1				
Urinary bladder									
Carcinoma		1							
Vascular system									
Hemangioendothelioma	1	1		3					
Bone									
Osteogenic sarcoma									1
Harderian gland									
Adenocarcinoma		3	1	1	1	1	2	1	1
Skin									
Squamous cell carcinoma				2		1	1	2	2
Salivary gland									
Adenocarcinoma						1	1		
Parotid gland									
Adenocarcinoma	1								
Total	33	41	25	28	24	24	26	20	27
Degenerative diseases									
Nephrosclerosis	15	9	14	3			1	6	20

[a] $0.02 < P < 0.05$ with Student's t test.
[b] $0.001 < P < 0.01$ with Student's t test.

phomas was confirmed to be high in control $BC3F_1$ mice (58%) (*22*) and tended to decrease when the doses increased. Myeloid leukemia was absent in the untreated control mice, as in a previous experiment (*22*). Therefore, each case occurring in irradiated groups can be considered to have been induced by radiation. Solid tumors of many types were also present, the most frequent being in the lung, liver, skin and soft tissues, including both benign and malignant forms. As far as degenerative diseases are concerned, severe nephrosclerosis appears only late in life at low frequency in the untreated mice (i.e. 8%) and at low doses, except at 0.71 Gy of fractionated neutrons.

Trend analysis. This analysis was made for selected categories of neoplastic diseases, and the results are summarized in Table III. The incidence of myeloid leukemia was significant in the range 0–0.17 Gy and above for both neutron-irradiation protocols. For solid tumors, a significant positive trend of the incidence was first found in the following dose ranges of fractionated neutrons: 0–0.17 Gy for lung and liver, 0–0.10 Gy for skin and 0–0.535 Gy for soft tissues. The

TABLE II
Survival and Pathology Data for BC3F$_1$ Male Mice Treated with Acute Doses of Whole-Body Fission Neutrons

Dose (Gy)	0	0.17	0.36	0.71	1.07	1.43	1.79	2.14
Survivors at 30 days	193	49	47	48	49	49	96	22
Mean survival	816	781	740[a]	710[b]	675[b]	652[b]	684[b]	645[b]
(±SD, days)	(±163)	(±186)	(±200)	(±180)	(±191)	(±160)	(±169)	(±187)
No. of autopsied mice	189	46	43	48	47	48	90	22
Malignant lymphoma	109	19	12	20	16	7	18	2
Myeloid leukemia		1	1	2	2	1	2	
Solid tumors: Site and type								
Lung								
Alveolar adenoma	4	1	3	2	2	2	3	
Alveolar adenocarcinoma	3	3	4	3	1		5	
Liver								
Hepatocellular adenoma		1	1	4		4	2	
Hepatocellular adenocarcinoma	15	7	4	6	9	5	3	
G.I. tract								
Adenocarcinoma			2			1		1
Adrenal gland								
Cortical adenoma			2	5	2	4	6	1
Cortical adenocarcinoma	2				1	1	1	
Kidney								
Adenoma				1	1	2	3	2
Soft tissues								
Fibrosarcoma	7		4	6	1	7	14	1
Brain								
Glioblastoma			1					
Vascular system								
Hemangioendothelioma	1				1			
Bone								
Osteogenic sarcoma						1	2	
Harderian gland								
Adenocarcinoma		1				2		
Skin								
Squamous cell carcinoma			1	2	5	1	3	1
Salivary gland								
Adenocarcinoma							1	
Testis								
Leydig cell tumor			1			1		
Parotid gland								
Adenocarcinoma	1					1		
Total	33	13	23	29	23	32	43	6
Degenerative diseases								
Nephrosclerosis	15	1	1	1	1	2	5	2

[a] $0.001 < P < 0.01$ with Student's t test.
[b] $P < 0.001$ with Student's t test.

same analysis was also carried out by pooling all benign and malignant epithelial tumors found in each experimental group. A significant increase in incidence is first observed in the dose range of 0–0.17 Gy of fractionated neutrons. For acute exposures a neutron dose of 0.17 Gy was already effective for induction of lung, liver and all epithelial tumors, while the minimum effective dose ranges were 0–0.36 Gy for the skin and 0–0.71 Gy for soft tissue tumors.

Age-adjusted tumor incidences. The adjusted percentage incidences of selected tumors in the dose range 0–0.71 Gy are shown in Fig. 1 (panels B to F) for the two different modalities of neutron irradiation. The data from fractionated and acute exposures at the doses of 0.17, 0.36 and 0.71 Gy, where measurements with both irradiation modalities were carried out, are also reported in Table IV.

After exposure to fractionated doses of neutrons, myeloid leukemia was not observed at doses below 0.17 Gy, and its incidence declined at doses higher than 0.25 Gy (Fig. 1, panel B). After acute exposure to neutrons, the level of induction was maximum at doses between 0.71 and 1.79 Gy with a decrease to zero incidence after exposure to 2.14 Gy (*22*). Tumors of the lung and of the liver peaked at fraction-

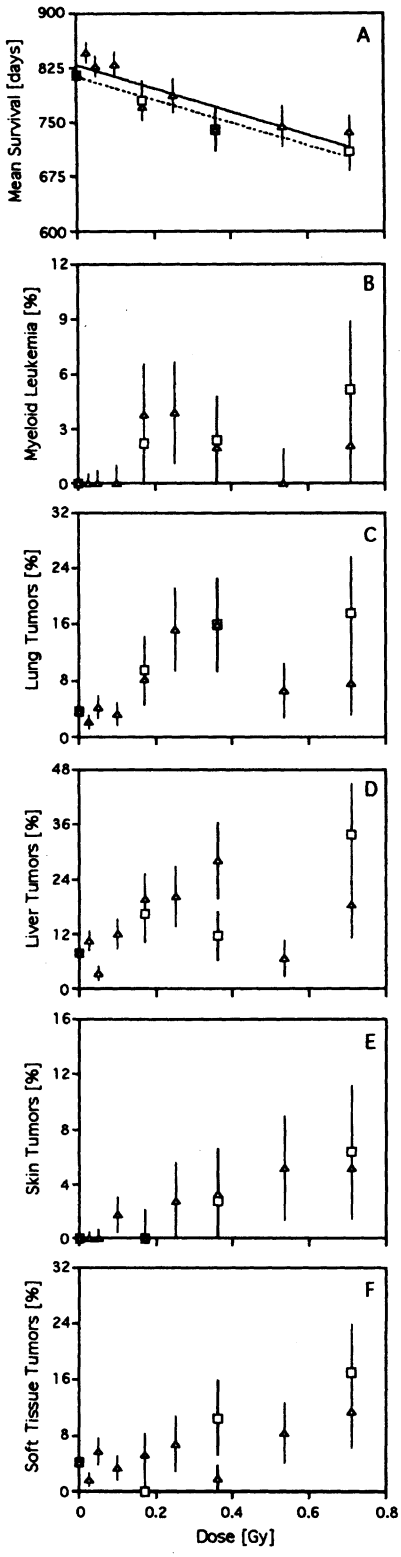

FIG. 1. Mean survival (Panel A) and age-adjusted percentage incidences of selected tumors (panels B to F) in $BC3F_1$ male mice irradiated whole-body with fractionated (△) or acute (□) fission-spectrum neutrons. Bars are SE. Solid and dotted lines are for fractionated and acute neutrons, respectively (see text).

ated doses of 0.36 Gy and declined afterward (Fig. 1, panels C and D); skin and soft tissue tumors exhibited an increasing incidence pattern in the dose range 0–0.71 Gy (Fig. 1, panels E and F).

DISCUSSION

The objectives of the present work were to study long-term survival and tumor induction in the low-dose region of fission-spectrum neutrons, and to provide information about the inverse dose-rate effect, based on a variety of end points in the same well-defined biological *in vivo* system, i.e., the $BC3F_1$ mice (*16, 17, 20, 22*).

The data for survival shown in Tables I and II and Fig. 1 (panel A) suggest that in our system there is no consistent increase in life-span shortening with neutron dose fractionation in comparison with acute irradiation within comparable dose ranges.

Myeloid leukemia induced by fractionated doses of neutrons was peaking at lower doses (Fig. 1, panel B) compared to acute exposures (*22*). However, in the present experiment the values for the incidence were too low (less than 6%) to provide convincing evidence about the influence of dose fractionation. It is worth mentioning that Huiskamp (*23*) found no change in the incidence of myeloid leukemia in CBA/H mice after 0.4 Gy of fission neutrons when the dose rate was reduced from 0.1 to 0.002 Gy/min.

The percentage of mice bearing an epithelial tumor is plotted in Fig. 2. Data of this type have shortcomings from a mechanistic point of view, but at the same time have the advantage of providing a straightforward and simple index of a major radiation risk. Another favorable aspect of this procedure of data collating is its ability to yield intrinsic robust results, as the frequency of events taken into account can be relatively high even when the sample size is limited. A similar approach was used by Grahn *et al.* (*24*). The data that they reported are consistent with an increase of about 40% in the linear risk coefficient of induction of epithelial tumors in $B6CF_1$ male mice, comparing single doses with 24 weekly doses of JANUS reactor fission neutrons and an even greater difference in the case of 60 fractions.

In our case, the results in Fig. 2 show that the risk of induction of epithelial tumors in $BC3F_1$ male mice by fission neutrons from the TAPIRO reactor is similar in the same range of doses for both neutron treatments. The dose responses between 0 and 0.71 Gy were fitted with a linear-quadratic model to a negative quadratic term (R = 0.95 and R = 0.99 for fractionated and acute neutron doses, respectively). The linear coefficients, describing the ability of the radiation to affect the mouse per Gy (±SE), were 87 (±34) for five daily doses of fission neutrons and 64 (±40) for acute doses of neutrons, with a ratio of 1.36 (±1.00).

TABLE III
One-Tailed *P* Values from Positive Trend Analysis on the Observed Incidence of Selected Tumors in BC3F$_1$ Mice after Exposure to Fission Neutrons

Irradiation protocol	Dose range (Gy)	Myeloid leukemia	Lung tumors	Liver tumors	Skin tumors	Soft tissue tumors	Epithelial tumors
Split doses	0–0.710	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
	0–0.535	0.006	<0.001	0.001	<0.001	0.028	<0.001
	0–0.360	<0.001	<0.001	<0.001	<0.001	ns	<0.001
	0–0.250	<0.001	<0.001	<0.001	0.002	ns	<0.001
	0–0.170	<0.001	0.043	0.001	0.024	ns	<0.001
	0–0.100	nc[b]	ns[c]	ns	0.002	ns	ns
	0–0.050	nc	ns	ns	nc	ns	ns
	0–0.025[a]	nc	ns	ns	nc	ns	ns
Acute doses	0–2.14	0.069	0.014	0.002	<0.001	<0.001	<0.001
	0–1.79	0.036	0.003	<0.001	<0.001	<0.001	<0.001
	0–1.43	0.013	0.012	<0.001	<0.001	<0.001	<0.001
	0–1.07	0.003	0.002	<0.001	<0.001	0.016	<0.001
	0–0.71	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
	0–0.36	0.024	<0.001	0.050	0.010	ns	<0.001
	0–0.17[a]	0.034	0.094	0.047	nc	ns	0.008

[a]Values of *P* are for the heterogeneity test.
[b]nc indicates that no such case was found within the dose range indicated.
[c]ns (not significant) indicates a $P \geq 0.1$.

This increase is similar to that found by Grahn *et al.* (*24*); however, the difference between the two linear terms is not significant (Student's *t* test = 0.67, df = 9, *P* = 0.5). In addition, due to the larger negative quadratic term in the case of dose fractionation compared to acute exposure, the overall enhancing effect proves to be virtually negligible.

It has also been pointed out that the life-span shortening in irradiated mice is associated primarily with an increased incidence and/or an acceleration of time of appearance of tumors (*25*). We have calculated the mean survival times of mice bearing epithelial tumors in each distinct experimental series and fitted them to a linear model of the dose in the dose range 0–0.71 Gy. The comparison of the linear coefficients might suggest an increased effectiveness of the fractionated exposure of 22%; however, this difference again is not significant (Student's *t* test = 0.50, df = 9, $P > 0.6$).

No evidence that fractionated doses of neutrons are more effective than acute irradiation could be drawn for lung, liver, skin and soft tissue tumors from trend analysis results (Table III) and age-adjusted incidences (Table IV; Fig. 1, panels C to F).

Fry (*26*) and Broerse *et al.* (*27*), reviewing the time–dose relationship for high-LET radiation, recently concluded that it is not possible to make generalizations about dose-rate or fractionation effects from data for either leukemogenesis or solid tumorigenesis. Our study indicates that a 5-day fractionation of the dose of fission neutrons does not enhance markedly the life-span shortening or the risk of tumor induction in BC3F$_1$ male mice. In the cases where an effect could be quantified, it reached a maximum of 40%.

TABLE IV
Age-Adjusted Percentage Incidences of Selected Tumors in BC3F$_1$ Mice after Fractionated or Acute Exposures to Fission Neutrons

Dose (Gy)	Myeloid leukemia	Lung tumors	Liver tumors	Skin tumors	Soft-tissue tumors	Epithelial tumors
0.00	0.0 (0.5)[a]	3.7 (1.4)	7.9 (2.0)	0.0 (0.5)	4.2 (1.5)	13.2 (2.6)
0.17	3.8 (2.8)	8.3 (3.7)	19.7 (5.5)	0.0 (1.4)	5.3 (3.1)	27.8 (6.4)
	2.2 (2.2)[b]	*9.6 (4.8)*	*16.6 (6.2)*	*0.0 (2.2)*	*0.0 (2.2)*	*25.0 (7.2)*
0.36	2.0 (2.0)	16.0 (6.6)	28.2 (8.3)	3.3 (3.3)	1.9 (1.9)	42.1 (9.3)
	2.4 (2.4)	*16.0 (6.1)*	*11.7 (5.3)*	*2.8 (2.8)*	*10.6 (5.3)*	*31.3 (8.3)*
0.71	2.1 (2.1)	7.7 (4.5)	18.5 (7.2)	5.2 (3.7)	11.6 (5.2)	35.8 (8.7)
	5.2 (3.7)	*17.6 (4.5)*	*33.9 (11.0)*	*6.4 (4.8)*	*17.0 (6.9)*	*46.1 (10.4)*

[a]Numbers in parentheses are standard errors.
[b]Values in italics refer to single acute doses.

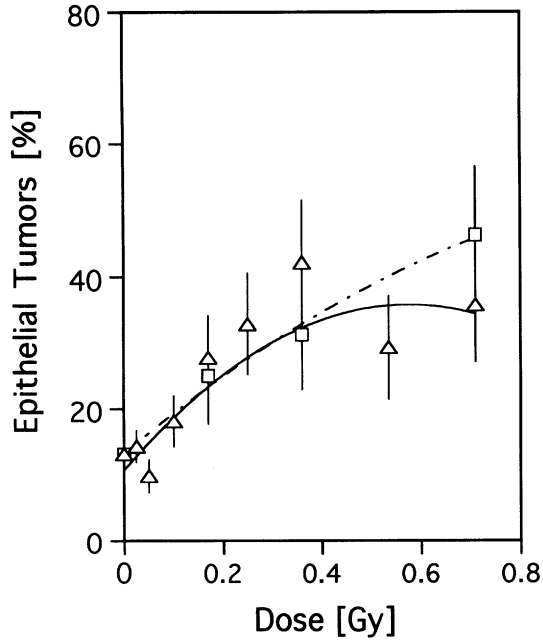

FIG. 2. Variations of age-adjusted percentage incidences of epithelial tumors in $BC3F_1$ male mice irradiated whole-body with fractionated (△) or acute (□) fission-spectrum neutrons. Bars are SE. Solid and dotted lines are for fractionated and acute neutrons, respectively (see text).

Therefore, while our results do not exclude that the biological effectiveness of fission neutrons *in vivo* might be enhanced slightly by dose fractionation, they show that such an enhancement, if existing, would be fairly limited. This is also in agreement with the results of our experiments on the effect of dose fractionation of fission neutrons on exponentially growing C3H 10T1/2 cells, carried out using the same number of fractions and the same interval between fractions in a comparable dose range (*28*).

Nevertheless, in view of the complex nature of cancer and the interplay of many biological factors, gaining more detailed knowledge of the inverse dose-rate effects for high-LET radiation may be useful for its fundamental more than its practical implications in radiological protection.

ACKNOWLEDGMENTS

This study was supported in part by CEC-Radiation Protection Action, Contract FI3P-CT92-0042, Contribution No. 2668. The authors are indebted to RSV-TAPIRO reactor technical staff for neutron irradiation. We express our thanks to Mr. Candido Troiani, Mrs. Marisa Manzotti and Mrs. Maria Frinconi for technical assistance.

Received: July 9, 1993; accepted: December 30, 1993

REFERENCES

1. ICRP, *1990 Recommendations of the International Commission on Radiological Protection*. Publication 60, *Annals of the ICRP* **21**(2), Pergamon Press, Oxford, 1991.
2. H. H. Vogel, Jr. and H. W. Dickson, Mammary neoplasia in Sprague-Dawley rats following acute and protracted irradiation. In *Neutron Carcinogenesis* (J. J. Broerse and G. B. Gerber, Eds.), pp. 135–154. Report EUR 8084, Commission of the European Communities, Luxembourg, 1982.
3. D. L. Lundgren, N. A. Gillett, F. F. Hahn, W. C. Griffith and R. O. McClellan, Effects of protraction of the α dose to the lungs of mice by repeated inhalation exposure to aerosols of $^{239}PuO_2$. *Radiat. Res.* **111**, 201–224 (1987).
4. R. L. Ullrich, Tumor induction in BALB/c mice after fractionated or protracted exposures to fission-spectrum neutrons. *Radiat. Res.* **97**, 587–597 (1984).
5. J. R. Maisin, A. Wambersie, G. B. Gerber, G. Mattelin, M. Lambiet-Collier, B. De Coster and J. Gueulette, Life-shortening and disease incidence in mice after exposure to γ rays or high-energy neutrons. *Radiat. Res.* **128**, S117–S123 (1991).
6. J. F. Thomson, F. S. Williamson and D. Grahn, Life shortening in mice exposed to fission neutrons and γ rays. IV. Further studies with fractionated neutron exposures. *Radiat. Res.* **103**, 77–88 (1985).
7. E. F. Riley, R. C. Miller and A. L. Lindgren, Recovery of murine lens epithelial cells from single and fractionated doses of X rays and neutrons. *Radiat. Res.* **114**, 567–578 (1988).
8. C. Medvedovsky and B. V. Worgul, Neutron effects on the lens. *Radiat. Res.* **128**, S103–S110 (1991).
9. D. Grahn, B. A. Carnes and B. H. Farrington, Genetic injury in hybrid male mice exposed to low doses of ^{60}Co γ-rays or fission neutrons. II. Dominant lethal mutation response to long-term weekly exposures. *Mutat. Res.* **162**, 81–89 (1986).
10. R. W. Hornung and T. J. Meinhardt, Quantitative risk assessment of lung cancer in U.S. uranium miners. *Health Phys.* **52**, 417–430 (1987).
11. J. H. Lubin, Y-L. Qiao, P. R. Taylor, S-X. Yao, A. Schatzkin, B-L. Mao, J-Y. Rao, X-Z. Xuan and J-Y. Li, Quantitative evaluation of the radon and lung cancer association in a case control study of Chinese tin miners. *Cancer Res.* **50**, 174–180 (1990).
12. C. K. Hill, B. A. Carnes, A. Han and M. M. Elkind, Neoplastic transformation is enhanced by multiple low doses of fission-spectrum neutrons. *Radiat. Res.* **102**, 404–410 (1985).
13. C. A. Jones, B. A. Sedita, C. K. Hill and M. M. Elkind, Influence of dose rate on the transformation of Syrian hamster embryo cells by fission-spectrum neutrons. In *Low Dose Radiation* (K. F. Baverstock and J. W. Stather, Eds.), pp. 539–546. Taylor & Francis, London, 1989.
14. J. L. Redpath, C. K. Hill, C. A. Jones and C. Sun, Fission-neutron-induced expression of a tumour-associated antigen in human cell hybrids (HeLa × skin fibroblast). Evidence for increased expression at low dose rate. *Int. J. Radiat. Biol.* **58**, 673–680 (1990).
15. C. K. Hill, A. Han and M. M. Elkind, Fission-spectrum neutrons at low dose rate enhance neoplastic transformation in the linear, low dose region (0–10 cGy). *Int. J. Radiat. Biol.* **46**, 11–14 (1984).
16. V. Covelli, V. Di Majo, B. Bassani, S. Rebessi, M. Coppola and G. Silini, Influence of age on life shortening and tumor induction after X-ray and neutron irradiation. *Radiat. Res.* **100**, 348–364 (1984).
17. V. Di Majo, M. Coppola, S. Rebessi and V. Covelli, Age-related susceptibility of mouse liver to induction of tumors by neutrons. *Radiat. Res.* **124**, 227–234 (1990).
18. P. Pihet, M. Coppola, T. Loncol, V. Di Majo and H. G. Menzel, Microdosimetry study of radiobiological facilities at the RSV-TAPIRO reactor. *Radiat. Prot. Dosim.* **52**, 409–414 (1994).
19. R. Peto, M. C. Pike, N. E. Day, R. G. Gray, P. N. Lee, S. Parish, J. Peto, S. Richards and J. Wahrendorf, Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. In *Long-Term and Short-Term Screening Assays for Carcino-*

gens: A Critical Appraisal, pp. 311–426. IARC Monograph, Suppl. 2, International Association for Cancer Research, Lyon, 1980.

20. V. Covelli, M. Coppola, V. Di Majo, S. Rebessi and B. Bassani, Tumor induction and life shortening in BC3F$_1$ female mice at low doses of fast neutrons and X rays. *Radiat. Res.* **113**, 362–374 (1988).
21. R. L. Ullrich, M. C. Jernigan and J. B. Storer, Neutron carcinogenesis: Dose and dose-rate effects in BALB/c mice. *Radiat. Res.* **72**, 487–498 (1977).
22. V. Covelli, V. Di Majo, M. Coppola and S. Rebessi, The dose–response relationships for myeloid leukemia and malignant lymphoma in BC3F$_1$ mice. *Radiat. Res.* **119**, 553–561 (1989).
23. R. Huiskamp, Acute myeloid leukemia induction in CBA/H mice by irradiation with fission neutrons as a function of exposure rate. *Radiat. Environ. Biophys.* **30**, 213–215 (1991).
24. D. Grahn, L. S. Lombard and B. A. Carnes, The comparative tumorigenic effects of fission neutrons and cobalt-60 γ rays in the B6CF$_1$ mouse. *Radiat. Res.* **129**, 19–36 (1992).
25. M. Coppola, V. Covelli and V. Di Majo, Analysis of mortality from different causes of death in mice exposed to fast neutrons. In *Proceedings of Fifth Symposium on Neutron Dosimetry* (H. Schraube, G. Burger and J. Booz, Eds.), pp. 35–43. Report EUR9762, Commission of the European Communities, Luxembourg, 1985.
26. R. J. M. Fry, Time–dose relationship and high-LET radiation. *Int. J. Radiat. Biol.* **58**, 866–870 (1990).
27. J. J. Broerse, D. W. van Bekkum, J. Zoetelief and C. Zurcher, Relative biological effectiveness for neutron carcinogenesis in monkeys and rats. *Radiat. Res.* **128**, S128–S135 (1991).
28. A. Saran, S. Pazzaglia, M. Coppola, S. Rebessi, V. Di Majo, M. Garavini and V. Covelli, Absence of a dose-fractionation effect on neoplastic transformation induced by fission-spectrum neutrons in C3H 10T1/2 cells. *Radiat. Res.* **126**, 343–348 (1991).