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BIOLOGICAL EFFECTS IN RODENTS EXPOSED TO 10^8 PULSES OF ELECTROMAGNETIC RADIATION

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Abstract—Rodents were exposed to electromagnetic pulse (EMP) radiation to test the hypothesis that rapid changes in electric and magnetic fields would induce injuries in biological systems with high cell turnover rates. The AFRRI EMP generator provided five pulses per second with a peak electric field intensity of 447 kV/m with a 5 nsec rise time and 550 nsec 1/e fall time. Exposures, totalling 10^8 pulses, were continuous except for approximately 2 hr, 5 days per week for biological sampling and animal care during 38 weeks. Biological assays were periodically conducted in exposed and nonexposed animals at appropriate intervals.

It was observed that the reticulocyte count in exposed rats was nearly always greater than in nonexposed rats. However, there were no concomitant differences in peripheral erythrocyte counts between the two groups, nor did radioactive iron incorporation indicate increased cellular production in the irradiated group. Platelet counts in exposed rats were decreased about 10% below those in the nonexposed group most of the time. Levels or relative counts of circulating leukocytes did not differ between the two groups. Bone marrow cellularity was not different between the two groups. Analysis of chromosomes from bone marrow cells showed no detectable increases of aberrations in EMP exposed rats. Routine chemical analysis of blood demonstrated similar values in the two groups. Histological studies indicated no effect of EMP. Observations of fetuses from pregnant rats showed no abnormalities. No incidence of mammary tumors was observed in the female Sprague–Dawley rats. In leukemia prone AKR/J male mice, leukemia did not occur earlier in EMP exposed animals, nor was the fraction of leukemic mice greater in this group when compared with the nonirradiated control mice.

The present experiment utilizing the above-described physical parameters represented a condition exceeding by several orders of magnitude that normally encountered by humans who operate EMP facilities. Exposures of rodents under these conditions indicated no apparent acute injuries.

INTRODUCTION

THE utilization of electromagnetic pulse (EMP) generators by industry and military establishments and the exposure of personnel during routine operation have occurred in recent years (BOWERS and FREY, 1972; HIRSCH and BRUNER, 1970). This potential hazard to man has been a matter of concern, and safety standards have been proposed (DEMOSS, 1971). However, there are not enough biological data to establish firm standards.

Basically, EMP consists of a pulse of radio-frequency waves with a nearly instantaneous rise in the electric and magnetic fields and a subsequent decline in the fields. EMP radiation may be represented as a traveling wave consisting of transverse electric and magnetic oscillating fields; the amplitude of the oscillations is directly related to the power density of the field. There could be an effective energy exchange from the electromagnetic field to the medium whenever these forces are sufficient to alter the kinetic or potential energy of the molecules in the medium. A contributing effect of heat is not predicted because of the low average power of the EMP. It may therefore be questioned whether exposure to EMP could present a thermal hazard to man.

However, at the molecular level in biological systems there are vital ionic and electrochemical processes which could be altered by rapid pulses of electric and magnetic fields. These altered processes could acutely affect biological systems with high cellular turnover, such as the hematopoietic and the reproductive

systems, and possibly introduce somatic changes which could result in fatal disease later in life. In addition, a transient derangement of the neurotransmitting apparatus cannot be excluded (HIRSCH *et al.*, 1968).

There are only a few reports dealing with the effects of EMP on biological systems. Some of these reports indicated a possible biological effect (HELLER, 1970; HOLM and SCHNEIDER, 1970; MICKEY and KOERTING, 1970; SHER *et al.*, 1970), while others detected none (HIRSCH *et al.*, 1968; TAKASHIMA, 1966).

In the present experiment, rodents were exposed to the AFRRI EMP generator utilizing a frequency of pulses and peak electric field strengths several magnitudes higher than existing operating conditions involving exposures of humans. Biological tests involved parameters from systems of high cellular turnover and from tumor inducers. It appears that exposure to 10^8 electromagnetic pulses during 38 weeks does not result in an acute biological hazard.

METHODS

EMP exposure

The AFRRI EMP generator employed in this study provided five pulses per second with a peak electric field intensity of 447 kV/m with a 5 nsec rise time and a 550 nsec 1/e fall time. The pulse generator fed a parallel plate transmission line structure with a provision for the placement of 200 nonmetallic cages between the plates. Other specifications can be found in the report by BRUNHART *et al.* (1973).

Biological parameters were periodically assayed in exposed and nonexposed animals at appropriate intervals during the nearly continuous EMP irradiation. Exposures were interrupted 5 days per week for about 2 hr for biological sampling and animal care. Food and water were supplied *ad lib.* to 700 male and 40 female Sprague–Dawley rats and to 100 male AKR/J mice.

Biological tests

A. *Bone marrow.* This study was designed to include 600 male Sprague–Dawley rats. Of this number, 12 were utilized for the determination of bone marrow cellularity and chromosome aberrations every 2 weeks after exposure to EMP. Half were exposed to EMP and half were not. To determine the proliferative capacity of rat bone marrow, the concentration of mitotic cells was determined 6 hr after the first injection intraperitoneally of colchicine (1 mg/kg) (HOST, 1966; PERRIS *et al.*, 1971). A second injection was made 3 hr after the first. Bone marrow differential counts and estimates of cellularity from one femur were obtained in each animal (SCHALM, 1965). For possible chromosomal aberrations, eight additional rats were used every 2 weeks. The mitotic bone marrow cells were arrested in metaphase using colchicine *in vitro* as prepared by the method previously reported (TJIO and WHANG, 1965).

B. *Blood.* Blood samples (0.2 ml) were obtained via the jugular vein from two groups of 10 continuously irradiated male Sprague–Dawley rats and from nonexposed groups so that one of the groups was utilized per week. The concentrations per mm^3 of erythrocytes, leukocytes, neutrophils, lymphocytes, reticulocytes and platelets were determined from these blood samples (SCHALM, 1965). A group of 30 male Sprague–Dawley irradiated rats and an equal number of nonirradiated controls were used for the measurement of ^{59}Fe incorporation into newly formed erythrocytes. The radioiron (1 μCi of ^{59}Fe in 0.01 μg of total iron) was injected via the tail vein into five groups of six rats each at 6 hr, 1, 7, 14 and 21 days after the onset of the EMP irradiation. Blood sampling and testing for radioactivity was identical to that described before and was conducted 7 days after ^{59}Fe injection (BAUM, 1967). In addition to the procedures described above, blood obtained from five irradiated and five control rats sacrificed for bone marrow was utilized for standard blood chemistry assays, as follows: protein, albumin, calcium, phosphorus, cholesterol, urea nitrogen, uric acid, creatinine, bilirubin and alkaline phosphatase.

C. *Histology.* Histological studies as well as postmortem examinations were performed on the animals sacrificed for bone marrow assays.

D. *Embryology.* Five pregnant rats were placed in the EMP irradiation facility and exposed to 7×10^6 pulses during 17 days of gestation. Five other pregnant animals were utilized as nonirradiated controls. At the end of the exposure time, the fetuses were removed and fixed in Bouin's solution. They were examined grossly for abnormalities and were saved for histological studies.

E. *Mammary tumors.* Twenty female rats were continuously exposed and observed for possible development of mammary tumors and were compared with an equal number of nonexposed animals.

F. *Leukemia.* Approximately 33 weeks after the onset of EMP exposure, the 42 surviving AKR/J leukemia-prone male mice which had been subjected to 8.6×10^7 pulses and 24 surviving nonirradiated controls were sacrificed. Histopathological studies were conducted to test for the presence of leukemia.

Statistics

The *t*-test was used to determine the significance of differences between groups.

RESULTS

A comparison of the total number of nucleated bone marrow cells and the number of mitotic rubricytes and myelocytes in Sprague–Dawley rats exposed to 10^8 electromagnetic pulses and in their nonexposed controls indicated that EMP irradiation did not alter the bone marrow cell concentrations (Fig. 1). Furthermore, there were no changes in the rate of cellular production as represented by the concentration of mitotic cells.

The reticulocyte count of the EMP exposed rats was often significantly greater than that of the nonirradiated animals (Fig. 2). However, no consistent differences in the concentration of circulating erythrocytes were observed between the two groups (Fig. 3). Furthermore, radioactive iron incorporation was similar in both the nonirradiated and the irradiated groups subjected from 0.1 to 7.7×10^6 pulses (Table 1). With the exception of some isolated instances, it did not appear that the number of circulating leukocytes (WBC) differed significantly between the two groups (Fig. 4). No consistent elevation in circulating neutrophils was measured in exposed animals (Fig. 5). Again, except for some isolated sampling periods, the number of circulating lymphocytes did not differ between the two animal groups (Fig. 6). There appeared to be several periods when platelets from irradiated animals were significantly lower than nonexposed groups (Fig. 7).

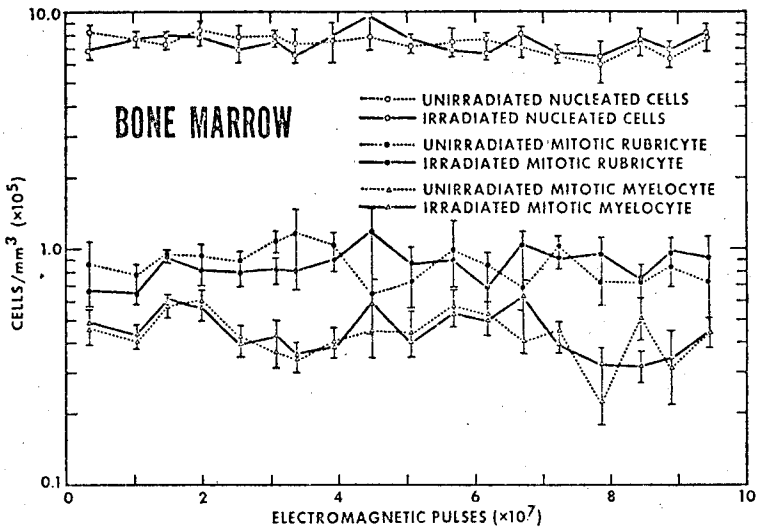

FIG. 1. Nucleated cells from the bone marrow of rat femurs during 38 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

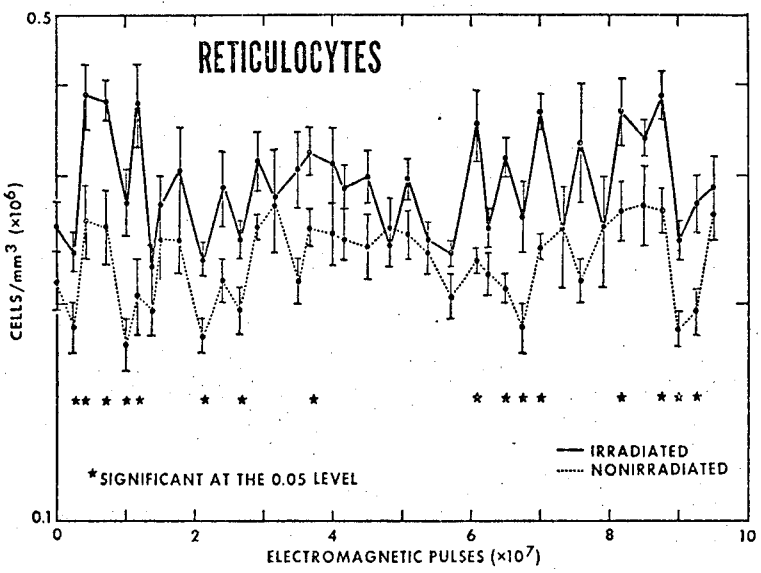

FIG. 2. Reticulocytes in peripheral blood from rats during 38 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

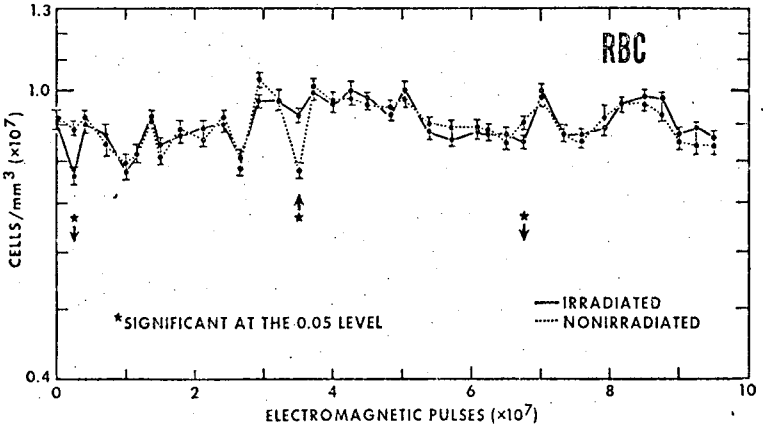

FIG. 3. Red cells in peripheral blood from rats during 38 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

In evaluating the results of other assays, analysis of chromosomes showed no detectable increases in aberrations in EMP exposed rats (Table 2). Histological reports indicated no effect of EMP. Chemical analysis of blood constituents indicated no differences between the two groups (Table 3). Observations of fetuses from rats showed no abnormalities (Table 4). Exposure to EMP did not induce an early onset of spontaneous leukemia in AKR/J mice (Table 5). There were no significant differences in the number of leukemic mice between the two groups nor in their thymic or splenic weights.

At 1 yr of age and after exposure to 10^8 electromagnetic pulses, no mammary tumors have been observed in the experimental female rats and in their nonirradiated controls.

As can be seen in the tabulated summary of the biological parameters tested, the assays

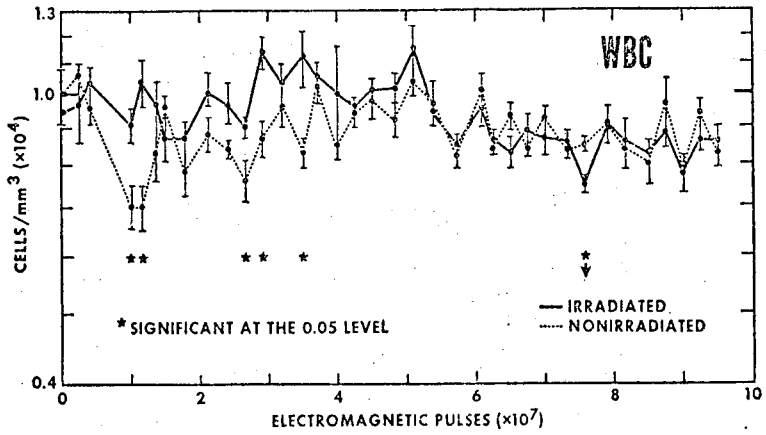

FIG. 4. White cells in peripheral blood from rats during 38 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

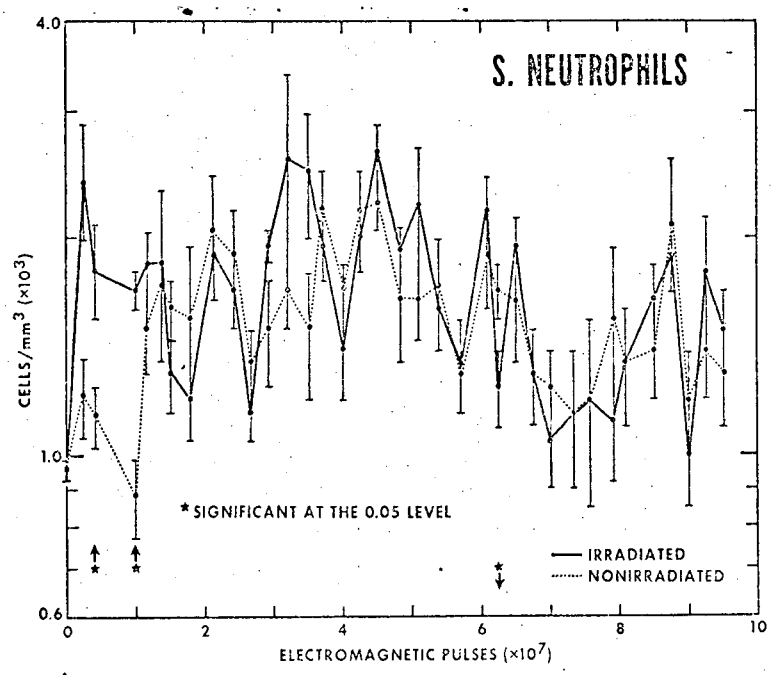

FIG. 5. Segmented neutrophils in peripheral blood from rats during 38 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

showed no acute injurious effects from exposure to 10^8 pulses during 38 weeks of irradiation.

DISCUSSION

Evaluation of the experimental results in the present experiment dealing with rodents exposed to 10^8 pulses of electromagnetic radiation clearly indicates that they did not experience acute biological injuries. At 1 yr of age, the animals had reached approximately half of their life span. Occasionally, significant differences between the irradiated and non-irradiated groups for hematological parameters were observed which never reached physiological abnormality. It is of interest to note that in a preliminary experiment conducted at

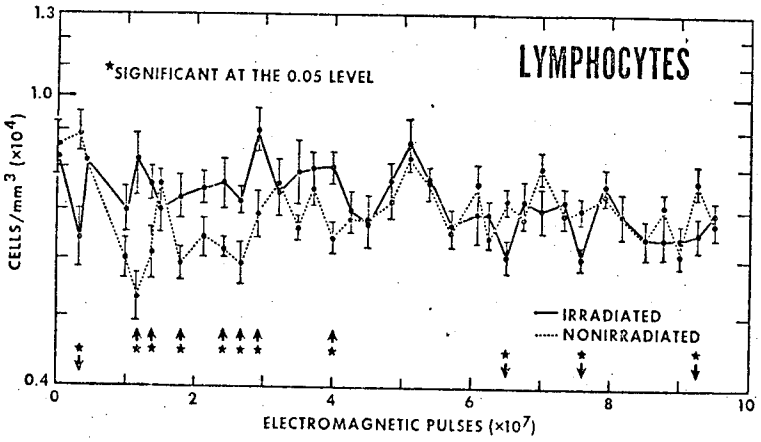

FIG. 6. Lymphocytes in peripheral blood from rats during 38 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

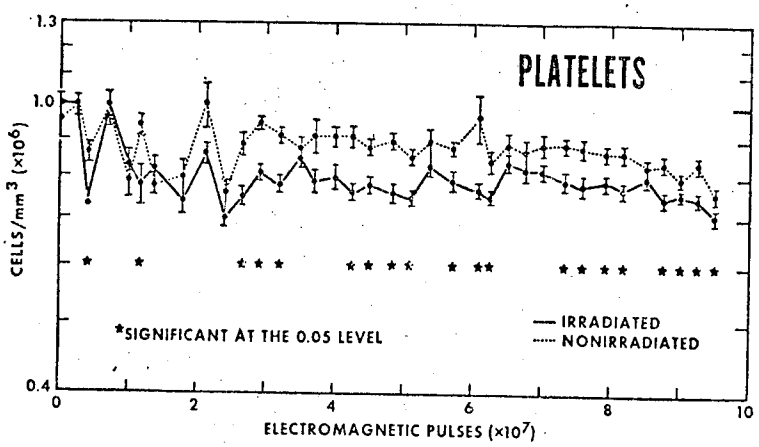

FIG. 7. Platelets in peripheral blood from rats during 38 weeks of EMP exposure. Each point shows a mean value with the associated standard error.

Table 1. Iron-59 incorporation into erythrocytic precursors of rats exposed to EMP radiation

Time group	Pulses ($\times 10^6$)	^{59}Fe uptake*(%)
6 hr	0	82.7 ± 2.1
	0.1	86.4 ± 4.0
1 day	0	81.7 ± 4.6
	0.4	77.9 ± 6.3
7 days	0	80.1 ± 5.3
	2.8	79.7 ± 2.3
14 days	0	72.4 ± 2.9
	5.0	64.8 ± 3.6
21 days	0	85.3 ± 5.2
	7.7	82.9 ± 3.3

* Mean ± S.E. (number = 6).

Table 2. Chromosome aberrations in rats exposed to 10^8 electrogmagnetic pulses during 38 weeks

Group	No. of rats	No. of cells	No. of aberrations
Nonirradiated	40	2.000	2
Irradiated	40	2,000	2

the Lovelace Foundation (HIRSCH and BRUNER, 1970), utilizing dogs, some inconsistent hematological differences were observed.

There appears to be no definite physiological explanation for the observed hematological changes which might propose the possibility

Table 3. Blood chemistry

Serum assay	Nonirradiated*	Irradiated* (5×10^7 pulses)
Protein (g/100 ml)	6.2 ± 0.15	6.2 ± 0.12
Albumin (g/100 ml)	2.8 ± 0.11	2.6 ± 0.11
Calcium (mg/100 ml)	9.3 ± 0.25	9.3 ± 0.23
Phosphorus (mg/100 ml)	5.0 ± 0.15	5.2 ± 0.10
Cholesterol (mg/100 ml)	105 ± 10.0	93 ± 7.2
Urea nitrogen (mg/100 ml)	21 ± 1.0	22 ± 1.0
Uric acid (mg/100 ml)	1.0 ± 0.31	1.3 ± 0.27
Creatinine (mg/100 ml)	0.5 ± 0.02	0.6 ± 0.05
Bilirubin (mg/100 ml)	0.3 ± 0.02	0.3 ± 0.03
Alkaline phophatase (units/100 ml)	177 ± 30	178 ± 39

* Mean ± S.E. (number = 5).

Table 4. Embryology

Group	Normal fetuses*	Resorptions*
Nonirradiated	13.0 ± 1.3	0 ± 0
Irradiated (7×10^6 pulses, 17 days)	13.4 ± 0.6	0.6 ± 0.4

* Mean ± S.E. (Number = 5).

Table 5. Incidence of spontaneous leukemia in AKR/J male mice

	Group	
	Nonirradiated	Irradiated
Electromagnetic pulses (33 weeks)	0	8.6×10^7
Surviving fraction	24/50	42/50
Leukemic fraction	11/24	9/42
Thymus weight (mg)	165 ± 39*	130 ± 27†
Spleen weight (mg)	181 ± 73*	115 ± 23†
WBC (cells/mm^3)	9500 ± 100*	10,000 ± 900†

* Mean ± S.E. (number = 24).
† Mean ± S.E. (number = 42).

Table 6. Summary after 10^8 pulses during 38 weeks of chronic EMP irradiation

Experiment	Biological effect
Blood chemistry	None
Blood count	Variable
Bone marrow	None
Chromosomal aberration	None
Embryology	None
^{59}Fe uptake	None
Histology	None
Leukemia	None
Mammary tumor	None

that they represent extremes of normal biological fluctuations. For example, increases in the number of reticulocytes normally indicate either a transient release of cells from bone marrow compartments or an increase in red cell production. The latter usually persists over a long period of time and eventually shows a corresponding increase in the circulating number of erythrocytes. The data in the present experiment show elevation of reticulocytes in the irradiated animals for several weeks without concurrent increases in peripheral erythrocytes. Furthermore, no increases in ^{59}Fe incorporation of erythrocytic precursors in the EMP exposed rats were measured, clearly indicating no increased cellular production. Although the concentration of platelets is consistently lower in irradiated rats, it is still quite within normal levels and again represents no apparent functional problem.

It was of concern in the present study to determine whether exposure of mice to an EMP field could trigger the onset of spontaneous leukemia. Such possibilities are indicated in rodents exposed to ionizing radiation and subsequently subjected to experimental bleeding (Gong, 1971). The AKR/J mice selected for the present study spontaneously develop leukemia between 6–12 months of age (Skipper *et al.*, 1972). It was proposed that if EMP exposure were inducive to leukemia development, it should be observable at an earlier time in the irradiated mice. However, the results do not demonstrate an earlier onset of the disease in EMP exposed mice. At the time of sacrifice, when the mice had been

subjected to 8.6×10^7 electromagnetic pulses, no differences were observed between them and their nonirradiated controls. It is therefore doubtful if exposure to EMP induces an accelerated onset of leukemia in animals particularly prone to this disease.

As had been emphasized earlier, the present experiment utilizing five pulses per second for 10^8 pulses and a peak electric field strength of 447 kV/m represents a condition in excess of that normally encountered by humans who operate EMP facilities (HIRSCH and BRUNER, 1970). Exposures of rodents under these conditions indicated no apparent acute injuries based on the biological assays employed. It is suggested that existing safety standards might be reevaluated, particularly in regard to acute effects (DEMOSS, 1971). However, since the present experiment has been conducted for only 38 weeks after the onset of EMP radiation exposure, no final assessment could be made for the appearance of late somatic effects (e.g. tumors and cancers) possibly induced early by damage at the molecular level. Such injuries usually would be manifested toward the latter part of the life-span in rodents (2nd yr).

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