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Results of lifespan exposure to continuous and intermittent extremely low frequency electromagnetic fields (ELFEMF) administered alone to Sprague Dawley rats

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ABSTRACT

Background: Up to now, experimental studies on rodents have failed to provide definitive confirmation of the carcinogenicity of extremely low frequency electromagnetic fields (ELFEMF). Two recent studies performed in our laboratory on Sprague-Dawley rats reported a statistically significant increase in malignant tumors of different sites (mammary gland, C-cells carcinoma, hemolymphoreticular neoplasia, and malignant heart Schwannoma) when ELFEMF exposure was associated with exposure to formaldehyde (50 mg/l) or acute low dose of γ-radiation (0.1 Gy) (Soffritti et al., 2016a) (Soffritti et al., 2016b). The same doses of known carcinogenic agents (50 mg/l formaldehyde, or acute 0.1 Gy γ-radiation), when administered alone, previously failed to induce any statistically significant increase in the incidence of total and specific malignant tumors in rats of the same colony.

Objectives: A lifespan whole-body exposure study was conducted to evaluate the possible carcinogenic effects of ELFEMF exposure administered alone to Sprague-Dawley rats, as part of the integrated project of the Ramazzini Institute (RI) for studying the effects on health of ELFEMF alone or in combination with other known carcinogens.

Methods: Male and female Sprague-Dawley rats were exposed 19 h/day to continuous sinusoidal-50 Hz magnetic fields (S-50 Hz MF) at flux densities of 0 (control group), 2, 20, 100 or 1000μT, and to intermittent (30 min on/30 min off) S-50 Hz MF at 1000 μT, from prenatal life until natural death.

Results: Survival and body weight trends in all groups of rats exposed to ELFEMF were comparable to those found in sex-matched controls. The incidence and number of malignant and benign tumors was similar in all groups. Magnetic field exposure did not significantly increase the incidence of neoplasias in any organ, including those sites that have been identified as possible targets in epidemiological studies (leukemia, breast cancer, and brain cancer).

Conclusions: Life-span exposures to continuous and intermittent sinusoidal-50 Hz ELFEMFs, when administered alone, did not represent a significant risk factor for neoplastic development in our experimental rat model. In light of our previous results on the carcinogenic effects of ELFEMF in combination with formaldehyde and γ-radiation, further experiments are necessary to elucidate the possible role of ELFEMF as cancer enhancer in presence of other chemical and physical carcinogens.

1. Introduction

Early epidemiological studies have shown that human exposure to ELFEMF was associated with increased risk of leukemia in children (Wertheimer and Leeper, 1979), as well as lymphomas/leukemias in adults (Milham, 1982), breast cancer in women aged less than 55 years (Wertheimer and Leeper, 1979) and also in men (Matanoski et al., 1991; Matanoski and Breysse, 1989; Demers et al., 1991; Tynes et al.,

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1992). A pooled analysis identified a significant increased risk of childhood leukemia at exposures greater than 0.4μT (Ahlbom et al., 2000). The epidemiological evidence led the International Agency for Research on Cancer (IARC) to classify ELFEMF as a possible carcinogen (IARC, 2002). Further case control studies (Draper et al., 2005; Kroll et al., 2010; Sermage-Faure et al., 2013) and a pooled analysis based on primary data (Kheifets et al., 2010) estimated an approximately two-fold increased risk of childhood leukemia at magnetic fields levels above 0.3–0.4μT.

Starting from the first case-control study performed in New Zealand on electricians employed in power transformer plants (Pearce et al., 1989), several publications have shown statistically significant increases in the incidence of lymphoma and leukemia in adult males exposed to ELFEMF at the workplace (Bastuji-Garin et al., 1990; Richardson et al., 1992; Milham, 1996; Villeneuve et al., 2000; Bethwaite et al., 2001; Lowenthal et al., 2007)

Despite the epidemiological evidence, up to now experimental long-term bioassays in which ELFEMF were administered alone to rodents have failed to confirm the carcinogenic potential of ELFEMF exposure (Mandeville et al., 1997; Margonato et al., 1995; Yasui et al., 1997; Boorman et al., 1999; McCormick et al., 1999; Qi et al., 2015). Studies to date have typically lacked the size to identify rare events and have not lasted long enough to track diseases in later life. Furthermore, these studies have not taken into account *in utero* exposure, with the exception of two studies, one conducted by Mandeville et al. (1997) on small groups of Fisher 344 rats and another study conducted by Qi et al. (2015) on small groups of B6C3F1 mice. Mechanistic evidence on the genotoxicity of ELFEMF is also inconclusive (IARC, 2002), although recently DNA strand breaks have been reported in Vero cells exposed to ELFEMF (100 Hz, 5.6 mT) for 45 min (Mihai et al., 2014). As IARC pointed out "it is also relevant to ask whether ELF electric and magnetic fields have effects similar to those of known 'non-genotoxic' carcinogens, 'tumor promoters' or 'cocarcinogens', i.e. agents that seem to enhance cancer by a mechanism other than that of direct DNA damage" (IARC, 2002). Several effects on molecular pathways that might be relevant to cell proliferation and transformation have been associated with the exposure to ELFEMF at cellular level, including changes of flux through voltage-gated calcium channels and resonant interactions with complex ion-biomolecules, but there is no compelling evidence for anyone of these mechanisms and their causal role in cancer (Liboff et al., 1989; Blackman et al., 1985; WHO, 2007).

This background motivated the RI to plan a project of life-span experimental studies on ELFEMF designed to evaluate the carcinogenic potentiality of ELFEMF alone, and also in association with other known carcinogenic agents, in order to simulate different possible human exposure situations (Soffritti et al., 1999). The distinctive characteristics of this project were: 1) the use of a large number of male and female rats per group in order to enhance the sensitivity and the statistical power of the studies; 2) starting the exposure to ELFEMF right from prenatal life and continuing until natural death; 3) inclusion of all rats of each litter in order to consider family effects; 4) the availability of a total of almost 20,000 male and female historical controls monitored over 40 years in a standardized way, including the same diet and same environmental conditions; 5) all rats submitted to complete necropsy and histological evaluation; 6) the experiments of the project started concurrently in order to compare the effects of the different exposure situations and use the same control group of animals; and 7) the animals were those born during the simultaneous breeding of 750 male and 750 female breeders, matched outbred.

The design of the project encompassed 4 experiments; one experiment included 5029 Sprague-Dawley rats exposed only to ELFEMF; in the other 3 experiments, groups of rats were exposed to ELFEMF plus γ-radiation (657 rats), or formaldehyde (805 rats), or Aflatoxin B1 (642 rats). Overall the project encompassed 7133 rats and all the experiments started concurrently and shared the same control group.

We recently reported the first results from our comprehensive experimental project, showing that life-span exposure to ELFEMF in combination with formaldehyde (50 mg/L, from 6 weeks of age until 104 weeks of age), or with an acute low dose γ-radiation (0,1 Gy at 6 weeks of age), induced significant specific carcinogenic effects in our Sprague-Dawley rats (Soffritti et al., 2016a, b). The same doses of known carcinogenic agents (50 mg/l formaldehyde, or acute 0.1 Gy γ-radiation), when administered alone, previously failed to induce any statistically significant increase in the incidence of total and specific malignant tumors in rats of the same colony (Soffritti et al., 1989, 2002, 2015).

In this paper we report the results of ELFEMF administered alone in Sprague-Dawley rats from prenatal life until natural death. The plan of the experiment is shown in Table 1. The results of the present study on ELFEMF alone are then compared with our previously reported results on the effects of ELFEMF in combination with formaldehyde or acute low dose γ-radiation.

Table 1
Long-term bioassay on S-50 Hz ELFEMF, administered alone to Sprague-Dawley rats, from prenatal life to spontaneous death: plan of the experiment.

Group	Treatment	Animals	
No.	S-50 Hz ELFEMF (μT)[a]	Sex	No.
I	0[b]	M	500
		F	501
		M + F	1001
II	2 C	M	500
		F	502
		M + F	1002
III	20 C	M	501
		F	502
		M + F	1003
IV	100 C	M	500
		F	500
		M + F	1000
V	1000 O/O	M	250
		F	250
		M + F	500
VI	1000 C	M	253
		F	270
		M + F	523
Total			5029

[a] The treatment with S-50 Hz for19 h/day, in continuous (C) or intermittent (O/O) mode.
[b] The control group is in common with the experiments on the effects of ELFEMF in combination with formaldehyde and γ-radiation (BT2CEM; BT3CEM).

2. Materials and methods

2.1. S-50 Hz MF exposure conditions and facilities

To ensure the experimental animals had the same environmental conditions (temperature 22 ± 3 °C, relative humidity 40–60% and 12 h/day homogeneous diffusion of light), the rats were located in a room of 60 × 15 × 4 m, in all more than 900sqm.

The ELFEMF exposure system was previously described in our papers (Soffritti et al., 2016a, b). The MF exposure system was constructed so as to satisfy a number of technical conditions, namely: (1) the magnetic field was linearly polarized; (2) the field lines were horizontal and parallel to the ground; (3) the field uniformity was better than 10%; (4) the current supply had a maximum harmonic distortion of 3%; (5) the field rise time at power-up was at least 10 periods (for 50 Hz, 200 ms); (6) the current generator was noiseless; (7) the joule effect on windings did not alter the environmental temperature, a maximum variation of 2 °C being tolerated near coils; (8) coil noise and vibration were absent; and (9) the natural field level was no more than 0.1 μT and all mutual interaction of the system was avoided, while in any case the

Fig. 1. RI studies on ELFEMF: exposure system. The exposure system was based on independent devices, each one serving at least 500 rats. The toroidal shaped device guaranteed the absence of interference between the structures. A wooden support structure for rat cages was mounted inside the toroidal magnet. Note that the thoroidal structures were all located in a unique room of more than 900 m^2, in order to ensure a homogeneous conduct of the experiments.

control group stayed in the same room.

The exposure system was based on independent devices. Each simple exposure device served at least 500 rats, leaving enough space to isolate ill/moribund rats. In order to satisfy stray field requirements, a good solution was obtained by using a toroidal-shaped device. Fig. 1

shows the device's magnetic structure. All the devices needed were identical and the different intensity of MF was obtained by properly tuning the power supplies which were of the current-controlled type. The toroidal shaped device guarantees the absence of interference between the structures. The fact is that, about 1 m away from the external torus boundary producing 1 mT, the field level is approximately 0.1 μT (Montanari, 2003).

The toroid was designed with 24 coils made of three turns of insulated copper cable, mounted on a superstructure of aluminum composed of two insulated parts in order to avoid a closed loop subject to total field. The total copper cross section was 11 × 28 mm^2, and the total current used for 1 mT level was 359.6 A. The electric power was supplied by low density current and the large amount of a good thermal-conducting insulation prevented heating, leaving the device at room temperature. Vibrations and noise were proved to be absent.

Mounted inside the toroidal magnet was a wooden support structure for rat cages. One of the toroids to be used was mounted and treated in order to verify the correctness of the computed parameters pertaining to the experiment. All results were in agreement with computed values.

A magnetic field probe was placed at a representative animal location to monitor the fields. An information system continuously stored the exposure data throughout the experiment. The details of the exposure system have been described by Montanari (2003).

2.2. Diet

All the animals received standard feed administered in pellets ad libitum and provided by the “Laboratorio Dottori Piccioni” (Milan, Italy), the formulation being certified for each supply used at the Cesare Maltoni Cancer Research Center of the RI (CMCRC/RI) over a period of more than 40 years. All the animals received tap water ad libitum. Both feed and water were periodically analyzed to exclude the presence of contaminants.

2.3. Experimental animals

Sprague-Dawley rats from the same colony used for more than 40 years at the CMCRC/RI were used as experimental animals. The basic expected tumorigram and its fluctuations were based upon data derived from more than 20,000 historical controls.

Generation of experimental animals was performed following the standard operating procedure of the CRCCM/RI already described in our previous work (Soffritti et al., 2016a).

All the male and female breeders were euthanized by CO_2 overexposure respectively 3 weeks after birth of offspring and 1 week after their weaning at 4–5 weeks of age.

The experimental animals were identified by ear punch (Jackson Laboratory method) and distributed by sex, litter by litter, until the planned number for each group was reached. After weaning, animals received ordinary feed and water ad libitum. Animals were housed 5 per cage, in polycarbonate cages (41 × 25 × 15) and a shallow layer of white wood shavings as bedding. All the animals were kept in a temperature-controlled environment at 22 ± 3 °C and 40–60% relative humidity, with 12 h/daylight/dark alternation.

The experiments were conducted according to the current (2001–2004) Italian law regulating at the time, the protection of animals used for experimental and other scientific purposes (Decreto Legislativo 116, 1992). The experiment was performed following the principles of Good Laboratory Practice (GLP), with the same standard operating procedure as in our concurrent combination studies BT2 CEM and BT3 CEM (Soffritti et al., 2016a, 2016b).

2.4. Treatment

Five groups of male and female Sprague-Dawley rats were exposed to ELFEMF continuously at 0, 2, 20, 100 or 1000μT, or intermittently at 1000μT of S-50 Hz MF (BT1 CEM). Treatment with S-50 Hz MF began during fetal life exposing the female breeders from the 12th day of pregnancy, and for the offspring it lasted until natural death. Daily duration of the exposure lasted 19 h/day throughout the study. The exposure system was deactivated (switched off) for 5 h a day in order to allow clinical observation of animals as well as room and animal cleaning. The animals treated with 1000μT were distributed into two groups receiving either continuous exposure or intermittent treatment consisting in 30 min on/ 30 min off.

2.5. Histopathology

All dead rats were submitted to necropsy and the following organs and tissues 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,

esophagus, stomach (fore and glandular), intestine (four levels), bladder, prostate, uterus, ovaries, testes, interscapular fat pad and subcutaneous, mediastinal and mesenteric lymph

nodes. The organs and tissues collected were preserved in a 70% solution of Solvanol (a mixture of ethyl and isopropyl alcohol respectively, approx. 60% and 40%, obtained from Vital srl, Bologna, Italy), and 30% distilled water, apart from bone tissues which were preserved in 10% formalin and then decalcified.

All lesions were trimmed so as to include a portion of adjacent normal tissue. As far as normal tissues and organs are concerned, trimming was performed according to standard laboratory procedures. The trimmed specimens were processed and embedded in paraffin blocks according to standard operating procedures (SOP) of the laboratory. Then 3–5 μm sections were cut and routinely stained with Hematoxylin-Eosin. A histopathology evaluation was performed in blind by at least two pathologists. At least one senior pathologist peer-reviewed all lesions of oncological interest as well as any lesion of dubious interpretation. In the pathological diagnosis, all the pathologists used the same evaluation criteria and the same classification based on international standard criteria (INHAND, NTP), described in the

specific SOP and long adopted at the CMCRC/RI. The diagnoses are reported in the experimental registries.

2.6. Statistical analyses

Statistical analysis for possible differences in survival times was based on Kaplan-Meier survival curves evaluated by Log-rank tests, as well as on the Cox proportional hazard regression model (Cox, 1972). To highlight possible differences in the incidence of tumors among treated groups and controls or among different treated groups, Chi-squared and Fisher tests were performed. The Chi-squared test was used when the number of tumors was higher than 5 in all groups; in all other cases Fisher's Exact test was used. The level of significance was set at $p \leq 0.05$. The statistically significant p-values found are reported in the tables. The presence of a linear trend in tumor incidences was evaluated by the Cochran-Armitage trend test with a level of significance set at $p \leq 0.05$. In the BT1CEM (S-50 Hz MF alone) experiment, the Cochran-Armitage trend test was performed both considering all groups, and omitting the group treated intermittently to ELFEMF (O/O group).

3. Results

3.1. Food and water consumption, body weight and survival

The experiment proceeded as planned and no unexpected alteration in the clinical status of the animals was observed in the various groups. The biophase parameters for control and treated groups are presented in Fig. 2. No differences were observed in mean water consumption (A and B), food consumption (C and D), mean body weight (E and F) or survival index (G and H), either in male or in female rats.

3.2. Carcinogenic effects of ELFEMF alone (BT1 CEM)

No statistically significant increase in the incidence of tumors was observed in animals exposed to ELFEMF alone. The overall incidence of benign and malignant neoplasms is reported in Table 2 and a list of all the neoplastic lesions is reported in the Supplementary Material. An increase, though not statistically significant, was observed in the total malignant tumors incidence of both male (39.0 vs 35.0%) and female (48.0 vs 42.5%) rats exposed intermittently to 1000μT compared to controls. In a period of over 15 years (1984–2001), the data on historical control rats of the RI showed that the incidence of malignant tumors out of 2415 untreated males was 38,0% and out of 2424 untreated females the incidence was 44,9%.

A broad spectrum of neoplastic and non-neoplastic lesions was observed in experimental rats. However, the pathological findings in both sexes were consistent with the expected pattern of age-related changes in this species and strain and provided no evidence of any carcinogenic effect related to the exposure to ELFEMF alone.

3.3. Comparison of the results of our studies on ELFEMF administered alone (BT1 CEM) or in association with chemical or physical agents(BT2 CEM and BT3 CEM)

The present results in terms of specific tumors incidences in rats exposed for their lifespan to different intensities of ELFEMF alone are compared in Table 3 with the results of the studies on lifespan exposures to ELFEMF in combination with formaldehyde (50 mg/L, from 6 weeks of age until 104 weeks of age), or with acute exposure of γ-radiation (0.1 Gy at 6 weeks of age) (Soffritti et al., 2016a, b) (Table 3).

4. Discussion

This article represents the conclusion of the RI project of life-span experimental studies on ELFEMF designed to evaluate the carcinogenic potentiality of ELFEMF alone and in association with other known carcinogenic agent. This extensive and comprehensive project took more than 15 years to be completed, studying over 7000 animals from prenatal life up to the point of their natural death, including routine examination of more or less 200,000 histological slides, for a financial commitment equivalent to 7,000,000 Euros. The RI project on ELFEMF was supported only by independent funds, the most part by citizens and associates of the RI. In consideration of the results presented in this article on ELFEMF alone and our previously published results on ELFEMF in combination with formaldehyde or with acute exposure of γ-radiation, we observe that ELFEMF alone did not represent a significant risk factor for neoplastic development in our experimental rat model. On the other hand, ELFEMF seem to act as cancer enhancer in presence of other chemical and physical carcinogens (formaldehyde and γ-radiation). We will now discuss in more detail the different carcinogenic effects of ELFEMF when administered alone or in combination with formaldehyde and γ-radiation, focusing on the different effects on the incidence total malignant tumors, mammary gland carcinoma, malignant heart Schwannoma, thyroid C-cell carcinoma and hemolymphoreticular neoplasia (Table 3). These experimental findings are of particular interest in light of the epidemiological evidence of rising incidences worldwide of thyroid cancer, breast cancer (also in males), lymphoma and leukemia (Ly et al., 2013; Fidler et al., 2017; Howlader et al., 2017). Since the experiments were performed at the same time with the same experimental conditions in all the treatment groups (ELFEMF administered alone or in combination with formaldehyde and γ-radiation) and the same concurrent control group was used, this study designs results adequate and relevant for comparing the effects between ELFEMF alone or in combination with other known carcinogens.

4.1. ELFEMF and total malignant tumors

No statistically significant differences in the incidence of total malignant tumors were observed in rats exposed to all doses of ELFEMF when administered alone. A statistically significant increased incidence of total malignant tumors was observed in male rats exposed to 1000μT plus 50 mg/L formaldehyde, in males and females exposed to 20 μT plus 0.1 Gy γ-radiation, and in males exposed to 1000 μT plus 0.1 Gy γ-radiation. A statistically significant positive trend in the incidence of total malignant tumors was observed, using the Cochran-Armitage test, both in male rats from the ELFEMF/formaldehyde experiment and male and female rats from the ELFEMF/γ-radiation experiment. Only non-statistically significant increase in the incidence of total malignant tumors were observed in female and male rats treated with formaldehyde and γ-radiation alone. The total malignant tumor incidence significantly increased only when a known carcinogenic physical or chemical agent was administered in association with ELFEMF (Table 3). Our results support the hypothesis that the combined exposure to ELFEMF and formaldehyde or γ-radiation might enhance the onset of malignant tumors.

4.2. ELFEMF and breast cancer

Breast cancer is the most prevalent malignant disease in women, and its incidence continues to increase. In 1987, Stevens suggested that ELFEMF and visible light at night (around 1015 Hz) might increase the long term risk of breast cancer (Stevens, 1987). To date, numerous laboratories around the world have examined the correlation between ELFEMF and the development of breast cancer. An enhancing effect of ELFEMF on the induction of breast cancer in the rat had been first observed in the second half of the 1990s (Löscher and Mevissen, 1995; Thun-Battersby et al., 1999) after co-treatment with DMBA. These effects have been repeatedly confirmed (Fedrowitz and Löscher, 2005, 2008).

In a review of the published literature on magnetic fields and mammary cancer in rodents (12 studies), Boorman et al. (2000) wrote that ‘when considered in total, the results of these studies demonstrated

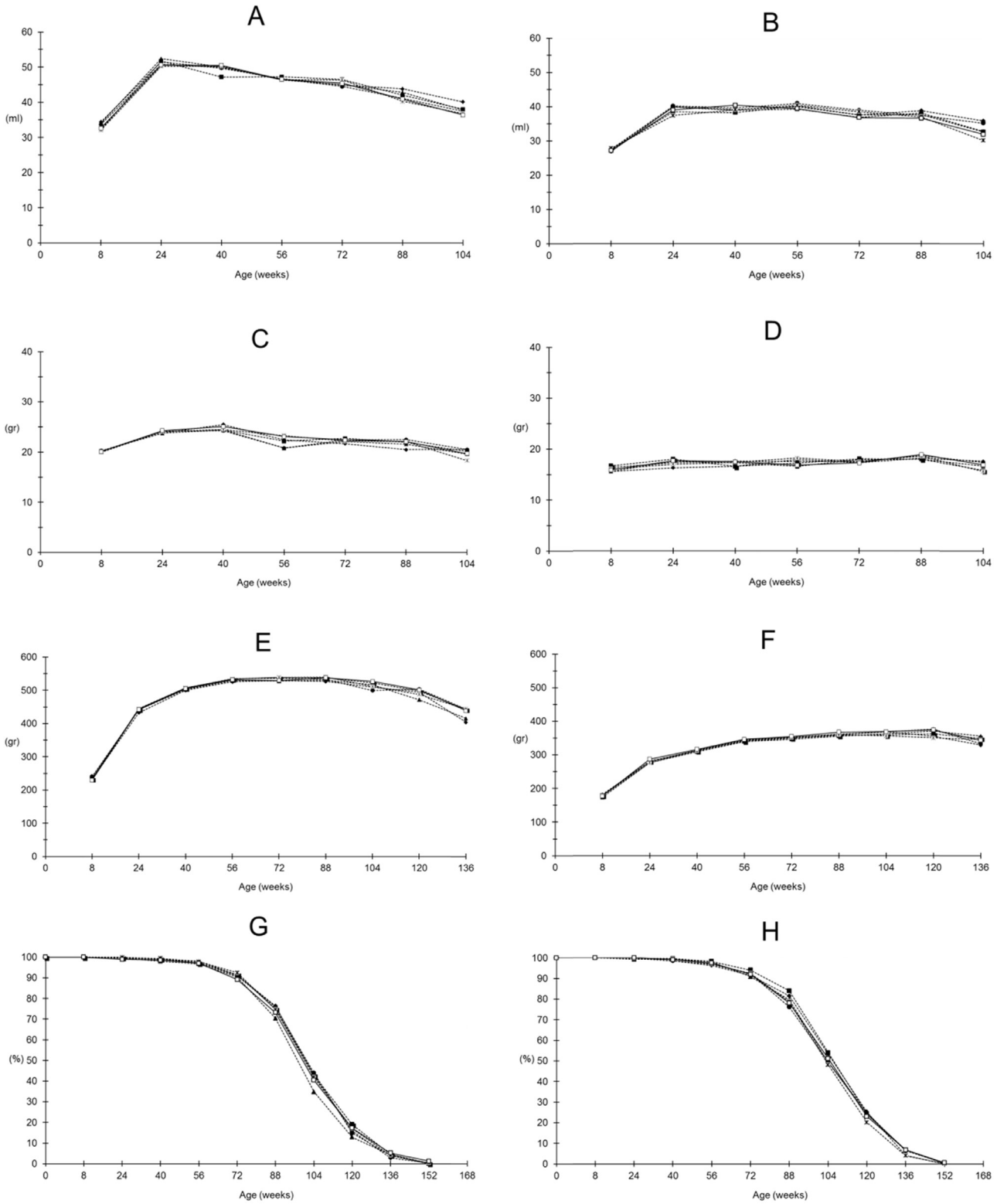

Fig. 2. Food and water consumption, body weight and survival. Male (A) and female (B) water consumption, and male (C) and female (D) food consumption from 8 to 104 weeks of age; male (E) and female (F) mean body weight from 8 to 136 weeks of age; male (G) and female (H) survival index from 0 to 152 weeks of age. Data shown refer to control group (□), C 2 μT CEMBF (*), C 20 μT CEMBF (•), C 100 μT CEMBF (■), O/O 1000 μT CEMBF (▲), and C 1000 μT CEMBF (♦) treated group.

either negative or inconsistent positive results across five endpoints' (among which there was tumor incidence) and he concluded with a citation of the U.S. National Institute of Environmental Health Science that 'the collection of studies provides strong evidence of no effect of magnetic fields on the promotional development of mammary cancer".

In our studies, no statistically significant increase in mammary adenocarcinomas was observed in rats exposed to all doses of ELFEMF administered alone. When the exposure occurred in conjunction with acute γ-radiation, males exposed to ELFEMF 20 μT plus 0.1 Gy showed a significant increased incidence of mammary carcinoma (2.9%) when

Table 2
Long-term bioassay on S-50 Hz ELFEMF, administered alone to Sprague-Dawley rats, from prenatal life to spontaneous death: incidence of benign and malignant tumors.

Group no. Treatment (μT)[a]	Animals		Tumor-bearing animals			
			Benign tumors		Malignant tumors	
	Sex	No.	No.	%	No.	%
I	M	500	253	50.6	175	35.0
(0)	F	501	341	68.1	213	42.5
	M + F	1001	594	59.3	390	39.0
II	M	500	251	50.2	175	35.0
(2 C)	F	502	357	71.1	207	41.2
	M + F	1002	608	60.7	380	37.9
III	M	501	225	44.9	185	36.9
(20 C)	F	502	350	69.7	210	41.8
	M + F	1003	575	57.3	395	39.4
IV	M	500	260	52.0	133	26.6[N]
(100 C)	F	500	365	73.0	203	40.6
	M + F	1000	625	62.5	336	33.6
V	M	250	128	51.2	98	39.2
(1000 O/O)	F	250	177	70.8	120	48.0
	M + F	500	305	61.0	218	43.6
VI	M	253	132	52.4	86	34.1
(1000 C)	F	270	196	72.6	118	43.7
	M + F	523	328	62.8	204	39.1

[a] The treatment with S-50 Hz MF for19 h/day, in continuous (C) or intermittent (O/O) mode.
[N] Significantly lower with respect to the control value using Chi-squared and Fisher tests ($p < 0.05$).

compared to untreated controls (0.2%); moreover, an increased incidence of mammary adenocarcinoma, though not statistically significant, was also observed in males from the group treated with ELFEMF 1000 μT plus 0.1 Gy (0.9%). Notably, a statistically significant increase in breast cancer among male rats exposed to higher doses of γ-radiation (1 Gy) was shown by our laboratory in a previous experiment, but not in male and female rats treated with the lowest dose of 0.1 Gy (Soffritti et al., 2015). Mammary cancer is a rare tumor in male rats. Our historical male controls of the same period showed an average mammary cancer incidence of 0.3% with a range between 0% and 1.3% which is similar to the incidence observed in all groups treated with ELFEMF alone (range 0–0.4). These experimental findings on the possible effects of ELFEMF as breast cancer enhancer in males are also consistent with the epidemiological evidence of an increased breast cancers incidence in men occupationally exposed to ELFEMF (Demers et al., 1991; Loomis, 1992; Sun et al., 2013).

Similarly, female rats exposed to 1000 μT plus 0.1 Gy showed a statistically significant increase in the mammary gland adenocarcinoma incidence (16.1%) when compared with both females exposed only to 0.1 Gy (positive controls), and untreated controls (7.6% and 6.4%, respectively). Moreover, female rats from the experiment in which ELFEMF are combined with acute exposure to γ-radiation showed a significant positive trend, using the Cochrane-Armitage test. No statistically significant differences in the incidence of mammary gland carcinoma were observed when rats were exposed to ELFEMF in combination with formaldehyde, in either males or females (Table 3).

4.3. ELFEMF and malignant Schwannoma of the heart

Malignant Schwannoma of the heart is a rare tumor in rodents, as it is in humans, comprising only 0.75% of all primary cardiac tumors (Morishita et al., 1988). In humans, genetic factors (e.g. neurofibromatosis) and environmental factors (e.g. γ radiation) can increase up to 10 fold the risk of malignant progression of Schwannoma (Seferis

Table 3
Compared incidence of specific malignant tumors related to the exposure to ELFEMF (S-50 Hz) administered alone (BT1CEM) or associated with formaldehyde (BT2CEM) or γ-radiation (BT3CEM) (Soffritti et al., 2016a, 2016b).

Experiment	Treatment		Animals at start		Tumor incidences				
	ELFEMF (μT)	Other	Sex	No.	Total malignant tumors	Mammary gland adenocarcinomas	Heart malignant Schwannomas	Thyroid C-cell carcinomas	Hemolymphoreticular neoplasias[a]
					%	%	%	%	%
Historical controls (up to 2001)			M	2415	38.0	0.5	0.7	1.0	20.5
			F	2424	44.9	8.9	0.4	1.4	12.8
Concurrent controls	0	/	M	500	35.0	0.2	0.2	1.0	16.6
			F	501	42.5	6.4	0	1.6	13.6
BT1CEM	2 C	/	M	500	35.0	0.4	0.6	1.4	14.8
			F	502	41.2	7.6	0.2	1.0	9.2
	20 C	/	M	501	36.9	0	0.4	1.8	19.6
			F	502	41.8	7.2	0.6	1.2	14.5
	100 C	/	M	500	**26.6**[N]	0.2	0	1.0	12.4
			F	500	40.6	6.6	0.8	1.4	11.2
	1000 O/O	/	M	250	39.2	0	0.4	2.0	18.0
			F	250	48.0	6.8	0	1.6	12.8
	1000 C	/	M	253	34.1	0	0.8	1.2	14.2
			F	270	43.7	8.1	0.4	1.1	16.3
BT2CEM	0	50 mg/L Formaldehyde	M	200	41.5	0	0	1.5	22.0
			F	202	47.0	6.9	0.5	2.5	11.4
	1000 C	50 mg/L Formaldehyde	M	200	**48.5****	0.5	0.5	**4.0***	**23.5***
			F	203	37.9	3.9	0.5	1.5	12.3
BT3CEM	0	0.1 Gy γ-radiation	M	118	39.0	0	**0**	0	15.3
			F	105	44.8	7.6	1.0	0	12.4
	20 C	0.1 Gy γ-radiation	M	105	**51.4****	**2.9***	1.9	2.9	18.1
			F	107	**59.8****	7.5	0.9	2.8	19.6
	1000 C	0.1 Gy γ-radiation	M	110	**54.5****	0.9	**2.7***	2.7	**25.5***
			F	112	51.8	**16.1****	0	2.7	14.3

*,** Significantly increased from control value by Chi-squared and Fisher tests: *$p < 0.05$; **$p < 0.01$.
[a] Hemolymphoreticular neoplasias include lymphomas, leukemias and histiocytic sarcomas.
[N] Significantly lower incidence with respect to control value using Chi-squared and Fisher tests ($p < 0.05$).

et al., 2014). In a period of over 15 years (1984–2001), the data on historical control rats of the RI showed that the incidence of Schwannoma out of 2415 untreated males was 0.7% and out of 2424 untreated females the incidence was 0.1%. The pathological diagnostic criteria of malignant glial tumors have been recently revised by the NTP and the pathological diagnosis of the RI were performed in blind and in compliance with the most recent NTP recommendations and the INHAND guidelines (Elmore et al., 2017; Kaufmann et al., 2012).

There was no statistically significant increase in schwannoma of the heart in male and female rats exposed to ELFEMF alone. When the administration of ELFEMF to male rats was associated with 0.1 Gy radiation, the incidence increased by up to 1.9% (not statistically significant) and 2.7% (statistically significant) in the 20 μT and 1000 μT treated groups respectively. These tumors proved positive to immunohistochemistry for S100. Several IHC markers have been studied for their potential use in the diagnosis of pre-neoplastic and neoplastic nervous system tumors. Among these, S100 is one of the most commonly used and validated ones (Fregoso and Hoover, 2012). Our results support the hypothesis that the combined exposure to ELFEMF plus 0.1 Gy radiation might enhance the onset of this rare tumor. No enhancing effects were observed when ELFEMF were combined with formaldehyde (Table 3). It is noteworthy that also exposure to electromagnetic fields of radiofrequency statistically significantly increased the incidence of these rare tumors in treated animals (Wyde et al., 2016; NTP, 2018; Falcioni et al., 2018).

4.4. ELFEMF and thyroid carcinoma

C-cell thyroid carcinomas are rare malignant tumors of the thyroid. In humans, C-cell carcinoma around 4% of all thyroid malignancies (ACS, 2017). In a period of over 15 years (1984–2001), the data on historical control rats of the RI showed that the incidence of C-cell carcinoma out of 2415 untreated males was 1% and out of 2424 untreated females the incidence was 1.4%. Among our untreated historical controls of the same period, out of 2415 male Sprague-Dawley rats we observed an overall incidence in C-cell carcinomas of 1.0% (0 − 2.1%).

Among the long-term carcinogenicity bioassays conducted up to now by the US National Toxicology Program (NTP), only two compounds, Ziram and 2,4 Diaminoanisole sulfate, have shown clear evidence of increasing C-cell carcinomas in rats (NTP, 1978, 1983). This is out of 589 studies, which means that this tumor type is very rarely linked with chemical treatment.

Two NTP studies were performed exposing rodents to ELFEMF alone (Boorman et al., 1999; McCormick et al., 1999). In male F344 rats exposed to a range of ELFEMF fields similar to the one used in our experiment, an increased incidence in C-cell tumors of the thyroid gland was seen in groups continuously exposed to 20 mG (2 μT) and 2 G (200 μT) ELFEMF (60 Hz magnetic fields for 18.5 h per day, 7 days per week, for 106 weeks). Because no such effect was seen in male rats exposed to higher field intensities (10 G = 1000 μT) or in female rats from any exposure group, this finding was judged to provide equivocal evidence of carcinogenicity in rats (Boorman et al., 1999). In the mouse study, only 1C-cell adenoma was diagnosed in a female from the 10 G intermittent (1 h on and 1 h off) group (McCormick et al., 1999). As such, the results from the NTP mouse study provided no evidence to support the biological significance of the equivocal findings in male rats.

In our experiment we observed that the administration of ELFEMF alone did not increase the incidence of C-cell thyroid carcinomas, confirming the previous results of the NTP. However, when the administration of 1000 μT ELFEMF occurred in combination with formaldehyde, the incidence of thyroid tumors in males significantly increased (4%), and a statistically significant positive trend was found in the incidence of C-cell thyroid carcinomas, while the administration of formaldehyde alone produced only non-significant increase in males (1.5%) and females (2.5%). Concerning the effect of ELFEMF plus ɣ-radiation, an increased incidence, though not significant, was found for males and females from all groups treated with both ELFEMF and ɣ-radiation. Our observations suggest that ELFEMF might have an enhancing effect on the onset of C-cell carcinomas of the thyroid, only when associated with exposure to formaldehyde or ɣ-radiation (Table 3).

4.5. ELFEMF and hemolymphoreticular neoplasias

As reported by Boorman et al. (2000), several animal studies in rats and mice have been conducted to evaluate the potential leukemogenic effects of 50/60 Hz MF. Four long-term bioassays published up to now failed to show any leukemogenic effects of ELFMF in experimental test conditions. In a large long-term bioassay in which, after exposure to ionizing radiation, groups of female mice were exposed to 60 Hz MF for 120 weeks, no effects on lymphomas and leukemias were observed (Babbitt et al., 1999). In another initiation/promotion study, groups of mice were exposed to DMBA by way of initiation and afterwards exposed to 50 Hz–1000 μT for 16 weeks. The overall incidence of lymphoma was similar among the groups (Shen et al., 1997). In the study performed on F344 rats by NTP, magnetic field exposure did not increase leukemia incidence in any treated groups. In fact, the only statistically significant difference observed in this study was a decreased incidence of leukemia in male rats exposed intermittently to the highest dose (Boorman et al., 1999). Other studies conducted using various transgenic models or transplanted tumor models did not show any leukemogenic effect. In our study, none of the groups of rats exposed to ELFEMF administered alone presented any significant increased incidence of hemolymphoreticular neoplasias confirming the previous results of the NTP (Boorman et al., 1999).

Formaldehyde is considered a well-known leukemogenic agent (IARC, 2012). In our past study on formaldehyde (Soffritti et al., 1989, 2002) and also in the present study, when formaldehyde was administered alone, at the dose of 50 mg/L only non-statistically significant increase in the incidence of hemolymphoreticular neoplasia were observed; but a statistically significant increased incidence was observed in our previous experiment in animals exposed to 100 mg/L of formaldehyde (Soffritti et al., 2002).

In our combination studies, in male rats treated with formaldehyde at 50 mg/L and 1000 μT ELFEMF, we observed a statistically significant increased incidence of hemolymphoreticular neoplasia, and a statistically significant positive trend, using the Cochran-Armitage test. Indeed the increase in hemolymphoreticular neoplasms in male rats exposed to 1000 μT plus 50 mg/L formaldehyde (23.5% versus 16.6% in concurrent controls) may be due in particular to the carcinogenic effects of formaldehyde, since the response in the formaldehyde alone group was 22%.

Gamma radiation exposures are also related to lymphomas and leukemia in both humans and experimental animals (IARC, 2012). Acute administration of γ- radiation at the same dose of 0.1 Gy used in the combination study, did not show any statistically significant increase in hemolymphoreticular neoplasias. By contrast, a significant increased incidence of hemolymphoreticular neoplasias was observed in male Sprague-Dawley rats exposed to 1000μT ELFEMF and 0.1 Gy γ-radiation (Soffritti et al., 2016b). Our observations suggest that ELFEMF might have an enhancing effect on the onset of hemolymphoreticular neoplasias, only when associated with exposure to formaldehyde or γ-radiation (Table 3).

5. Conclusions

The RI has been conducting an integrated experimental project on ELFEMF which comprised life-span studies, including exposure to ELFMF alone and in combination with carcinogenic chemical and physical agents, designed to evaluate the possible role of ELFEMF in the carcinogenic process. In this article we reported that exposure to

ELFEMF alone from prenatal life until natural death did not produce any statistically significant increase in total malignant tumors or any specific tumor in our experimental rat model, confirming previous results of the NTP (Boorman et al., 1999; McCormick et al., 1999). However, when administered in combination with formaldehyde or γ-radiation, ELFEMF seemed to enhance their carcinogenic power and the development of different kind of malignant tumors (mammary gland carcinoma, C-cell carcinoma, hemolymphoreticular neoplasia, and malignant heart Schwannoma). In fact, when administered alone to our rats from the same colony, also the doses of the known carcinogenic agents used for the ELFEMF combination studies (50 mg/l formaldehyde, or 0.1 Gy γ-radiation) failed to induce any statistically significant increase in the incidence of total and specific malignant tumors (Soffritti et al., 2016a, b).

These findings are relevant in term of public health because, in all industrialized countries, exposure to ELFEMF always combines with concurrent exposures to other chemical or physical agents, at low or high doses, in the workplace or in the general environment.

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Ethics review and approval

The experiments were conducted according to the Italian law regulating, at the time, the protection of animals used for experimental and other scientific purposes (Decreto Legislativo 116/1992).

Disclosure statement

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper. They also declare that their funding sources had no direct role in the study design, data collection, analysis and interpretation of the data, in the writing of the manuscript, or in the decision to publish the work.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2018.02.036.

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