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Report of final results regarding brain and heart tumors in Sprague-Dawley rats exposed from prenatal life until natural death to mobile phone radiofrequency field representative of a 1.8 GHz GSM base station environmental emission

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ABSTRACT

Background: In 2011, IARC classified radiofrequency radiation (RFR) as possible human carcinogen (Group 2B). According to IARC, animals studies, as well as epidemiological ones, showed limited evidence of carcinogenicity. In 2016, the NTP published the first results of its long-term bioassays on near field RFR, reporting increased incidence of malignant glial tumors of the brain and heart Schwannoma in rats exposed to GSM – and CDMA – modulated cell phone RFR. The tumors observed in the NTP study are of the type similar to the ones observed in some epidemiological studies of cell phone users.

Objectives: The Ramazzini Institute (RI) performed a life-span carcinogenic study on Sprague-Dawley rats to evaluate the carcinogenic effects of RFR in the situation of far field, reproducing the environmental exposure to RFR generated by 1.8 GHz GSM antenna of the radio base stations of mobile phone. This is the largest long-term study ever performed in rats on the health effects of RFR, including 2448 animals. In this article, we reported the final results regarding brain and heart tumors.

Methods: Male and female Sprague-Dawley rats were exposed from prenatal life until natural death to a 1.8 GHz GSM far field of 0, 5, 25, 50 V/m with a whole-body exposure for 19 h/day.

Results: A statistically significant increase in the incidence of heart Schwannomas was observed in treated male rats at the highest dose (50 V/m). Furthermore, an increase in the incidence of heart Schwann cells hyperplasia was observed in treated male and female rats at the highest dose (50 V/m), although this was not statistically significant. An increase in the incidence of malignant glial tumors was observed in treated female rats at the highest dose (50 V/m), although not statistically significant.

Conclusions: The RI findings on far field exposure to RFR are consistent with and reinforce the results of the NTP study on near field exposure, as both reported an increase in the incidence of tumors of the brain and heart in RFR-exposed Sprague-Dawley rats. These tumors are of the same histotype of those observed in some epidemiological studies on cell phone users. These experimental studies provide sufficient evidence to call for the re-evaluation of IARC conclusions regarding the carcinogenic potential of RFR in humans.

1. Introduction

Early warnings on the potential carcinogenic risks of mobile phone radiofrequency radiation (RFR) raised in the early 2000 when, for the first time, it was published that people using mobile phones had a significant increased risk to develop vestibular Schwannoma and brain tumors (Hardell et al., 2003, 2002). In 2011, the International Agency for Research on Cancer (IARC) classified RFR as possible human carcinogen (Group 2B) based on limited evidence both in humans and experimental animals (Baan et al., 2011; IARC, 2013). Two epidemiological case-control studies resulted more informative for the IARC evaluation, showing that the risk to develop brain tumors and vestibular Schwannoma was increased in people with the highest cumulative use of mobile phones, in people who had used mobile phones on the

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same side of the head as that on which their tumor developed, and in people whose tumor was in the temporal lobe of the brain (the area of the brain that is most exposed to RFR when a wireless phone is used at the ear) (Hardell et al., 2011; Interphone study group, 2010). Another small case series study contributed to the IARC evaluation of evidence for an association of vestibular Schwannoma with mobile phone (Sato et al., 2011). The IARC Working group also noted that well conducted mechanistic studies showed that RFR induced aneuploidy, spindle disturbances, altered microtubule structures or DNA damage in several in vivo and in vitro models (IARC, 2013). Nevertheless, the IARC Working Group evaluated the overall evidence from mechanistic studies as inconclusive (IARC, 2013).

Experimental studies defining the potential carcinogenic effects of exposure to RFR have been largely inadequate because of the exposure conditions applied, because of the limited number of animals used in each experimental group and because of the short duration of the experiments. Since the late 90's, the need for well-conducted studies on laboratory animals has been identified by several public health institutions, including the World Health Organization and the US Food and Drug Administration (FDA, 1999; Repacholi, 1997). Indeed the conduct of cancer bioassays with RFR presents challenges that are not ordinarily met in studies with chemical or other physical agents. For example, the radiation frequency is an important determinant of the specific absorption rate (SAR). The whole-body SAR provides little information about spatial or organ-specific energy deposition, as it strongly depends on field polarization and animal posture. Furthermore, long-term exposure to RF radiation at a fixed frequency and power density will result in substantial changes in SAR over time as an animal gains body weight. Even if the power is adjusted for body weight changes, the spatial distribution can vary (IARC, 2013). Although SAR is a key parameter for thermal RFR effects, several other parameters of RFR exposure such as exposure duration, frequency, polarization, modulation, and environmental magnetic fields are of importance for biological RFR effects (IARC, 2013; Belyaev, 2010). In addition physiological parameters, which may vary in development and between individuals, are of importance (IARC, 2013; Belyaev, 2010). Variability of physiological parameters need to be addressed in long-term bioassays using a large group of animals adequately randomized.

Following the nomination to study cell phone radiofrequency radiation made by the U.S. Food and Drug Administration, the US National Toxicology Program (NTP) started a large systematic and integrated experimental project on RFR, including in vivo long-term bioassays in Harlan Sprague-Dawley (HSD) rats and B6C3F1/N mice exposed to RFR from prenatal life up to 2 years in the situation of near field, reproducing the exposure to RFR generated by the antenna of mobile phone (Wyde et al., 2016).

In 2005, the Ramazzini Institute (RI) started a life-span carcinogenic study on Sprague-Dawley rats to evaluate the carcinogenic effects of RFR in the situation of far field, reproducing the environmental exposure to RFR generated by an 1.8 GHz GSM antenna of the radio base stations of mobile phone (Soffritti et al., 2006, 1999). This is the largest long-term study ever performed in rats on the health effects of RFR, including 2448 animals. The plan of the experiment is reported on Table 1.

The elaboration of the NTP studies have been already completed and a report of partial findings has been recently published (Wyde et al., 2016). The communication of the first important findings of the study was urged by two factors: 1) the fact that also a small increase of the incidence of tumors induced by the exposure to RFR could have great impact for public health; and 2) because the tumors of the brain and heart observed at low incidence in male rats exposed to Global System for Mobile Communications (GSM) – and Code Division Multiple Access (CDMA) – modulated cell phone RFR in the NTP study are of the type similar to the ones observed in some epidemiological studies of cell phone users. Interim cohorts were also examined for evidence of RFR-induced genotoxicity: DNA damage was significantly increased in the frontal cortex of male mice (both CDMA and GSM), peripheral leukocyte of female mice (CDMA only) and hippocampus of male rats (CDMA only) (Smith-Roe et al., 2017). Previous studies have also shown that RFR might disrupt the blood-brain barrier. (Nittby et al., 2008).

Table 1
Long-term bioassay on 1.8 GHz base station RFR, administered at different doses to Sprague-Dawley rats, from prenatal life to spontaneous death: plan of the experiment (Experiment BT 1CEMRF).

Group	Treatment	Animals	
No.	GSM-RFR 1.8 GHz (V/m)[a]	Sex	No.
I	0	M	412
		F	405
		M+F	817
II	5	M	401
		F	410
		M+F	811
III	25	M	209
		F	202
		M+F	411
IV	50	M	207
		F	202
		M+F	409
Total			2448

[a] Treatment with GSM-RFR 1.8 GHz for 19 h/day started on the 12th day of pregnancy and lasted until natural death for groups I, II, III, IV.

The elaboration of the RI study data is still ongoing. However, partial findings are now available and, for the same reasons reported by the NTP, we felt motivated to publish urgently the final results on brain and heart tumors.

2. Materials and methods

2.1. 1.8 GHz base station exposure system and facilities

In order to expose the animals to a mobile phone radiofrequency field representative of a 1.8 GHz base station, a specific radiation system, totally representative of the real environmental situation present in geographic areas close to GSM base station radiation emissions (Fig. 1). The exposure system was designed and constructed by TESEO S.P.A. Company, Turin, Italy. The field generation, in order to be representative of a real GSM field emission, has been modulated in GMSK mode, in CalI operating mode and with the complete Time Slot

assignment. The field emission has been determined in the frequency of 1835 MHz, normally used for GSM services. The intensity ofthe fields generated in the test areas can be defined in the 1–50 V/m range. The RF generation units regulates the output RF levels using a closed loop control system, able to stabilize the generated RF level in an uncertainty level of 1 dB range.

The rats were located in 4 rooms with the same environmental conditions (i.e. temperature of 22 ± 3 °C, a relative humidity of 40–60% and 12 h/day homogeneous diffusion of light). The exposure rooms were totally shielded with RF absorbing material (Hyfral APM12) in order to minimize the effect of field non-uniformity due to reflections and consequent interferences caused by the walls. The shielded rooms ensured a minimum insulation of 30 dB. The rat cages were located in wooden circular-shaped devices. Each single exposure devices served at least 400 rats. All devices were identical and a different intensity of RFR was provided as reported by the experimental design. The exposure system included the following parts: 1) main generator unit; 2) external control panel; 3) main radiator system (transmitting antenna); 4) feedback probe

1) The main generator unit was assembled in a metallic crack to

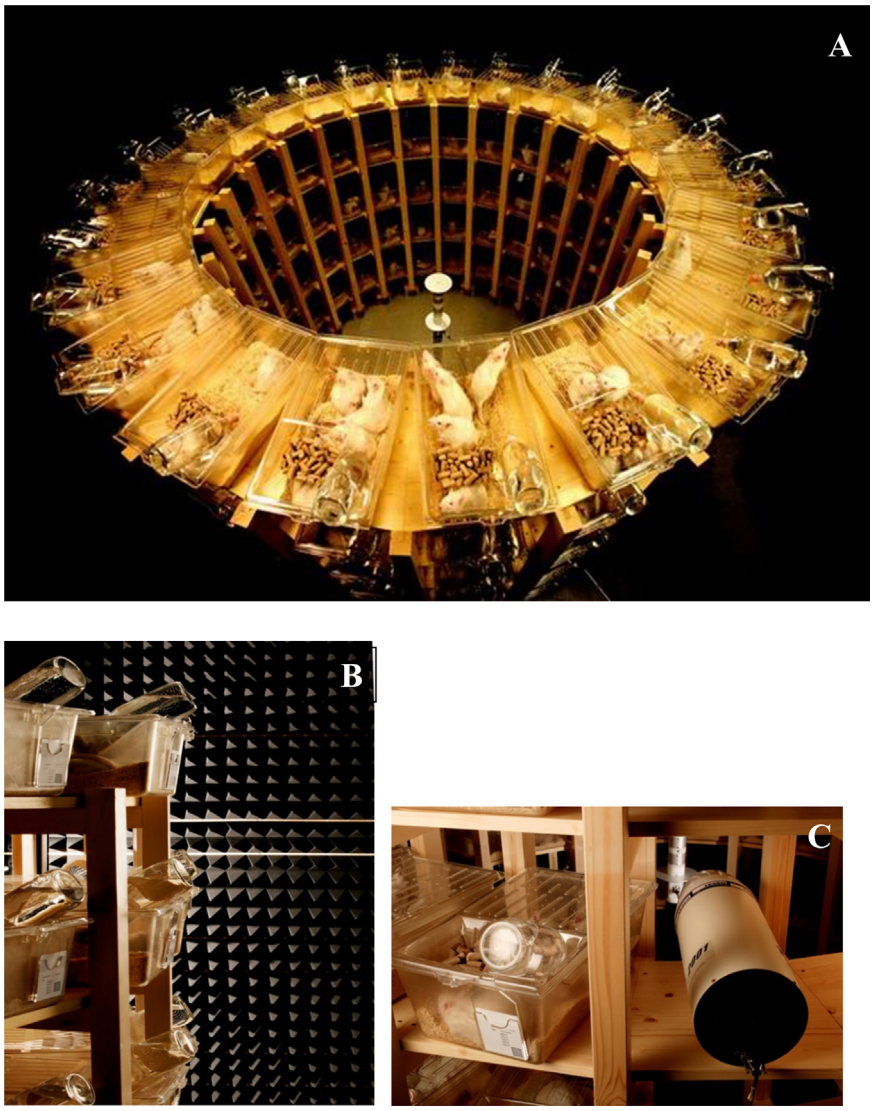

Fig. 1. RI study on 1.8 GHz base station RFR: exposure system. The rat cages were located in wooden circular-shaped devices, as in a sort of condominium. Each single exposure device served at least 400 rats (A). The exposure rooms were totally shielded in order to minimize the effect of field non-uniformity due to reflection and consequent interference caused by the walls (B). Detail of the RFR feedback probe used to measure the field (TESY2001 field sensor) and of the animal cage with methacrylate markers, cover and mangers (C).

produce the following functions: a) generate a GSM signal, with complete channel simulation capability and frequency preselection; b) signal pre – amplifications – age, with gain regulation input; c) final stage, dimensioned for a total power output of plus 50 dBm; d) reference signal loop back input, for closed loops power control; the control was closed at the antenna connection level in order to stabilize at the maximum level the radiation unit RF feed; e) power supply unit; f) cooling unit for continuous use of the device.

2) The external control panel was installed to ensure the personnel health. The exposure system status was controlled using an external control panel installed on the wall in proximity of the room door. The panel was connected to the main unit using a multiple wire, terminated with connectors.
3) The main radiation system was connected to the main generator unit through a low loss coaxial cable. The RFR emission was radiated to the cage using a collinear antenna (a phased array of stacked dipoles) installed in the center of the cage devices. The about radiator was able to transmit an homogeneous RFR far field (with cylindrical distribution of the strength) and shape able to cover the complete height of the cage devices. A reflector, installed on the top of the radiator reduced the vertical emission lobe. Part of the RF power feeding the radiator unit was coupled using a splitter; this signal was used to feed the closed loop level control system installed in the tower rack. The rat cages were distributed on dielectric structures with circular profile; the RF radiator has been installed in the center of each structure. Animals were located on five levels up to a height of about 1.6 m. Each level forms a ring within the toroid at a distance of about 2 m from the toroid center. The horizontal radiation polar diagram of the system allow a total field uniformity better than 3 dB on the total exposure surface. The radiation pattern on the vertical section of the exposure area has a cardioids shape; the vertical opening angle allow a field uniformity better than 3 dB on the exposed area
4) Monitoring probe. The method applied for the measurement of the RFR was completely in compliance with the measurement standards generally applied during “on–site” GSM measurement and evaluation. The about mentioned standard in Italy has been defined in detail in the D.L. 381/98 that outlines the timing and procedures to apply during this measure. The probe used to measure the field was the TESY2001 field sensor. The probe was linked to a personal computer and it was able to show continuously the field intensity value updated every 10 s. The TESY2001_WIN software was used to control the probe, to download the recorded information and produce diagrams of thee field values of the day, week or month.

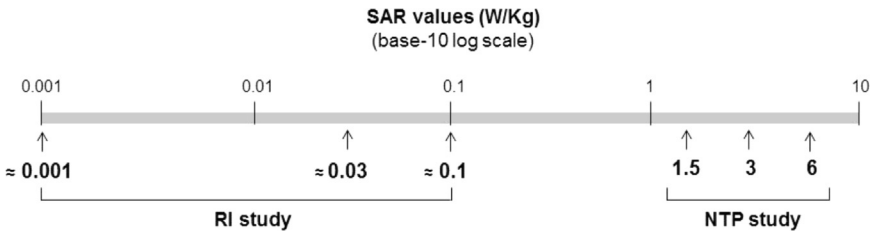

Fig. 2. Comparison between the estimated SAR levels of the RI study (far-field RFR) and the SAR levels of the NTP study (near-field RFR). The estimated exposure SAR levels of the Ramazzini Institute study (0.1 W/Kg, 0.03 W/Kg, 0.001 W/Kg) are significantly lower than the ones used by the NTP (6 W/Kg, 3 W/Kg, 1.5 W/Kg).

The exposure system was positively evaluated by representatives of the US National Institute of Standards and Technology and the US National Institute of Environmental Health Sciences.

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 spontaneous tumor incidence and its fluctuations were based upon data derived from more than 20,000 historical controls.

The animals in experiment were generated in the following way: 1) inbred males and females of the experimental animals used to generate the breeders, were randomized in 4 groups avoiding to have more than 1 brother or sister per group. The size of the breeder groups was proportional to the member of offspring required for the experiment; 2) mating of the studies who generated the experimental animals was strictly outbred (mode possible by pedigree identification number of each animal); 3) all offspring of each litter of these breeders were assigned to the respective planned experimental group.

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. In order to minimize dispersion and interferences, no metal cage accessories were used and instead methacrylate markers, cover and mangers were adopted. 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 (2005–2008) Italian law regulating at the time, the protection of animals used for experimental and other scientific purposes (Legislativo, 1992). The experiment was performed following the principles of Good Laboratory Practice (GLP), with the same standard operating procedure described in our previous studies (Soffritti et al., 2016a, 2016b).

2.4. Treatment

Four groups of 817, 811, 411, 409 male and female Sprague-Dawley rats of our colony were exposed from prenatal life (12th day of mother gestation) until natural death to a 1.8 GHz GSM far field respectively of 0 (control, sham exposure), 5, 25, 50 V/m with a whole-body exposure for 19 h/day, using the remaining 5 h for maintenance purposes, like feed and water refill, cage cleaning, test system verification and check of the health of animals. The plan of the experiment is reported on Table 1.

2.5. 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 $p \leq .05$. 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 $p \leq .05$.

2.6. SAR estimates

SAR estimation has been performed in collaboration with Dr. Franco Maroglio (TESEO S.p.A. Company, Turin, Italy.) and Dr. Perry Wilson (US National Institute of Standards and Technology). The SAR estimate was obtained multiplying a far-field coupling factor (F) with the power density (E^2/η_0). A far-field coupling factor of 0.18 W/Kg/(mW/cm^2) for the rat whole body SAR was derived from previous estimates (Anderson et al., 2004), while η_0 is the free-space impedance ($\eta_0 = 377\,\Omega$). For E = 50 V/m, we get a power density ($E^2/\eta 0$) of 0.66 mW/cm^2 and a whole body SAR of 0.1 W/Kg (0.18 × 0.66). For 25 V/m, we get a power density of 0.17 mW/cm^2 and a whole body SAR of 0.03 W/Kg. For 5 V/m, the power density will be 0.07 mW/cm^2 and the expected whole body SAR 0.001 W/Kg.

In Fig. 2a comparison between the estimated SAR levels of the RI study (far-field RFR) and the SAR levels of the NTP study (near-field RFR).

3. Results and discussion

3.1. Food and water consumption, body weight and survival

The experiment proceeded smoothly 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. 3. 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. Neoplastic lesions

In this article we are reporting the final results from the histopathological evaluation of all brains and hearts of treated and untreated animals. The estimated exposure SAR levels of the RI study (0.1 W/Kg, 0.03 W/Kg, 0.001 W/Kg) are significantly lower than the ones used by the NTP (6 W/Kg, 3 W/Kg, 1.5 W/Kg), but the time and length of exposure of the RI study (19 h/day, continuous exposure, 7 days/week, life-span) was longer than in the NTP study (18 h/day, 10 min on/10 mins off, 7 days/week, 104 weeks). The number of rats analyzed by the RI study (> 200 animals/sex/group, 4 groups, total 2448 animals) is also higher than the NTP study (90 animals/sex/group, 4 groups,

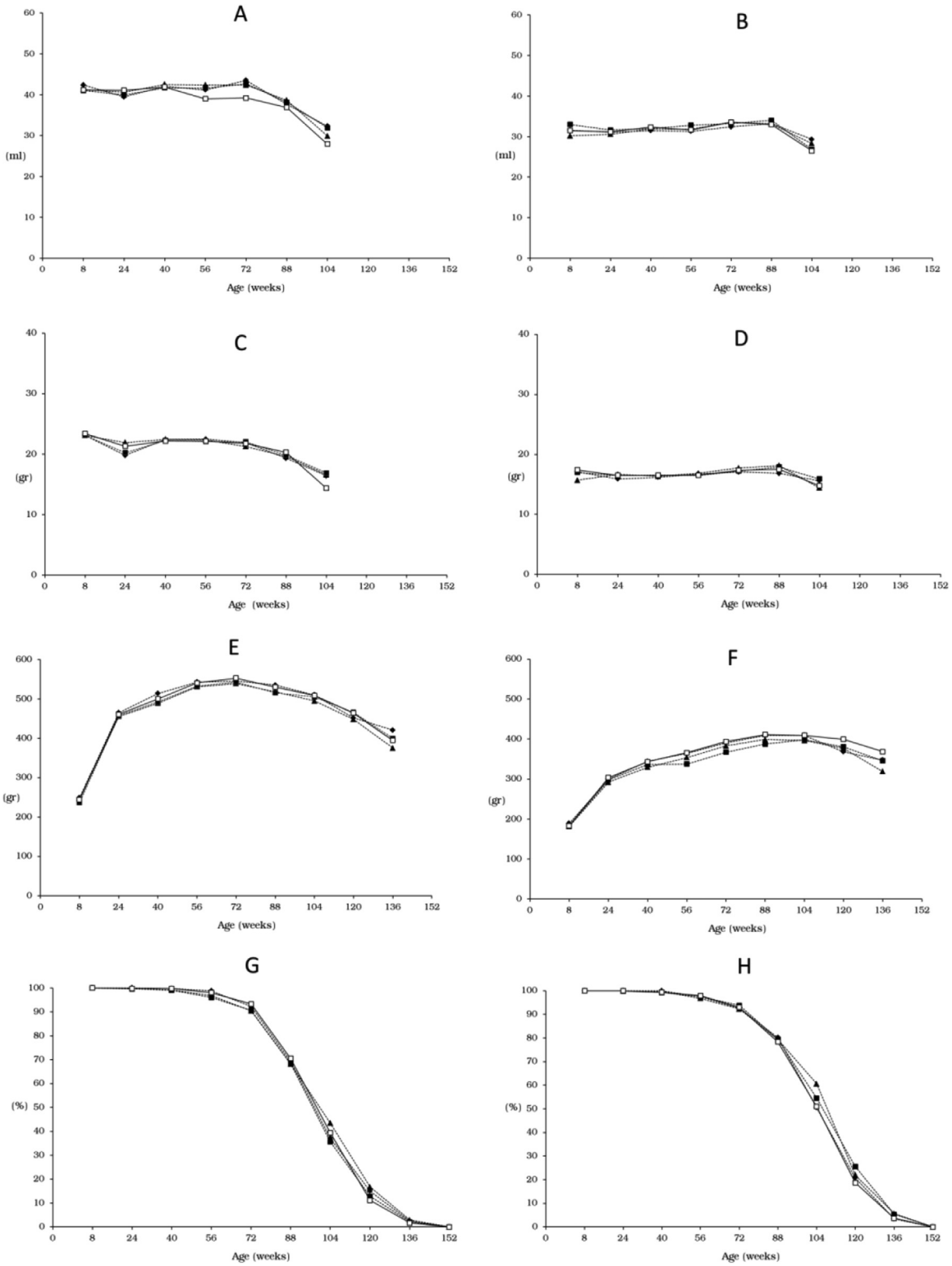

Fig. 3. Male (A) and female (B) water consumption, and male (C) and female (D) food consumption from 8 to 104 weeks of age; male (E) and female (F) mean body weight from 8 to 136 weeks of age; male (G) and female (H) survival index from 0 to 152 weeks of age. Data shown refer to control group (▢), 5 V/m (■), 25 V/m (▲), and 50 V/m (◆) treated group.

total 720 animals).

3.2.1. *Pre-malignant and malignant lesions of the heart*

The incidence of pre-neoplastic and neoplastic lesions of the heart are reported in Table 2. A statistically significant increase in the incidence of heart Schwannoma was observed in treated male rats at the highest dose: 0/412, 3/401 (0,7%), 1/209 (0,5%), 3/207 (1,4%) ($P < .05$; Fisher). Furthermore, an increase in the incidence of Schwann cells hyperplasia was observed in treated male rats at the highest dose, although this was not statistically significant: 3/412 (0,7%), 2/401 (0,5%), 1/209 (0,5%), 5/207 (2,4%). An increase in the incidence of Schwann cells hyperplasia was observed in treated female rats at the highest dose, although this was not statistically significant: 2/405 (0,5%), 0/410, 0/202, 2/202 (1,0%). Schwann cell hyperplasia or

Table 2
Long-term bioassay on 1.8 GHz base station RFR, administered at various doses to male (M) and female (F) Sprague-Dawley rats (Experiment BT 1CEMRF): results on pre-neoplastic and neoplastic lesions of the heart.

Group No.	Dose GSM-RFR	Animals		Hyperplasia Schwann cells		Endocardial Schwannoma		Intramural Schwannoma		Total Schwannoma	
	1.8 GHz (V/m)	Sex	No.	No.	%	No.	%	No.	%	No.	%
I	0	M	412	3	0.7	0	0.0	0	0.0	0	0.0
	(control)	F	405	2	0.5	0	0.0	4	1.0	4	1.0
		M+F	817	5	0.6	0	0.0	4	0.5	4	0.5
II	5	M	401	2	0.5	2	0.5	1	0.2	3	0.7
		F	410	0	0.0	2	0.5	7	1.7	9	2.2
		M+F	811	2	0.2	4	0.5	8	1.0	12	1.5
III	25	M	209	1	0.5	1	0.5	0	0.0	1	0.5
		F	202	0	0.0	0	0.0	1	0.5	1	0.5
		M+F	411	1	0.2	1	0.2	1	0.2	2	0.5
IV	50	M	207	5	2.4	2	1.0	1	0.5	3	1.4*
		F	202	2	1.0	1	0.5	1	0.5	2	1.0
		M+F	409	7	1.7	3	0.7	2	0.5	5	1.2

* Statistically significant $p \leq .05$ using Fisher exact test.

Schwannoma are two proliferative lesions of cardiac Schwann cells in rats (Alison et al., 1987; Novilla et al., 1991). In Sprague-Dawley rats, Schwannoma of the heart is a rare malignant tumor and it occurs more frequently in males rather than females. There are subendocardial and intramural variants of heart Schwannoma, with local invasion more common than distant metastases (Giovannini et al., 1999). Heart Schwannoma occurs in a variety of rat strains and has not been described in mice (Elmore et al., 2017). In a period of over 20 years (1984–2004), the data on historical control rats of the RI show that only 19 cases of Schwannoma have been reported out of 3160 untreated males (incidence 0,6%) and only 10 cases of Schwannoma have been reported out of 3165 untreated females (incidence 0,3%). The pathological diagnostic criteria of Schwann cell hyperplasia and Schwannoma of the heart 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 International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) guidelines (Berridge et al., 2016; Elmore et al., 2017). Furthermore, in order to harmonize the diagnostic criteria, the pathological lesions of the heart observed by the RI, on April 2017 have been screened for second opinion diagnosis in blind by NTP pathologists.

The RI findings on heart tumors are consistent with the results reported by the NTP (Wyde et al., 2016). In the NTP study, for both modulations (GSM and CDMA), there was a significant positive trend in the incidence of Schwannomas of the heart in rats with respect to exposure SAR (6 W/Kg, 3 W/Kg, 1.5 W/Kg). Additionally, the incidence of Schwannomas in the 6 W/Kg males was significantly higher in CDMA-modulated RFR-exposed males compared to controls. In the 6 W/Kg GSM-modulated RFR-exposed males the incidence was higher, but not statistically significant ($p = .052$) compared to controls. Schwann cell hyperplasia of the heart was also observed in three males exposed to 6 W/Kg CDMA-modulated RFR (Wyde et al., 2016).

Our findings are also consistent with the epidemiological evidence, where an increased incidence of tumors of the same cells, vestibular Schwannoma, had been associated with the use of mobile phones (Hardell et al., 2013). Schwannomas in humans might present pre-malignant characteristics: they can progress to malignant lesions (Hasegawa et al., 2013) and they often present molecular characteristics that a typical of pre-malignant lesions, such as aneuploidy (Warren et al., 2003). In particular, genetic factors (e.g. neurofibromatosis) and environmental factors (e.g. gamma radiation) can increase up to 10 fold the risk of malignant progression of vestibular Schwannoma (Seferis et al., 2014). The statistically significant increase in the incidence of heart Schwannomas observed in male rats in the late part of their life, both in the RI and NTP studies, are consistent with the epidemiological findings, where the highest increase in risk of vestibular Schwannoma among humans exposed to RFR was observed in men over 50 years of age with the highest cumulative exposure (Hardell et al., 2013, 2003).

3.2.2. Pre-neoplastic and neoplastic lesions of the brain

The incidence of pre-neoplastic and neoplastic lesions of the brain are reported in Table 3. No statistically significant increase in the incidence of pre-neoplastic and neoplastic lesions of the brain was observed. However, a non-statistically significant dose dependent increase in the incidence of malignant glial tumors was observed in treated female rats: 2/405 (0,5%), 3/410 (0,7%), 2/202 (1,0%), 3/202 (1,5%). No malignant glial tumors were observed in male controls (0/412) and only 2 malignant glial tumors were observed in female controls (2/405, incidence 0,5%). In a period of over 20 years (1984–2004), the data on historical control rats of the RI show that only 15 cases of malignant glial tumors have been reported out of 3165 untreated females (incidence 0,5%) (and 41 cases of malignant glial tumors have been reported out of 3160 untreated males, incidence 1,3%). Therefore, the incidence of malignant glial tumors observed in treated female rats is slightly increased, in particular at the highest dose, if compared with our historical controls. 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). Furthermore, in order to harmonize the diagnostic criteria, the pathological lesions of the brain observed by the RI, on April 2017 have been screened for second opinion diagnosis in blind by NTP pathologists.

The RI findings on brain tumors are consistent with the results reported by the NTP (Wyde et al., 2016). In the NTP study, a statistically significant positive trend in the incidence of malignant glial tumors was reported only in male rats ($p < .05$) for CDMA-modulated RFR exposures. A low incidence of malignant glial tumors was observed in all groups of male rats exposed to GSM-modulated RFR and in different groups of female rats exposed to GSM-modulated RFR and CDMA-modulated RFR exposures. No malignant glial tumors were observed in controls (0/180). Also in the RI study, only 2 malignant glial tumors were observed among controls (2/817, incidence 0.2%), while a slightly overall increased incidence was observed in male and female treated rats (13/1631, incidence 0.8%). It is noteworthy that the estimated exposure SAR levels of the RI study (0.1 W/Kg, 0.03 W/Kg, 0.001 W/Kg) are significantly lower than the ones used by the NTP (6 W/Kg, 3 W/Kg, 1.5 W/Kg).

The increase in the incidence of malignant glial tumors observed in

Table 3
Long-term bioassay on 1.8 GHz base station RFR, administered at various doses to male (M) and female (F) Sprague-Dawley rats (Experiment BT 1CEMRF): results on pre-neoplastic and neoplastic lesions of the brain.

Group	Dose	Animals		Meninges[a]				Glia[b]			
No.	EMF-GSM			Benign Tumors		Malignant Tumors		Glial cells hyperplasia		Malignant Tumors	
	1.8 GHz (V/m)	Sex	No.	No.	%	No.	%	No.	%	No.	%
I	0	M	412	2	0.5	1	0.2	0	0.0	0	0.0
	(control)	F	405	0	0.0	1	0.2	1	0.2	2	0.5
		M+F	817	2	0.2	2	0.2	1	0.1	2	0.2
II	5	M	401	4	1.0	4	1.0	0	0.0	3	0.7
		F	410	4	1.0	1	0.2	0	0.0	3	0.7
		M+F	811	8	1.0	5	0.6	0	0.0	6	0.7
III	25	M	209	1	0.5	1	0.5	1	0.5	2	1.0
		F	202	2	1.0	0	0.0	0	0.0	2	1.0
		M+F	411	3	0.7	1	0.2	1	0.2	4	1.0
IV	50	M	207	2	1.0	0	0.0	0	0.0	0	0.0
		F	202	2	1.0	0	0.0	0	0.0	3	1.5
		M+F	409	4	1.0	0	0.0	0	0.0	3	0.7

[a] Benign and malignant tumors of the meninges include meningioma and granular cell tumors benign and malignant.
[b] Tumors of the glia include oligodendroglioma, astrocytoma, mixed glioma.

the RI experimental study, is consistent with the epidemiological evidence, where an increased incidence of brain tumors of a similar histotype, glioma, had been associated with the use of mobile phones (IARC, 2013; Carlberg and Hardell, 2017). Central nervous system (CNS) tumors are rare in rats(< 0,1%), nevertheless their importance as sentinel tumors in carcinogenesis bioassays has been proved fundamental, since different substances are able to induce increased incidence of these malignancies (Elmore et al., 2017).

4. Conclusions

In 2005, the RI started a life-span carcinogenic study on Sprague-Dawley rats to evaluate the carcinogenic effects of RFR in the situation of far field, reproducing the environmental exposure to RFR generated by 1.8 GHz GSM antenna of the radio base stations of mobile phone. This is the largest long-term study ever performed in rats on the health effects of RFR, including 2448 animals. In this article, we report the final results regarding brain and heart tumors. A statistically significant increase in the incidence of heart Schwannoma was observed in treated male rats at the highest dose (50 V/m). Furthermore, an increase in the incidence of Schwann cells hyperplasia was observed in treated male and female rats at the highest dose (50 V/m), although this was not statistically significant. An increase in the incidence of malignant glial tumors was observed in treated female rats at the highest dose (50 V/m), although this was not statistically significant. Similarly to the NTP (Wyde et al., 2016), the communication of the first important findings of the RI study was urged by different factors: 1) the fact that also a small increase of the incidence of tumors induced by the exposure to RFR could have great impact for public health; 2) The RI findings on far field exposure to RFR are consistent with the results of the NTP study on near field exposure to RFR (Wyde et al., 2016), as both reported an increase in the incidence of tumors of the brain and heart in RFR-exposed Sprague-Dawley rats;and 3) because the tumors of the brain and heart observed at increased incidence in rats exposed to RFR generated by an 1.8 GHz GSM antenna in our study are of the same cytological origin of those observed in some epidemiological studies of cell phone users. These experimental studies provide sufficient evidence to call for the re-evaluation of IARC conclusions regarding the carcinogenic potential of RFR in humans.

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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, 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.

References

Alison, R.H., et al., 1987. Morphology and classification of 96 primary cardiac neoplasms in fischer 344 rats. Vet. Pathol. 24, 488–494.
Anderson, L.E., et al., 2004. Two-year chronic bioassay study of rats exposed to a 1.6 GHz radiofrequency signal. Radiat. Res. 162, 201–210.
Baan, R., et al., 2011. Carcinogenicity of radiofrequency electromagnetic fields. Lancet

Oncol. 12, 624–626.
Belyaev, I.Y., 2010. Dependence of non-thermal biological effects of microwaves on physical and biological variables: implications for reproducibility and safety standards. Eur. J. Oncol. - Libr. 5, 187–218.
Berridge, B.R., et al., 2016. Non-proliferative and proliferative lesions of the cardiovascular system of the rat and mouse. J. Toxicol. Pathol. 29, 1S–47S.
Carlberg, M., Hardell, L., 2017. Evaluation of mobile phone and cordless phone use and glioma risk using the bradford hill viewpoints from 1965 on association or causation. Biomed. Res. Int. 2017, 9218486.
Elmore, S.A., 2017. Proceedings of the 2016 national toxicology program satellite symposium. Toxicol. Pathol. 45, 11–51.
FDA, 1999. Letter of Nomination of Agents to the National Toxicology Program. Available at: 〈https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/wireless051999_508.pdf〉.
Giovannini, M., et al., 1999. Schwann cell hyperplasia and tumors in transgenic mice expressing a naturally occurring mutant NF2 protein. Genes Dev. 13, 978–986.
Hardell, L., et al., 2002. Case-control study on the use of cellular and cordless phones and the risk for malignant brain tumours. Int. J. Radiat. Biol. 78, 931–936.
Hardell, L., et al., 2003. Vestibular schwannoma, tinnitus and cellular telephones. Neuroepidemiology 22, 124–129.
Hardell, L., et al., 2013. Pooled analysis of case-control studies on acoustic neuroma diagnosed 1997–2003 and 2007–2009 and use of mobile and cordless phones. Int. J. Oncol. 43, 1036–1044.
Hardell, L., Carlberg, M., Hansson Mild, K., 2011. Pooled analysis of case-control studies on malignant brain tumours and the use of mobile and cordless phones including living and deceased subjects. Int. J. Oncol. 38 (5), 1465–1474. http://dx.doi.org/10.3892/ijo.2011.947. PMID:213314 4 6.
Hasegawa, T., et al., 2013. Long-term safety and efficacy of stereotactic radiosurgery for vestibular schwannomas: evaluation of 440 patients more than 10 years after treatment with Gamma Knife surgery. J. Neurosurg. 118, 557–565.
IARC, 2013. Non-ionizing Radiation, Part 2: Radiofrequency Electromagnetic Fields. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans/World Health Organization 102. International Agency for Research on Cancer, pp. 1.
INTERPHONE Study Group, 2010. Brain tumour risk in relation to mobile telephone use: results of the INTERPHONE international case-control study. Int. J. Epidemiol. 39, 675–694. http://dx.doi.org/10.1093/ije/dyq079. PMID:20483835.
Kaufmann, W., et al., 2012. Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems. Toxicol. Pathol. 40, 87S–157S.
Legislativo, Decreto, 1992. Decreto Legislativo 116, 1992. Attuazione della direttiva. 86/609/CEE in materia di protezione degli animali utilizzati a fini sperimentali o ad altri fini scientifici [in Italian]. Gazz. Uff. Suppl. Ordin. 5–25.
Nittby, H., Grafström, G., Eberhardt, J.L., Malmgren, L., Brun, A., Persson, B.R., Salford, L.G., 2008. Radiofrequency and extremely low-frequency electromagnetic field effects on the blood-brain barrier. Electromagn. Biol. Med. 27 (2), 103–126.
Novilla, M.N., et al., 1991. A retrospective survey of endocardial proliferative lesions in rats. Vet. Pathol. 28, 156–165.
Repacholi, M.H., 1997. Radiofrequency field exposure and cancer: what do the laboratory studies suggest? Environ. Health Perspect. 105 (Suppl 6), S1565–S1568.
Sato, Y., et al., 2011. A case-case study of mobile phone use and acoustic neuroma risk in Japan. Bioelectromagnetics 32, 85–93.
Seferis, C., et al., 2014. Malignant transformation in vestibular schwannoma: report of a single case, literature search, and debate. J. Neurosurg. 121, Suppl, 160–6.
Smith-Roe S.L., Stout, W.M. Winters, M.D. Hobbs, J. Shepard, C.A. Green, K.G. Kissling, A. Tice, G.E. Bucher, R.R. Witt, J.R., 2017. KL, Evaluation of the genotoxicity of cell phone radiofrequency radiation in male and female rats and mice following subchronic exposure. Environmental Mutagenesis and Genomics Society Proceedings of the 48th Annual Meeting Abstracts September 9–13, 2017, P. 36.
Soffritti, M., et al., 1999. Mega-experiments to identify and assess diffuse carcinogenic risks. Ann. N. Y Acad. Sci. 895, 34–55.
Soffritti, M., et al., 2006. Cancer prevention: the lesson from the lab. In: Biasco, G., Tanneberger, S. (Eds.), Cancer Medicine at the Dawn of the 21st Century: The View from Bologna. Bononia University Press, Bologna, Italy.
Soffritti, M., Tibaldi, E., Padovani, M., Hoel, D.G., Giuliani, L., Bua, L., Lauriola, M., Falcioni, L., Manservigi, M., Manservisi, F., Belpoggi, F., 2016a. Synergism between sinusoidal-50 Hz magnetic field and formaldehyde in triggering carcinogenic effects in male Sprague-Dawley rats. Am. J. Ind. Med. 59 (7), 509–521.
Soffritti, M., Tibaldi, E., Padovani, M., Hoel, D.G., Giuliani, L., Bua, L., Lauriola, M., Falcioni, L., Manservigi, M., Manservisi, F., Panzacchi, S., Belpoggi, F., 2016b. Life-span exposure to sinusoidal-50 Hz magnetic field and acute low-dose γ radiation induce carcinogenic effects in Sprague-Dawley rats. Int. J. Radiat. Biol. 92 (4), 202–214.
Warren, C., et al., 2003. Identification of recurrent regions of chromosome loss and gain in vestibular schwannomas using comparative genomic hybridisation. J. Med. Genet. 40, 802–806.
Wyde, M., et al., 2016. Report of partial findings from the National toxicology program carcinogenesis studies of cell phone Radiofrequency radiation in Hsd: Sprague Dawley® SD rats (whole body exposure). bioRxiv 055699.