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FEDERAL COMMUNICATIONS COMMISSION
OFFICE OF THE SECRETARY

Before the
FEDERAL COMMUNICATIONS COMMISSION
Washington, DC 20554

In the Matter of	)	
	)	
Complaint of Discrimination on the Basis of Handicap Filed by the Cellular Phone Taskforce on February 2, 1997	)	Disabilities Issues Task Force
	)	
Guidelines for Evaluating the Environmental Effects of Radiofrequency Radiation	)	ET Docket No. 93-62

COMMENTS AND ERRATA

In support of the Cellular Phone Taskforce's Appeal in the above matter, a new study published in Bioelectromagnetics 18:455-461 (1997) provides evidence of biological harm at exposure levels of between 0.1 and 1 microwatt per square centimeter in the VHF and UHF bands. It is attached as an Exhibit. The article is by Magras and Xenos, and entitled "RF Radiation-Induced Changes in the Prenatal Development of Mice." This is further evidence that the electrically sensitive will be discriminated against by radiofrequency radiation permitted at these power levels, since the electrically sensitive are more sensitive to the biological effects than the rest of the population.

Errata

The Cellular Phone Taskforce's Appeal of October 6, 1997 contains an inadvertant typographical error. The date in the next to last line of section 5 ("Conclusion") on page 4 should read "August 6, 1996." In addition, for clarity, that line should

0+6

read, "adopted <u>into</u> <u>effect</u> by the Commission on August 6, 1996" (underlined words added).

Respectfully submitted,

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Bioelectromagnetics 18:455–461 (1997)

RF Radiation–Induced Changes in the Prenatal Development of Mice

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The possible effects of radiofrequency (RF) radiation on prenatal development has been investigated in mice. This study consisted of RF level measurements and in vivo experiments at several places around an "antenna park." At these locations RF power densities between 168 nW/cm^2 and 1053 nW/cm^2 were measured. Twelve pairs of mice, divided in two groups, were placed in locations of different power densities and were repeatedly mated five times. One hundred eighteen newborns were collected. They were measured, weighed, and examined macro- and microscopically. A progressive decrease in the number of newborns per dam was observed, which ended in irreversible infertility. The prenatal development of the newborns, however, evaluated by the crown-rump length, the body weight, and the number of the lumbar, sacral, and coccygeal vertebrae, was improved. *Bioelectromagnetics 18:455–461, 1997.* © 1997 Wiley-Liss, Inc.

Key words: RF radiation effects; prenatal development; mice development

Five years ago the "antenna-park of Thessaloniki" progressively developed on the top of the nearby mountain Chortiatis, 1.5 km away from a small village of the same name. Today, almost 100 commercial TV and FM-radio broadcasting transmitters in the VHF and the UHF bands are situated there. The antennas are installed on towers well visible from a large part of the village. Living so close to the antennae and the vast amount of RF power they transmit, which is of the order of 300 kW, the people of the village Chortiatis, anxious for their health, encouraged the author to undertake a research program.

The hypothesis that RF radiation may adversely affect the health of the animal organism is still under consideration in public and scientific forums. One of the critical issues seems to be the RF effects on the reproductive process [Chernoff et al., 1992]. Numerous studies dealing with this subject ended up with seemingly contradictory results. Therefore, an "in vivo" study on experimental animals sensitive to RF radiation, was chosen. Based on the relevant literature, this research investigated RF radiation effects on the reproductive system, particularly on prenatal development. The mouse was selected as the experimental animal, because it is easily manipulated in the environment in which the experiments had to take place. Of course, experimenting at the mountain sites, far from the easily controlled laboratory conditions, might add a certain amount of uncertainty; therefore, these experiments should be considered preliminary.

MATERIALS AND METHODS

We used a total of 36 mice (18 females and 18 males), 2 months old and sexually mature (BALB/c/f breed colony). Breeding colony virgin males and females were obtained from the "Theageneion Anticancer Institute of Thessaloniki." The use of these experimental animals was approved by the Veterinary Service of the Municipality of Thessaloniki, according to the provisions of the laws 1197/81 and 2015/92 and the Presidential Decree 160/91 of the Greek Democracy. Upon arrival, all experimental animals were quarantined for 2 weeks to discover and to allow them to acclimatise the mountain environment, an altitude ranging between 570 (position h) and 730 m (position d) above sea level. All the mice were healthy at the end of this period and showed no signs of illness during

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TABLE 1. Light–Dark Cycle during the Experimental Matings

Gestation	Date	Day		Night	
		Min	Max	Min	Max
1st	25.5–16.6	14.28	14.47	09.13	09.32
2nd	21.6–12.7	14.37	14.48	09.12	09.23
3rd	6.9–29.9	11.54	12.45	11.15	12.06
4th	7.10–28.10	10.45	11.35	12.25	13.15
5th	23.11–13.12	09.34	09.55	14.05	14.26

the course of the study. Tap water and certified feed (Greek Sugar Factory) were freely available.

The mice were maintained under natural lighting, both during the daytime and at the night (Table 1). Twelve Plexiglas cages transparent to RF radiation, were placed at several locations with one female in each cage. Each female was caged with one male for 12 h. Vaginal smears were taken the next morning and successful mating was identified by the presence of sperm. The day on which evidence of mating was observed was considered to be the first day of gestation. The litters were collected in the first 2 h after delivery and were moved to the laboratory for examination. After a period of recovery, the same mating procedure was repeated for each dam. Five experimental pregnancies were carried out in a period of almost 6 months.

The first pregnancy of the experimental animals took place in eight selected positions (a–h, Fig. 1), some close to the "antenna-park" and some near the village of Chortiatis. Then the experimental animals were moved to two positions, because these positions presented almost the same RF radiation levels with those initially selected and the experiment could be managed more effectively. Six dams (labelled as group A), initially placed at positions a, b, c, and d, with their males, were moved to the position d (Refuge of Hypaithrios Life). The other six dams (labelled group B), with their males, initially placed at positions e, f, g, and h were moved to position h (Public Primary School of Chortiatis). These two positions were selected because the most important living conditions, i.e., light, temperature, ventilation, food, etc., were the same.

Finally, all the experimental animals were moved to position i (Laboratory of Anatomy, School of Veterinary Medicine, University of Thessaloniki) about 10 km away from the Mountain Chortiatis, in the city of Thessaloniki, for the fifth pregnancy. This relocation was done to seek an indication of a possible reversibility of the observed phenomena. In fact, we wanted to repeat the experiment in an environment almost free of RF. An extra group of six couples of mice were mated once and used as controls in the laboratory (posi-

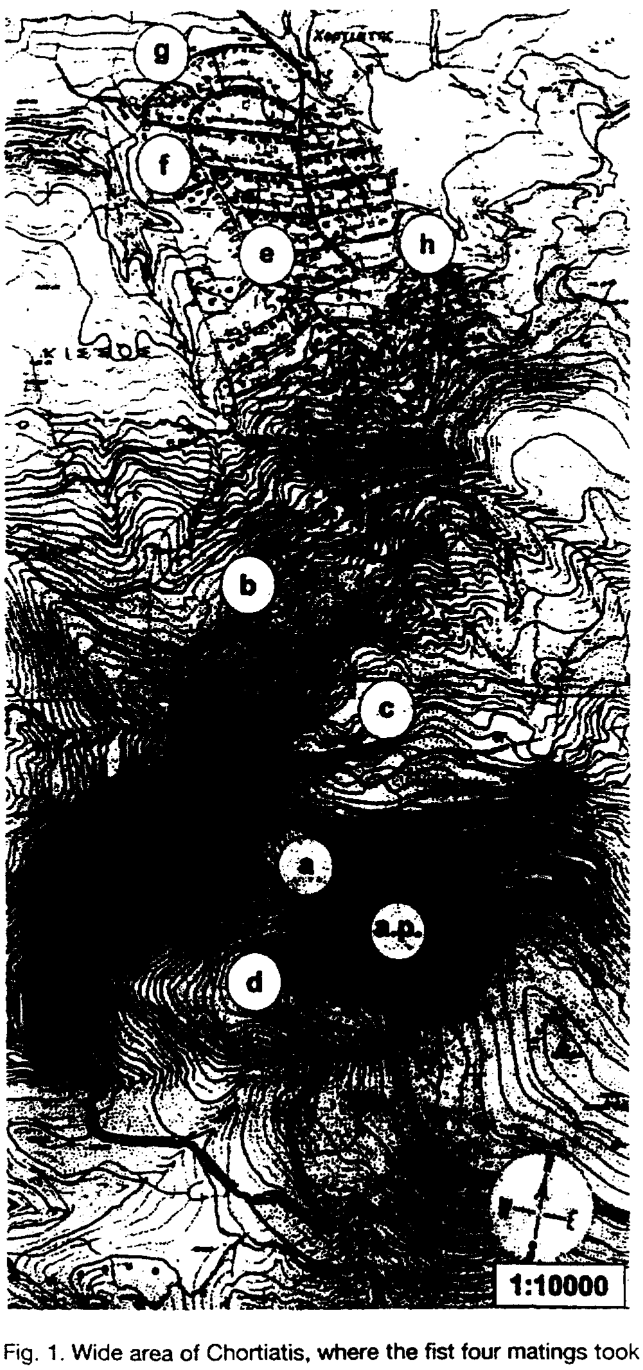

Fig. 1. Wide area of Chortiatis, where the fist four matings took place.

tion i), far from the "antenna park" in a more or less free-of-RF radiation environment.

It was extremely difficult to use RF-free controls at the mountain sites, because it was almost impossible to make "electromagnetically screened cages." Such a cage should ideally provide high (of the order of 30 dB) screening at the frequency range between 88.5 and 950 MHz (Commercial Radio FM band, UHF TV band, and Mobile Communication band), and therefore would require a very dense and well-grounded, highly conductive external metal grid. Obviously, mice could hardly survive in such cages for about 5 months.

The litter was considered to be the experimental unit for the analysis of data. We measured the crown-rump length, the body weight, the number of the posterior (lumbar, sacral, and coccygeal) vertebrae, the congenital malformations, and the ossification of the skeleton.

The RF power was measured in each position, using an electric field meter and a low gain (4 dB) wide-band (80–900 MHz) log-periodic antenna and spectrum analyser. To obtain comparable results the "IEEE std. C95.3.1991" was used. On the third floor of the public school, where the mice were situated, a 360 degree integration was also performed, due to the directivity of the measuring antenna together with the close proximity of the walls and metal furniture. Wherever iron bars or metal screens existed in front of the windows, two series of measurements were carried out; one on each side of the screen.

The collected newborns were killed for examination. Their crown-rump length was measured, and they were weighed and inspected under the dissecting microscope for external congenital malformations. Then they were fixed and subsequently cleared and stained in toto by a double staining of their skeleton [Peters, 1977]. The procedure was lightly modified as follows:

The newborns were fixed with alcohol 86% for 3 days; their skin, eyes, and viscera were removed; then they were immersed for 3 days in alcohol 100% and for 4 days in a mixture of alcohol 100% and ether 1:1. They were stained for 1–2 days with blue alcyan coloration [alcohol 86% 80 ml, acetic acid 20 ml, alcyan blue 20 mg] until the nonmineralised cartilagenous parts of the bones became blue. They were immersed in alcohol 100% for 4 days. Then they were stained for 12–24 days with red alizarin coloration [KOH 1 g, H_2O 100 ml, alizarin solution (alcohol 86% saturated with alizarin red S) 0.1 ml] until the ossified parts of the bones became red. They were immersed in solution Mall I (KOH 1 g, distilled water 80 ml, glycerine 20 ml) until the transparency of their body was completed. Finally, they were stored in a conservation solution (distilled water and glycerine 1:1, with

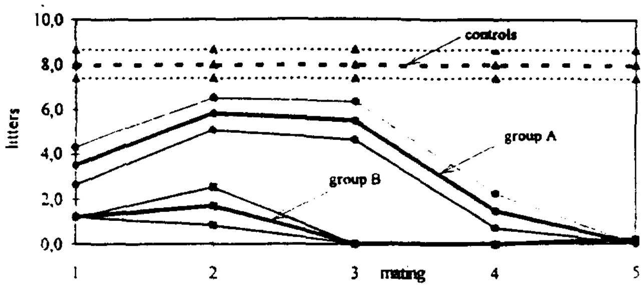

Fig. 2. Comparison of the mean values ± standard deviation of number of newborns per dam and mating from all experimental groups.

some thymol crystals as contamination prevention). The stained newborns were inspected for skeletal defects as well as for the degree of ossification of their bones. The ossification of the skeleton and particularly of the vertebrae is an excellent and creditable indicator of the prenatal exposure to noxious agents and can be a measure of development delay.

RESULTS

The RF power levels measured, although below the limits proposed by the "ENV50166-2" and the "IEEE C95.1.1991" standards, are high and well above the power levels that are likely to be measured in other European or U.S. residential areas. In fact, on the third floor of the public primary school (position h), an average power density of 1.053 $\mu W/cm^2$ was found, equivalent to a specific absorption rate of 1.935 mW/kg. In the Hypaithrios Life Refuge (position d) the average power density in which the mice were located was of the order of 168 nW/cm^2. This reduced level was due to the screening effect of the iron bars in front of the windows, which gave an 8–10 dB RF-power decrease. The average power density levels in position i (Laboratory of Anatomy, School of Veterinary Medicine, University of Thessaloniki), where the controls were placed and the fifth experimental matings were performed, was 40 dB weaker.

The number of the littered newborns by the experimental dams of groups A and B were, compared with those littered by the controls, progressively reduced from the first to the fifth pregnancy. This reduction is more evident in group B and is clearly shown in Table 2 and in Figure 2.

On the other hand, the rest of the four measured parameters, i.e., the crown rump length and the weight and the number of the lumbar, sacral, and coccygeal vertebrae increased in the newborns from groups A and B compared with the controls. This was more evident in group A than in group B (Table 2 and Fig. 3). A

TABLE 2. Statistical Characteristics of All Four Measurable Parameters per Dam, per Group, and per Gestation

Mating	Litters per dam mean ± s.d. median	Length (cm)	Weight (gr)	Vertebrae
Group A (6 dams)				
1st (25.05.1995)	3.5 ± 0.9	1.47 ± 0.13	2.71 ± 0.09	31.48 ± 1.43
	4.0	1.44	2.69	32.07
2nd (21.06.1995)	5.8 ± 0.7	1.25 ± 0.06	2.55 ± 0.05	24.28 ± 0.97
	7.0	1.22	2.50	24.29
3rd (08.09.1995)	5.5 ± 0.9	1.72 ± 0.25	2.71 ± 0.13	28.72 ± 1.92
	6.5	1.72	2.60	28.71
4th (07.10.1995)[a]	1.5	1.10	2.47	23.22
	0.0	1.10	2.47	23.22
5th (23.11.1995)[a]	0.0			
	0.0			
Mean value	3.3	1.39	2.61	26.93
Group B (6 dams)				
1st (25.05.1995)[a]	1.2	1.19	2.53	28.57
	0.0	1.19	2.53	28.57
2nd (21.06.1995)	1.7 ± 0.9	1.25 ± 0.04	2.60 ± 0.06	28.55 ± 1.14
	1.5	1.26	2.58	27.26
3rd (08.09.1995)[a]	0.0			
	0.0			
4th (07.10.1995)[a]	0.0			
	0.0			
5th (23.11.1995)	0.2	1.05	2.50	30.00
	0.0	1.05	2.50	30.00
Mean value	0.6	1.16	2.54	29.04
Controls (6 dams)				
1st (23.11.1995)	8.0 ± 0.07	0.96 ± 0.15	2.38 ± 0.02	19.59 ± 0.47
	7.5	0.97	2.37	19.52
Mean value	8.0	0.96	2.38	19.59

[a]Single or no gestation.

thorough external and internal examination under the dissecting microscope revealed only one case of extensive and two cases of limited malformation. No retardation of skeletal ossification worth mentioning was observed; only five cases out of 116 showed limited retardation. It has to be noted here, that the evaluation of the skeleton ossification was focused in the bones of the forelimbs and hindlimbs and in the lumbar, sacral, and coccygeal vertebrae.

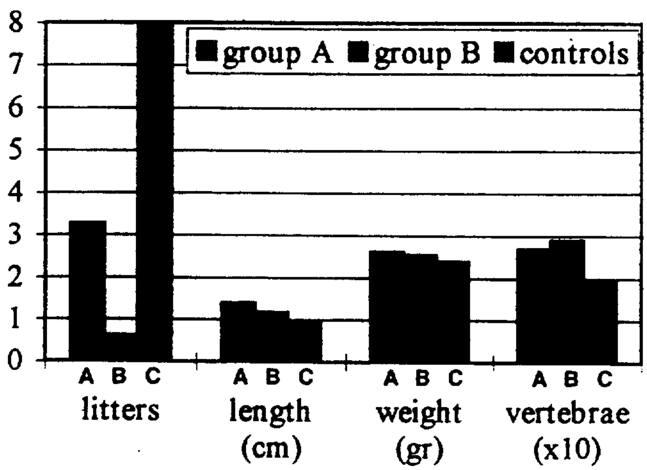

Fig. 3. Comparison of the mean values of all four measurable parameters for all gestations. Controls (C).

DISCUSSION

To study effects of a possibly noxious agent on a mammalian embryo, three groups should be considered: the embryos, the dams, and the males. In this work, all three have been studied: the infertility for dams and males, the lethality for embryos, the teratogenicity or the reduction in deformity for foetuses, or any combinations of them. They all have been considered by exposing male and female mice (before and during pregnancy) to an RF-radiation environment close to the "antenna park."

Infertility and lethality were assessed by counting the number of their newborns, whereas the possible teratogenicity and the reduction deformity by autopsy was considered by the study of the embryonic skeletons. An important stage in this study was the examination of the skeletons, since the ossification of the bones is considered an excellent and creditable indicator of the prenatal exposure to noxious agents and can be a measure of development delay. In the beginning of organogenesis, the neural tube functions as a precursor of the cartilages and bones of the developing skeleton [Noden and Delahunta, 1985]. Teratogenic factors of any kind, that affect the embryonic nervous system, result in structural defects of the skeletal components. Therefore, to detect the teratogenic action of a factor on the embryonic nervous system, it is technically convenient to study the foetal skeleton rather than the embryonic nervous system itself.

A very important result of this experimental study (Table 2 and Fig. 2) is a progressive decrease of the number of the size of the litters of the dams of group A (position d) and group B (position h), compared with the controls (position i) and with the breeding history of these mice. Mice from the BALB/c/f breeding colony obtained from the "Theageneion Anticancer Institute of Thessaloniki" have been used for years in our laboratory for reproduction. Repeated pregnancies with a recovery period of 1–4 weeks for over a year, had never affected the fertility of the dams or any morphological parameters of the offspring, a fact that to our knowledge has not been questioned in the available literature.

It is worth noting that the RF power density levels, although very different from place to place, were very low and well below the CENELEC and IEEE relevant standards. Yet, it should be pointed out that:

(a) the experimental animals lived in this environment for 6 months, which is a long period of time,

(b) there was a considerable difference in power density levels of the order of 10 dB between the two main positions d and h and almost of 40 dB between d and i,

(c) there is a considerable difference between the volumes and consequently the body mass of the adult mouse and other experimental animals used as models in the international standards applied to humans.

The interpretation of our observations could follow various directions. The most popular view in numerous studies of the relevant literature, that this is a consequence of the overheating of the irradiated testis [Lary et al., 1986, 1987; O'Connor, 1980)] could be considered. On the other hand, the assumption that RF and microwave radiation effects are limited to heating has been questioned in a series of studies [Cleary, 1988, 1990]. The exposure conditions in these "in vivo" studies may suggest a thermal component of RF-induced testicular damage. However, interpretation of these data with respect to damage thresholds or interaction mechanisms is difficult. This difficulty is due to a number of factors, including the time, intensity, or both, the variations in species sensitivities, and the frequency-dependent non-uniform microwave energy absorption in tissue. Consequently, although these findings seem to be consistent with a hypothesis that the RF-induced heating is associated with testicular damages, the borderline between the "direct" effects of radiation and the effects that are indirectly associated with the tissue heating is not very clear.

Our observations could also be attributed to an intra-uterus death of the irradiated embryos in the early stages of the prenatal development, a speculation that could not be investigated in our experimental design because it required a postmortem autopsy of the dam. On the other hand, the prerequisite to these scenarios is a large RF power density, whereas the power densities we measured were of the order of $\mu W/cm^2$ or nW/cm^2, rather than mW/cm^2, or in terms of specific absorption rate (SAR), mW/kg rather than W/kg. Therefore, we cannot exclude the possibility of an indirect nonthermal mechanism focused on the endocrinological axon hypophysis-gonads that causes infertility to the males or the females [Thuery, 1991].

It should be noted here that the male experimental animals progressively developed a very bad physiological condition (rough hair, emaciation, etc.), not correlated to any other sickness symptoms, during their stay at the experimental positions a–g. Therefore, despite of the limited amount of data, the duration of the exposure to low intensity RF electromagnetic fields seems to be a repression parameter. In fact, chronic or long-term exposure to low intensity electromagnetic fields is generally associated with adverse results [Lary et al., 1983]. The most peculiar findings of this study were the increases in the crown-rump length, the body weight, and the number of the posterior vertebrae (lumbar, sacral, and coccygeal) of the experimental offsprings compared with the controls (Table 2, Fig. 3).

It must be noted that a study of mice [Jensh et al., 1977; 1978a; 1978b] under low levels of irradiation during the whole period of a single gestation (10 and 20 mW/cm^2) had no effect on maternal, foetal, or placental masses and no effect on the frequency of resorption, foetal death rate, size of litter, sex of the newly born, and their ability to perform. Other studies [Michaelson et al., 1976] reported a faster development of rat foetuses. This finding agrees with another report [Johnson et al., 1977] that noted an increase in the weight of newly born rats and a premature opening of the eyes after prenatal irradiation (5 mW/cm^2 at 918 MHz, for 380 h), as well as an impaired ability to learn. On the other hand, other studies found lower average weight at birth. At medium power density levels (10, 20, and 50 mW/cm^2, at 2375 MHz), which are above the limits imposed by CENELEC and the relevant IEEE standard, the reproductive capacity of mice was somewhat impaired, with smaller litter size and a rise in neonatal mortality, which is a direct function of the power flux density [Il'Cevic and Gordodeckaja, 1976; McRee, 1980].

Although it is difficult to explain this foetal development increase, we believe that it could be due to a favourable placental nourishment of the foetuses during the pregnancy. In fact, this finding could be associated with:

(a) reproductive causes, i.e., blood-flow to a smaller number of foetuses, because of the reduction of the fertility of the irradiated males or females,

(b) thermal causes, i.e., possible increase of the blood flow of the dams, directly due to the RF irradiation,

(c) endocrinological causes, i.e., increase of the somatotrophic hormone because of the RF irradiation and

(d) environmental causes, i.e., the vasodilatation and partial increase of the blood pressure of the experimental dams because of the mountain altitude.

Of course combinations of these possibilities cannot be excluded.

According to various references [Tell and Harlen, 1979; Lu et al., 1980; Deschaux et al., 1983] discrepancies between the results of experiments may be due to different experimental conditions, random formation of hot spots in the glands and the hypothalamus, or a variety of other factors, as the cicardian rhythm and differences between species. With the exception of the high power effects on testicles, that do not belong to the endocrine ensemble, the interaction seems to involve the pituitary gland or even the central nervous system rather than the terminal glands.

We would close this discussion with what Jacques Thuery wrote (1991), that the true state of affairs is probably far more complex, but the available data are not sufficient to allow us to outline it more clearly, and that all attempts to extrapolate these results to humans lead to very high power densities, partly because geometric resonance effects are very significant in small animals. Consequently, taking into account the constant exposure of the human population living close to the "antenna park" to low intensity RF radiation, these adverse health effects in mice resulting from chronic or prolonged exposure may prove of importance in the near future. Indeed, there is evidence that chronic exposure to low-intensity RF radiation may be associated with health effects different to embryo-toxicity [Salford et al., 1992; Cleary, in press].

The findings of this preliminary experimental study have led to several conclusions. Of course, the final word to the problem in question has not been said as yet. Therefore, more work is called for; laboratory-based simulation might provide valuable information.

ACKNOWLEDGMENTS

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